Supercritical CO_2 impregnation of α-tocopherol into PET/PP films for active packaging applications

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Abstract

Supercritical carbon dioxide impregnation technique was used to adsorb α-tocopherol (TOC), a natural antioxidant, on monolayer and multilayer polyethylene terephthalate (PET)/polypropylene (PP) films to obtain active packaging. Supercritical impregnation experiments were performed at 17 MPa and 40 °C using different supports: PP films, PET films (ut-PET), corona discharge treated PET surface (ct-PET) films, PET/PP films. Supercritical carbon dioxide (SC-CO_2) impregnation revealed to be an effective technique in the attainment of active packaging films. Indeed, very high amounts of TOC were impregnated (up to 3.2 mg_{TOC}/cm^2_{film} considering a monolayer PP film and up to 2.66 mg_{TOC}/cm^2_{film} considering a multilayer film). Field emission scanning electron microscopy (FESEM) showed how the impregnation with TOC modified the film surface (which became heterogeneous). Fourier transform infrared spectroscopy (FT-IR) revealed the presence in the impregnated films of both TOC and polymers characteristic bands. Differential scanning calorimetry (DSC) indicated that the presence of SC-CO_2 induced a slight reduction in the crystallinity percentage of the polymers. Moreover, migration of α-tocopherol from the packaging films in a food simulant was studied using UV–vis spectroscopy, and the effectiveness of the supercritical impregnation to obtain the controlled-release of the active agent was verified. Finally, antioxidant activity tests confirmed the preservation of TOC antioxidant power after its impregnation on monolayer and multilayer films.

Keywords: PET/PP multilayer film; supercritical impregnation; migration tests; antioxidant activity.

1. Introduction

In recent years, the development of advanced packaging solutions gained considerable attention to prolong food shelf-life and preserve its quality and safety. One of the most attractive strategies turned out to be the active packaging, which provides the controlled release of antioxidant or antimicrobial agents included in some kinds of active packaging [1-4]. The active packaging is considered an effective response against lipid oxidation [5], which is one of the key causes of food deterioration.

A monolayer or a multilayer film containing the antioxidant can constitute the active packaging. In particular, a multilayer can consist of two layers (i.e., outer layer and active matrix layer) or of four layers (i.e., outer layer, barrier layer, active layer and control/inner layer) [5, 6]. Briefly, the barrier layer is placed between the outer layer and the active layer, and it allows to reduce the undesired migration of the active agent towards the outside of the package. The active layer, instead, incorporates the active compound. If a four-layer film is used, the inner layer, directly joined to the active layer, permits to control the active agent's migration towards the food surface.

Active packaging films can be carried out through many conventional techniques, such as extrusion or solvent casting, and the active agent can be contained into the plastic film or coated onto it. Up to now, different routes were attempted to reach the controlled-release of the active compound, as, for example, its incorporation into porous matrices [7, 8] or its encapsulation in a polymeric shell [9-12]. However, traditional methods for active packaging production have some drawbacks, mainly the deterioration of the active agent due to the high temperatures used during the process, that can also lead to the partial volatilization of the active compound with the solvent used. As a result, low penetration of the active agent in the polymer substrate and reduced loading efficiencies are generally obtained [13, 14]. An alternative solvent-free process is the impregnation using supercritical carbon dioxide (SC-CO_2) [15, 16] since it is possible to operate at low-temperature thanks to SC-CO_2 mild critical conditions (T_c=31.1 °C, P_c=7.38 MPa) [17, 18]. SC-CO_2 allows solubilizing and, then, incorporating different kinds of organic compounds into polymer matrices because it has high diffusivity and acts as a plasticizing and a swelling agent for polymers [19]. However, the impregnation of a specific compound into a polymer matrix is strongly affected by the interaction between an active solute/SC-CO_2/polymer substrate [19].

In the last few years, some articles were published on this subject, showing the effectiveness of the supercritical impregnation for food packaging applications [14, 20-24]. For example, Milovanovic et al. [14] loaded thymol into poly(lactic acid) (PLA)/poly(ε-caprolactone) (PCL) films obtaining a strong bactericidal action against *Escherichia coli* and *Bacillus subtilis*. Villegas et al. [22] impregnated cinnamaldehyde, a flavonoid present in the cinnamon essential oil, into PLA films. The loaded films exhibited good antibacterial activity and excellent thermal and mechanical properties, considering that they showed higher flexibility and greater resistance with respect to the unprocessed PLA films. Belizón et al. [23] produced active multilayer polyethylene terephthalate (PET)/polypropylene (PP) films containing antioxidant mango polyphenols. The latter two polymers are among the most commonly used ones for the development of active packaging [25]; the active films obtained by Belizón et al. [23] through supercritical impregnation preserved foods from degradation over an extended period. The same multilayer substrate was chosen by Cejudo Bastante et al. [24] that studied the supercritical impregnation of caffeic acid and of an olive leaf extract in PET/PP films, evaluating the antioxidant activity using the DPPH assay. In that paper, the potential of supercritical impregnation was highlighted, as well as the difficulty of optimizing the operating conditions of the process because of the numerous variables, often linked one to each other.

Categorized as GRAS ("Generally Recognized As Safe") by the US Food and Drug Administration, α-tocopherol (TOC) was widely proposed as a natural antioxidant for the production of controlled-release packaging [7, 8, 26-29], in order to reduce food oxidation, but also as a stabilizer for polymer processing [5]. For example, Chen et al. [28] used the cast film extrusion to produce ethylene vinyl alcohol and low-density polyethylene films containing 1500 ppm tocopherol and 1500 ppm quercetin, for the long-term inhibition of lipid oxidation; whereas, Hwang et al. [30] added TOC and resveratrol at various concentrations (0.01-0.04 $mg_{antioxidant}/mg_{film}$) to PLA film by a melt compounding and compression molding process for packaging applications. On the other hand, to our knowledge, there are no studies in the literature on supercritical impregnation of TOC for these purposes (reduction of food oxidation and stabilizing polymer processing), although it was applied in other fields, such as pharmaceutical one [31, 32]. For example, De Marco and Reverchon [31] impregnated TOC into maize starch aerogel to enhance the vitamin bioavailability, improving its dissolution rate, whereas Yokozaki and Shimoyama [32] loaded TOC into silicone aerogel to reduce the diffusivity of timolol maleate, an ocular drug used in the treatment of glaucoma.

This work aims at obtaining an active packaging, using SC-CO_2 that incorporates TOC in multilayer PET/PP films. To optimize the active packaging, a comparison between a film in which TOC is impregnated on the surface of untreated PET (ut-PET) and a film in which TOC is adsorbed on the surface of PET subjected to corona discharge treatment (ct-PET) was performed. The confirmation of the attainment of an active agent controlled-release system was given by migration tests in food simulant and by the antioxidant activity quantification.

2. Materials and methods

2.1 Materials

Ethanol (EtOH, purity 99.9 %) and α-tocopherol (TOC, purity 96%) were purchased by Carlo Erba (Cornaredo, MI, Italy) and Sigma-Aldrich (Milan, Italy), respectively. Morlando Group S.r.l. (Naples, Italy) supplied carbon dioxide (CO_2, purity 99 %) . The distilled water was obtained using a laboratory water distiller, purchased by ISECO S.p.A. (Aosta, Italy).

Bi-oriented Polyethylene Terephthalate (PET) film, with 23 μm thickness, was supplied by Nuroll S.p.a. Polypropylene (PP) film, with 33 μm thickness, was provided by Ifis S.p.A (Marcianise, Italy).

Multilayer PET/PP films were obtained by lamination technique, joining the single layers PP and PET films through the two-component polyurethane solvent based adhesive Polurene FP44A/58-01, supplied by Sapici S.p.a (Cernusco sul Naviglio, Italy), with an application weight of 3 g/m^2 by dry content. For the preparation of multilayer films, both monolayer PET and PP films were subjected to corona discharge treatment on the surface, to obtain sufficient wettability and adhesion capability [33]. The obtained multilayer film has a thickness of approximately 60 μm.

2.2 Corona discharge treatment

A corona discharge treatment was carried out in line during the film production. The treatment was applied to PET films (and to one side of the PP film when the multilayer film has to be prepared). Briefly, the electric discharge causes the oxidation of the polymeric surface through the generation of free radicals, resulting in the formation of additional polar groups on the surface that involve a strong adhesion of adhesives, dyes, coatings, etc. [33, 34]. It is worth noting that this treatment acts on small depths (nanometers) of the treated surface conferring it the desired properties, but leaving the bulk properties of the polymer unchanged.

High voltage and frequency discharge (corona discharge) were applied at atmospheric pressure with two treater machines, operating at a maximum output power of 8 kW set at an operating power of 3 kW to ensure a surface tension of 55 dyne/cm.

2.3 Apparatus and procedure for supercritical impregnation

The impregnation tests were performed employing a laboratory-scale apparatus (in Figure 1), which consists in a cylindrical stainless steel autoclave (NWA GmbH, Lorrach, Germany) with an internal volume of 100 mL, closed with two clamps on the top and the bottom. CO_2 was slowly fed at a constant flow rate (10 g/min) through a high-pressure pump (Milton Roy, mod. Milroyal B, Pont-Saint-Pierre, France), whose head was cooled by a refrigerating bath (Julabo, Seelbach, Germany). When the working pressure was reached, the operating conditions were maintained for a fixed time. An impeller, located on the top cap and driven by a variable velocity electric motor, ensured good stirring. Thin band heaters were used to heat the autoclave, whose internal temperature was measured by a K-type thermocouple (accuracy ±0.1 °C). The thermal control was guaranteed by a PID controller (Watlow, mod. 93, Toledo, OH, USA), whereas pressure was measured by a digital manometer (Parker, Minneapolis, MN, USA). A micrometric valve (Hoke, mod. 1315G4Y, Spartanburg, SC, USA), preceded by an on/off valve, allowed a slow depressurization. Through a rotameter at the exit of the cylinder, it was possible to evaluate the CO_2 flow rate .

Impregnation experiments were carried out using a static method [35, 36]. A small film piece (about 2.5 cm x 2.5 cm) was accurately weighed and put in a small stainless steel container, packed into filter paper (to avoid its contact with the liquid TOC); then, the sample was placed on the bottom of the cylindrical autoclave. In particular, the weights of PP and PET monolayer films were respectively 23.5 ± 1.5 mg and 24.5 ± 2.5 mg, whereas, for the multilayers PET/PP film, they were in the range 45.0 ± 3.0 mg. A weighed amount of TOC approximately equal to 0.80 g, corresponding to its saturation concentration in SC-CO_2 at the given pressure and temperature (10 mg_{TOC}/g_{CO2}) [31], was instead placed inside a small opened container, coaxially located on the impeller, to allow its contact with SC-CO_2. The chosen pressure assured a high solubility of TOC in

$SC\text{-}CO_2$ [31]. The amount of CO_2 inside the cylinder, which was about 80.8 g, was calculated from its density value at the operating temperature and pressure. Carbon dioxide density was evaluated using the Bender equation of state [37]. Once the autoclave was closed, CO_2 was slowly delivered to the cylinder and simultaneously heated up to the required temperature. When the desired pressure (17 MPa) was reached, CO_2 flow was stopped, and the operating conditions were maintained for a fixed time. At the end of the experiment, the autoclave was depressurized with a constant flow rate (about 0.1 MPa/min). Once the depressurization ended, the loaded film was recovered and weighed. The weight increase of the sample was related to the quantity of impregnated TOC, which was also checked using UV/vis spectrophotometer, as reported below in the analytical methods section. In order to assure that the impregnated films did not contain adsorbed carbon dioxide in the polymer, each loaded sample was weighted an hour after the end of the experiment. Impregnation tests were repeated twice; the difference in weight between the experiments conducted at the same operating conditions was less than 5%.

Impregnation kinetics were determined to find out the time required by TOC to reach the equilibrium concentration at the chosen value of temperature and pressure. Kinetic data were obtained at different contact times (2- 48 h), at 17 MPa and 40 °C.

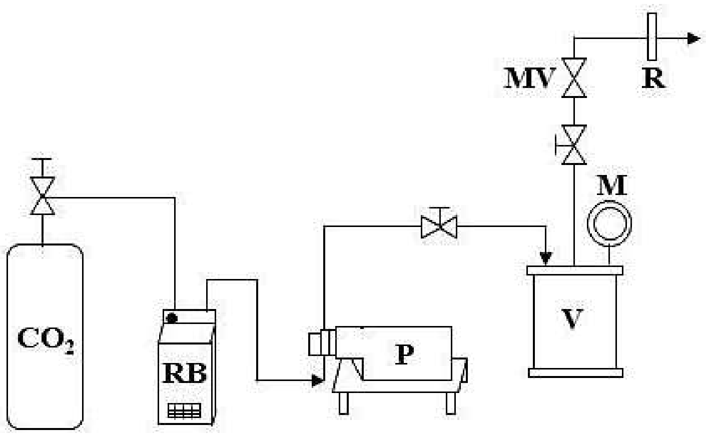


Figure 1 Schematic representation of the impregnation laboratory plant. CO_2: carbon dioxide supply; RB: refrigerating bath; P: pump; V: vessel; M: manometer; MV: micrometric valve; R: rotameter.

2.4 Analytical methods

Films were observed by Field Emission Scanning Electron Microscopy (FESEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany) before and after impregnation of TOC with SC-CO_2. Small film pieces were placed on a carbon tab previously attached to an aluminum stub (Agar Scientific, Stansted, United Kingdom) and, then, covered with gold-palladium (layer thickness 250 Å) using a sputter coater (mod. 108 A, Agar Scientific, Stansted, United Kingdom). Many FESEM images were taken for each sample to verify its uniformity.

Fourier transform infrared (FT-IR) spectra were obtained through an FTIR spectrophotometer (IRTracer100, Shimadzu Italia, Milan, Italy) at a resolution of 0.5 cm^{-1} in a scan wavenumber range from 4000 to 450 cm^{-1}, as the mean of 16 measurements. Potassium bromide (KBr) was used as an infrared transparent matrix and well-mixed in an agate mortar with small pieces of each film that has to be analyzed. Then, a hydraulic press (gradually increasing the pressure up to 30 MPa) compressed the samples blended with KBr in the form of disks [38].

Differential scanning calorimetry (DSC) measurements were carried out by a differential scanning calorimeter (mod. TC11, Mettler-Toledo International Inc., Columbus, OH, USA) using Mettler STARe system. Each film (about 5 mg) was crimped into an aluminum pan. For both PP and PET, the analysis was conducted under a nitrogen gas flow equal to 100 mL/min. PP samples were heated from -35 °C to 200 °C at a rate of 10 °C/min, and then held at 200 °C for 5 min to ensure the complete melting of crystallites. Then, they were cooled from 200 °C to -35 °C at a rate of 10 °C/min and, after, heated up again from -35 °C to 200 °C (10 °C/min). To better detect the PP glass transition temperature, DSC was repeated in the range from -50 °C to 20 °C at a rate of 5 °C/min.

PET samples were, instead, heated from 25 °C to 300 °C at a rate of 10 °C/min, held at 300 °C for 5 min, then cooled from 300 °C to 25 °C (10 °C/min) and finally heated up again from 25 °C to 300 °C (10 °C/min). The glass transition temperature (T_g), the melting temperature (T_m) and the melting enthalpy (ΔH_m) were determined from the second heating scan, according to the ASTM D3418-12 method [39]. A comparison with the melting enthalpy of pure crystalline PP and PET (207 J/g and 140 J/g, respectively) allowed to calculate the crystallinity degree (%) of pure and loaded films.

TOC loadings, evaluated to assess the weight increase of the impregnated samples, and *in vitro* migration studies were performed by a UV/vis spectrophotometer (model Cary 50, Varian, Palo Alto, CA, USA) at a wavelength of 295 nm [28]. Each analysis was repeated twice, and the mean release profiles were proposed in this work, plotting the percentage of released TOC as a function of time.

The migration tests were conducted at 40 °C in food simulant D1 (50 % EtOH), according to the European Commission Regulation No. 10/2011 [40]. In detail, small film pieces containing an equivalent amount of TOC (5 mg) were immersed in 70 mL of food simulant D1, continuously stirred at 100 rpm. The absorbance was monitored until the steady state was reached. Then, the maximum absorbance measured at the end of the release; i.e., when the film released all TOC to the outer ethanolic solution, was converted into TOC concentration using a calibration curve, obtained using very diluted standards at different concentrations of TOC in the release medium (0.2850, 0.1425, 0.0713 and 0.0356 mg/mL). In particular, the percentage of TOC released at any time was determined as follows:

$$TOC\ release, \% = \frac{the\ amount\ of\ TOC\ \ measured\ at\ a\ certain\ time}{the\ maximum\ amount\ of\ released\ TOC}\ x\ 100 \qquad (1)$$

To eliminate a possible influence of other polymer additives, release tests were also performed on pure single layer PP and PET films and on multilayer PET/PP films before supercritical impregnation; however, no significant absorbance value was detected at 295 nm.

The antioxidant activity of TOC impregnated into the different films was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as stable free radical, according to the method described by Brand-Williams [41]. Tests were carried out in triplicate.

Ethanolic solutions at different concentrations of pure TOC (30-800 ppm) were tested. Firstly, 0.1 mL of each solution was added to 3.9 mL of a 6 x 10^{-5} mol/L solution of DPPH. The decrease in the absorbance was monitored using a UV/vis spectrophotometer (model Cary 50, Varian, Palo Alto, CA) at 515 nm (characteristic wavelength of DPPH) every 5 minutes for two hours under dark at room temperature until the steady state was achieved. The initial DPPH concentration was measured at time zero. From these kinetic results, Efficient Concentration (EC_{50}) of TOC; i.e., the concentration of antioxidant that decreased the initial DPPH one by 50%, was graphically calculated and it was found to be equal to 191.3 µg/mL. Moreover, this preliminary study allowed to select the amount of TOC to be considered for the impregnated films analyses to avoid a total reaction of the DPPH and, at the same time, to apply the DPPH protocol optimized for the activity of the impregnated films by Cejudo Bastante et al. [24].Indeed, the DPPH protocol was slightly modified to evaluate the antioxidant activity of the impregnated films [24]. An amount of sample was submerged in 4 mL of 6 × 10^{-5} mol/L DPPH in ethanol. The absorbance was measured at 515 nm every 5 minutes for two hours under dark at room temperature. To have an accurate comparison, the antioxidant activity tests were performed considering the same amount of TOC (0.7 mg/mL) for all the samples.

The percentage inhibition (%I), which represents the amount of DPPH that reacts with a given concentration of TOC, was calculated as follows:

$$\%I = \left(1 - \frac{A_i}{A_0}\right) * 100 \quad (2)$$

where A_0 is the initial DPPH absorbance at 515 nm and A_i is the final absorbance of each sample measured at 515 nm after two hours of reaction.

To ensure that the adhesive or other polymer additives did not distort the measurements of antioxidant activity, a DPPH assay was also performed on the film before impregnation without observing the antioxidant activity.

3. Results and discussion

The work can be divided into four main steps:

(1) study of impregnation kinetics of TOC into single layer PET and PP films;

(2) single layer loaded films characterization to choose the best conditions to develop active multilayer PET/PP films;

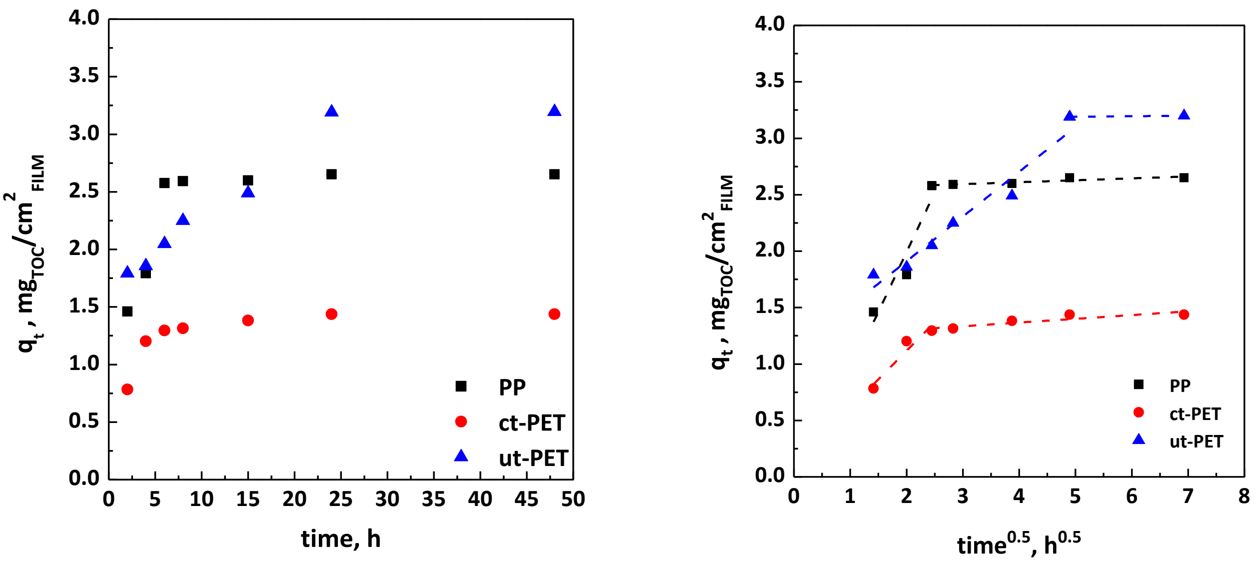
(3) study of impregnation kinetics of TOC into multilayer PET/PP film;

(4) multilayer PET/PP loaded films characterization to investigate the potential as controlled-release packaging.

3.1 Kinetics of TOC impregnation into single-layer films

Firstly, the impregnation of TOC into single layer films was studied at 17 MPa, 40 °C in correspondence of various contact times (from 2 to 48 h). In particular, the impregnation kinetics of TOC into PP film, on the side of ut-PET and ct-PET were determined and compared, as shown in Figure 2a. Uptake was expressed per unit area of film as q_t; i.e., mg of impregnated TOC per cm^2 of film (mg_{TOC}/cm^2_{PP}, $mg_{TOC}/cm^2_{ut\text{-}PET}$ and $mg_{TOC}/cm^2_{ct\text{-}PET}$, respectively). From the kinetic curves in Figure 2a, an increase of the loaded TOC can be observed with the increase of contact times, up to a maximum value, which was found at 24 h for all the films. Moreover, comparing the impregnation kinetics obtained for the different substrates, the lowest quantity of impregnated TOC was achieved with ct-PET, whereas the amount of loaded TOC into PP and ut-PET was more similar. TOC uptake is different considering different polymers because swelling capability as well as diffusivity [42] of CO_2 in the matrix vary from polymer to polymer. These properties influenced the amount of an active substance that can be charged into a polymer. As previously discussed, the high-frequency discharge used during the corona treatment causes the formation of additional polar groups on the polymer surface that probably have a weak attraction for fat-soluble TOC, leading to the low penetration of the active compound into the ct-PET surface [33, 34].

From a quantitative point of view, it is possible to note that the obtained loading is very high, if compared with literature results [19, 28, 30]; indeed, the amount of loaded TOC, expressed in terms of uptake, reached a very high value equal to 3.20 mg_{TOC}/cm^2_{FILM} in case of ut-PET after 24 h of impregnation. This achievement allows to overcome the main drawbacks of conventional techniques used to obtain active packaging; i.e., low penetration into the polymer and low loading efficiencies [19, 28, 30]. The high loadings reached confirmed the potential of the supercritical impregnation of active compounds into polymeric films with loadings greater than the ones obtained in previous papers focused on the same technique; for example, the loading of thymol into PLA/PCL films obtained by Milovanovic et al. [14] was equal to 35.8 wt %. Therefore, the loadings shown in Figure 2a obtained using supercritical impregnation are 2-3 orders of magnitude higher than the values reported in previous works [14, 28]. To determine which is, among "film diffusion" and "intraparticle diffusion", the limiting step of the process, the adsorption of TOC into the different films were plotted in terms of q_t versus $t^{0.5}$ (Figure 2b), according to the Weber and Morris approach [43]. Indeed, it is well known that q_t vs. $t^{0.5}$ plots can be characterized by a multilinearity, ascribable to the different steps that occur in the adsorption process. It is possible to note from Figure 2b that, for all the films, in correspondence of lower values of $t^{0.5}$, the plot is sharper because of a boundary layer effect due to the adsorption of TOC on the exterior part of the film: film diffusion is the governing step at the earlier stages of the process. The second part of the plot is linear and ascribable to the adsorption stage: at these stages, the adsorption is governed by the intraparticle diffusion.



(a) (b)

Figure 2 Impregnation of TOC into PP film, corona discharge treated and untreated surface of PET film: (a) kinetic curves; (b) intraparticle diffusion plot.

3.2 Characterization of single-layer films after impregnation

FESEM images of films before and after TOC impregnation (17 MPa, 40 °C and 6 h) are reported in Figure 3 for PP, ut-PET and ct-PET samples. Comparing the FESEM images of the unloaded films (Fig. 3a, 3b and 3c) with the ones of the impregnated films (Fig. 3d, 3e and 3f), it is possible to observe that, in the case of the unloaded films, the observed surface is smooth, whereas, in the case of the impregnated films, the film surfaces appear discontinuous because of the presence of TOC.

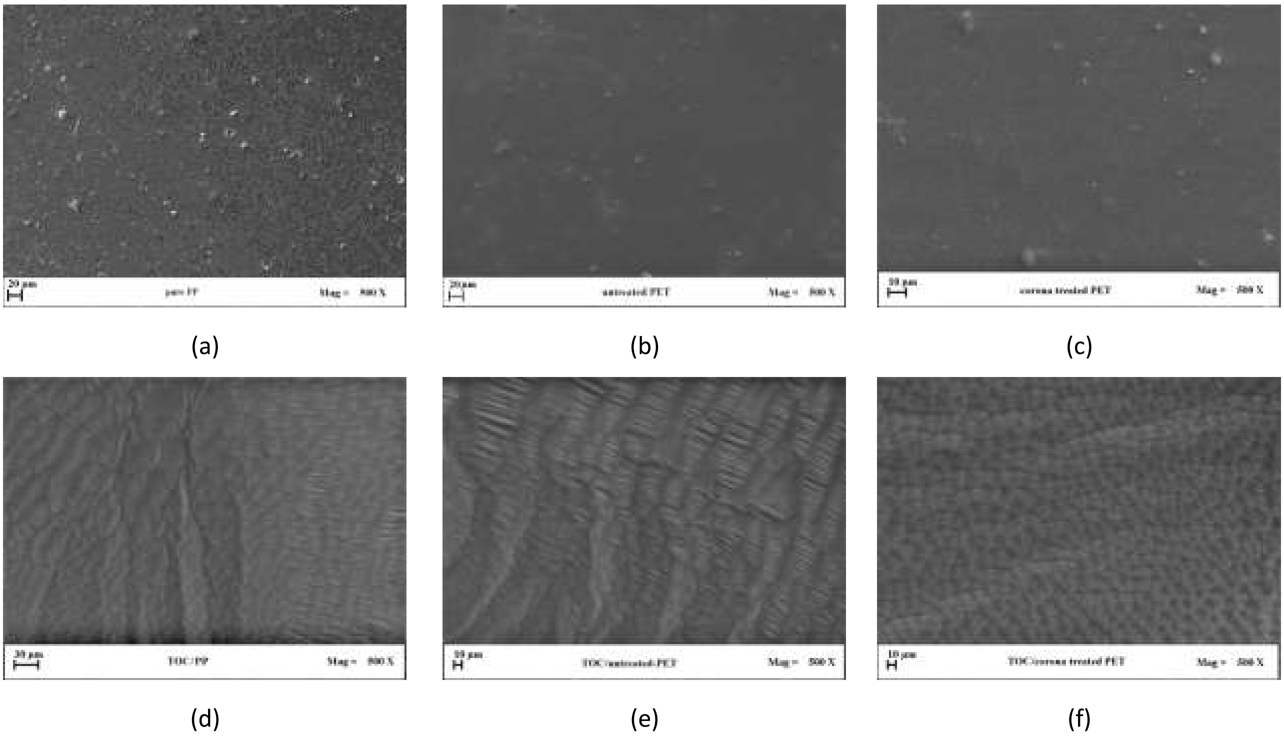


Figure 3 FESEM images of films: (a) pure PP; (b) pure ut-PET; (c) pure ct-PET; (d) TOC impregnated in PP; (e) TOC impregnated in ut-PET; (f) TOC impregnated in ct-PET.

FT-IR analyses were performed for pure TOC, pure films, and TOC impregnated into each monolayer, as reported in Figure 4. The spectra of the impregnated samples exhibited both the characteristic bands of the polymer and the active compound; in particular, all of them showed peaks ascribable to TOC at 1262 cm^{-1} for CH_2 and at 1086 cm^{-1} for plane bending of phenyl [44]. The increase of the absorbance peaks at 1378 and 1460 cm^{-1}, related to the methyl symmetric and asymmetric bending [44], observed in the spectra of all loaded films with respect to the ones of pure films, confirmed the presence of TOC. Indeed, the percentage increase of the absorbance at 1378 and 1460 cm^{-1} was significant, ranging from 63 % for loaded PP to 91 % for loaded ut-PET in the case of the first mentioned peak and from 33% for loaded PP to 84% for loaded ut-PET in the case of the second one. Moreover, an increase of the peak at around 500 cm^{-1} corresponding to the C-C bending was found in the spectrum of loaded ut-PET.

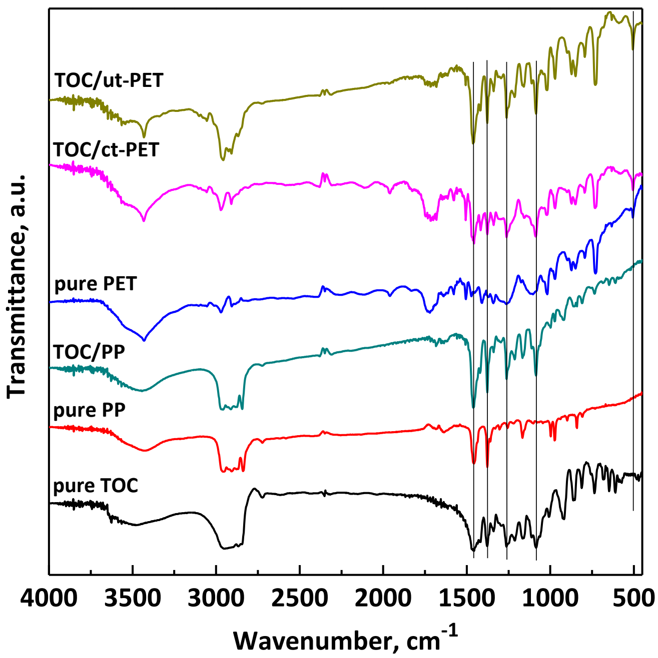
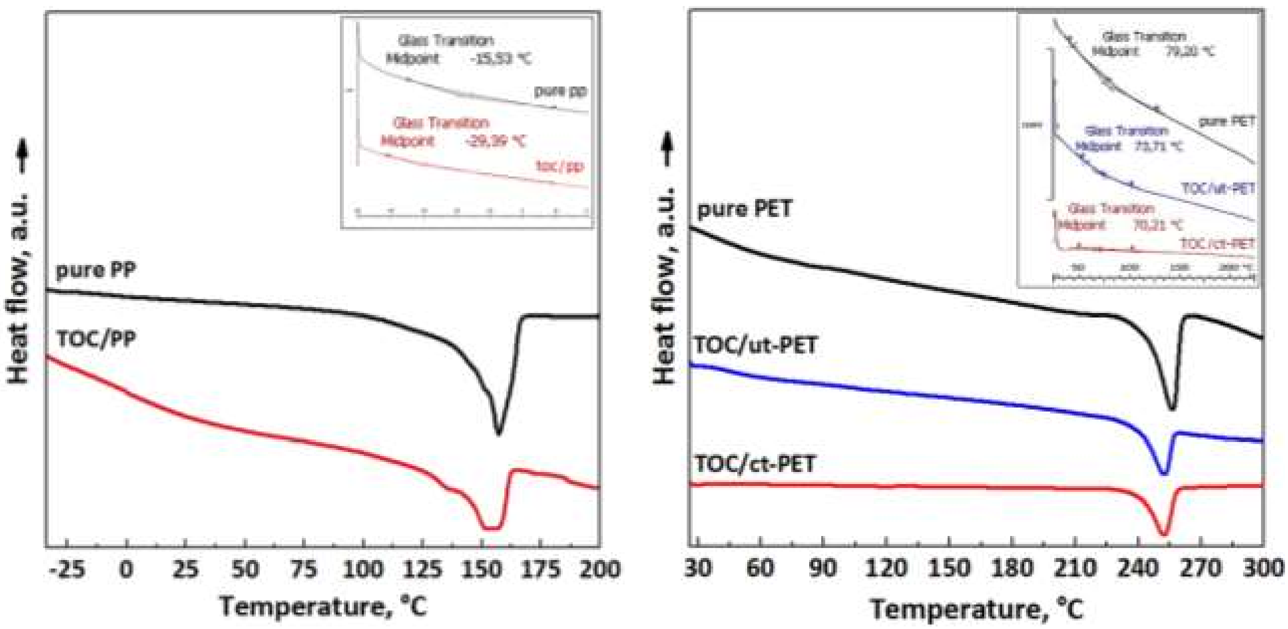


Figure 4 FT-IR spectra of pure TOC, pure PP and PET films, TOC impregnated into PP, ut-PET and ct-PET. Vertical lines are referred to the peaks at 1460, 1378, 1262, 1086 and 500 cm^{-1}.

Figure 5 showed DSC thermograms of films before and after TOC impregnation (17 MPa, 40 °C and 6 h). In agreement with the literature [45], in Figure 5a it is possible to observe that PP curve shows a peak at about 157 °C corresponding to its melting temperature, slightly shifted at a lower temperature after supercritical impregnation. Moreover, the T_g of pure PP was observed at about -16 °C [46], and it shifted at a lower temperature equal to -29 °C for impregnated PP. In Figure 5b, the PET curve exhibits a peak at around 256 °C, which corresponds to the T_m, according to literature data [47]; the T_m slightly shifted at a lower temperature in the case of PET films processed by supercritical impregnation (ut-PET, ct-PET). The corona discharge treatment does not influence the thermal properties, being a surface treatment; i.e., it only modifies the film electrostatic surface charge (and not the bulk properties) [33].

Moreover, PET thermogram shows a T_g at about 79 °C [47], less prominent and shifted at lower temperatures in impregnated samples; i.e., about 74 °C for impregnated ut-PET and 70 °C for impregnated ct-PET. The shifts of melting and glass transition temperatures may be ascribed to the well-known plasticizing effect of compressed fluids in the presence of semicrystalline polymers [19, 48]. Moreover, some literature studies show that a plasticizing effect can be also caused by active substances, including TOC, leading to an improvement in the flexibility and fatigue strength of polymers [49, 50]. The melting enthalpy (ΔH_m) and the crystallinity degree (%) for pure and loaded films were reported in Table 1. Comparing the results of pure and impregnated films, it is possible to observe that the presence of SC-CO_2 induced a slightly reduction in the crystallinity percentage of the polymers.



(a) (b)

Figure 5 Entire DSC thermograms and enlargement of a part of curves (with the indication of *Tg*) for pure and impregnated films: (a) PP samples; (b) PET samples.

Table 1 Melting enthalpy (ΔH_m) and crystallinity degree (%) of pure and loaded films.

Sample	**ΔH_m [J/g]**	**Crystallinity degree [%]**

unprocessed PP	88.97	42.98
TOC/PP	64.03	30.93
pure PET	42.15	30.10
ct-PET	34.71	24.79
ut-PET	39.83	28.45

The migration of TOC from the packaging films into the food simulant D1 [23] was studied using UV–vis spectroscopy. Release profiles of TOC incorporated into the films, processed by supercritical impregnation for 24 h, at 17 MPa and 40 °C, are shown in Figure 6. Unprocessed TOC was wholly dissolved in the release medium in almost 45 minutes, whereas the release of TOC was significantly delayed by its impregnation into films with SC-CO_2. In particular, all impregnated TOC migrated in 16 h from corona discharge treated PET surface (ct-PET), whereas it took 22.8 h and 23.3 h respectively from untreated PET surface (ut-PET) and PP film, which showed the slowest release profile. Release profiles revealed the presence of an initial burst-like effect, which was about 10 % in case of PP and ut-PET and 20% for ct-PET, due to the percentage of TOC located on/near the film surface, which dissolved quickly. In agreement with results about impregnation kinetics, the faster release of TOC from ct-PET is related to the lower penetration of TOC into the corona treated surface due to a low affinity with the additional polar groups formed during the treatment.

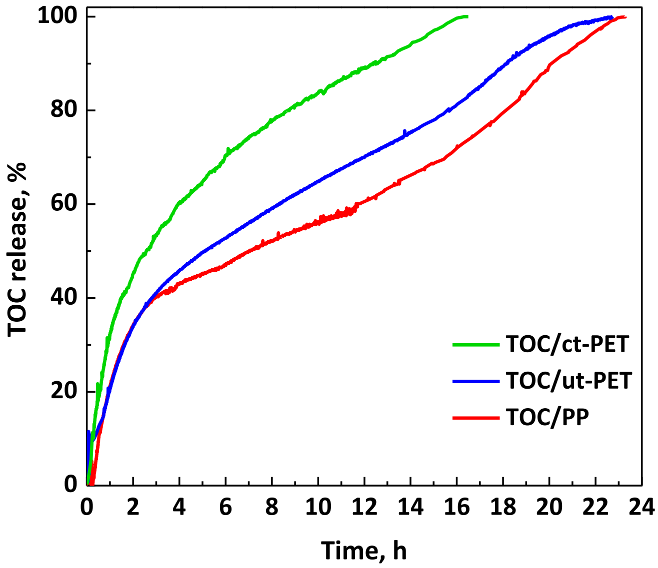


Figure 6 Release kinetics of TOC in food simulant D1 from single-layer films.

3.3 Kinetics of TOC impregnation into multilayer PET/PP film

Since the best results, from the release kinetics point of view, were obtained considering TOC release from PP film, the supercritical impregnation of TOC into a multilayer PET/PP film was studied by exposing the PP surface to the TOC dissolved in SC-CO_2. Indeed, the use of PP/PET multilayer film may be more favorable than the monolayer film. Indeed, the inner active layer (PP in this specific case) allows the migration of the active compound (TOC) towards the food surface. On the contrary, the outer layer (PET) is in contact with the external environment, offering more protection for the active matrix layer and reducing the unwanted migration of the active compound towards the outside of the package.

Even in the case of the multilayer film, the uptake (q_t) was expressed per unit area of film, as the ratio between the amount of impregnated TOC per cm^2 of film (mg_{TOC}/cm^2_{FILM}). The impregnation kinetics of TOC into a multilayer PET/PP film, reported in Figure 7, showed that the quantity of loaded TOC increased with the contact time, up to a maximum value reached in about 48 h equal to 2.66 mg_{TOC}/cm^2_{FILM}. This maximum amount of TOC loaded in the multilayer film, in terms of

mg_{TOC}/cm^2_{FILM}, was similar to the one obtained using the monolayer PP film. From the kinetics results, it is possible to deduce that a sufficient loading is reached after 24 h.

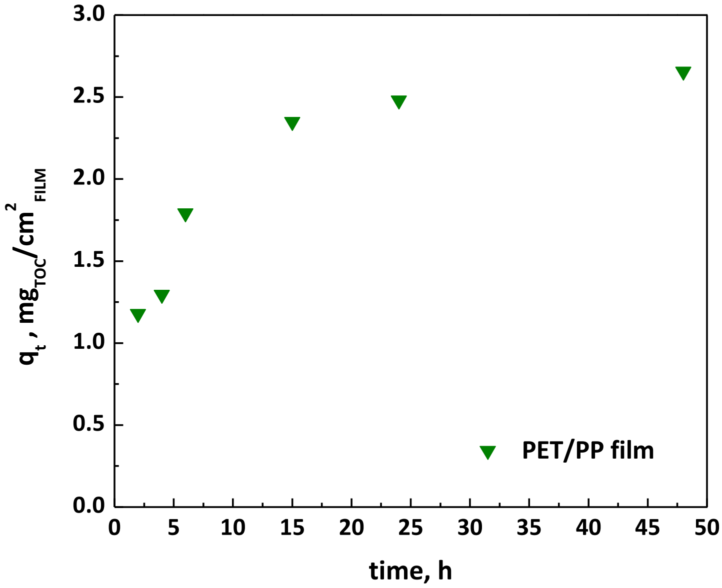


Figure 7 Kinetic curve at 17 MPa and 40 °C for the impregnation of TOC into multilayer PET/PP film.

3.4 Characterization of multilayer PET/PP films after impregnation

FT-IR spectra of pure TOC, pure multilayer PET/PP film, and TOC impregnated into multilayer PET/PP film are reported in Figure 8. The spectrum of multilayer PET/PP film exhibited both the characteristic peaks of monolayer PET and PP films. In addition to these, the spectra of the impregnated sample showed the typical bands of TOC, such as the peaks at 2927 and 2868 cm^{-1} related to the asymmetric and symmetric stretching vibrations of the CH_2 and CH_3, at 1262 cm^{-1} for CH_2 and at 1086 cm^{-1} for plane bending of phenyl [44]. Moreover, the presence of TOC was confirmed by an increase of the absorbance peaks at 1378 and 1460 cm^{-1}, respectively associated to the methyl symmetric and asymmetric bending [38], observed in the spectrum of loaded multilayer PET/PP film with respect to the pure one. The percentage increase of the absorbance at 1378 and 1460 cm^{-1} was found to be about 79% and 83%, respectively. Moreover, the presence of TOC peaks at 2927 and 2868 cm^{-1} was less evident in monolayer films, slightly noticeable in the case of ut-PET monolayer film, while it is more accentuated in the spectrum of multilayer film, as shown in Figure 8.

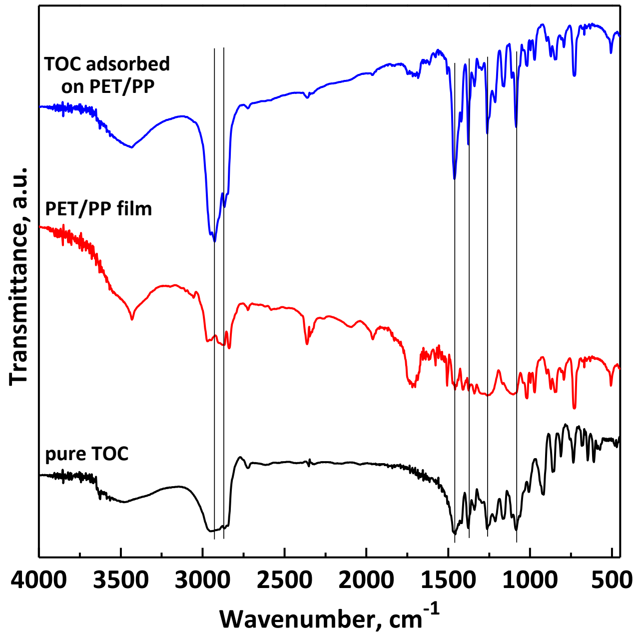


Figure 8 FT-IR spectra of pure TOC, pure multilayer PET/PP film and TOC impregnated into multilayer PET/PP film. Vertical lines are referred to the peaks at 2927, 2868, 1460, 1378, 1262 and 1086 cm^{-1}

Migration tests of TOC in food simulant D1 were performed on multilayer PET/PP film to study its possible use as active packaging. The release profile of TOC impregnated into PET/PP film (17 MPa, 40 °C and 24 h) is reported in Figure 9. It is possible to observe that TOC completely migrated from the multilayer film into food simulant D1 in 14 hours. . In conclusion, the impregnation with SC-CO_2 into polymeric films has excellent potential to develop controlled-release packaging.

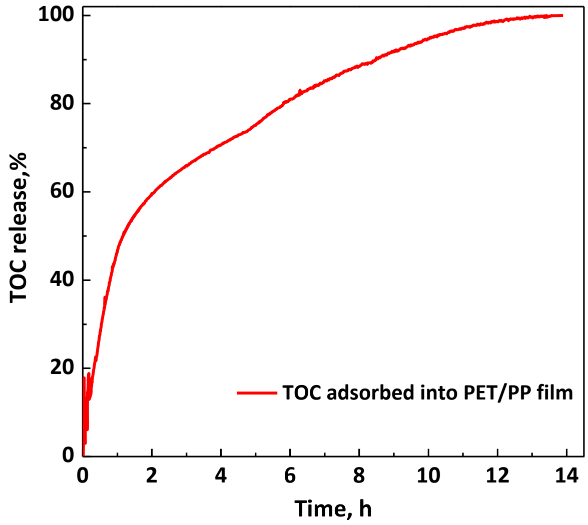


Figure 9 Release kinetics of TOC in food simulant D1 from multilayer PET/PP film.

The antioxidant activity was determined for unprocessed TOC, TOC impregnated on each monolayer (PP, ut-PET and ct-PET) and multilayer PET/PP films at 17 MPa and 40 °C for 24 h. An equivalent amount of TOC was considered to compare unprocessed TOC and impregnated films.

In particular, the antioxidant activity was checked to understand if TOC was damaged by thermal and oxidative degradation during the impregnation process. Results of percentage inhibition (%I) of the loaded films are similar to those of pure TOC, as demonstrated in Table 2 by reporting the data obtained after two hours of reaction with DPPH radical. Quantitatively, the reduction of the percentage inhibition with respect to the %I of unprocessed TOC was included between a minimum of 0.11 % for TOC impregnated into the ut-PET film to a maximum of 0.43 % for TOC loaded on PP film. These results confirmed that, in all cases, the antioxidant power of the active compound is preserved after the impregnation with SC-CO_2.

Table 2 Antioxidant activities in terms of percentage inhibition (%I) for pure TOC and loaded films.

Sample	**%I**
unprocessed TOC	92.4

PP	92.0
ct-PET	92.2
ut-PET	92.3
multilayer PET/PP	92.1

4. Conclusions

In the present work, the adsorption of TOC into polymeric films using supercritical impregnation was studied, firstly considering single-layer PP and PET films and, then, multilayer PET/PP films. The influence of corona discharge treatment on supercritical impregnation was also investigated comparing the PET untreated surface with the treated one. PET surface with corona discharge treatment showed the worst results both concerning the amount of impregnated TOC and the release profile, whereas the attainment of TOC loaded into PP films was the best option to produce a controlled-release packaging, with high values of loaded TOC. According to the literature, the shifts of melting and glass transition temperatures of polymers was observed from DSC analyses due to the well-known swelling and plasticizing effects of SC-CO_2 that allow a better penetration of the active compound into the polymer substrate, resulting in high loadings. Based on the results obtained for monolayers, to create active multilayer films, the impregnation of TOC with SC-CO_2 was studied considering the PP surface of PET/PP film. Migration tests in food simulant D1 (50% EtOH) demonstrated that the impregnation of TOC into polymeric films using SC-CO_2 induced a prolonged release of the vitamin. These results are promising from an industrial point of view, confirming the effectiveness of this process to produce controlled-release packaging. Moreover, the antioxidant activity tests confirmed that the impregnation process did not damage the TOC structure.

Besides, supercritical impregnation has proved to be an effective route to overcome the main limitations associated with the production of active packaging with conventional techniques. Indeed, high loading values, up to 3.20 mg_{TOC}/cm^2_{film} and 2.66 mg_{TOC}/cm^2_{film}, for monolayer and multilayer films respectively, were obtained. This result can be ascribed to the low temperature used and to the high diffusivity of SC-CO_2, which allows to avoid the volatilization/degradation of the active substance and, at the same time, to have a rapid penetration of the active principle into the polymer matrix.

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