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Abstract

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Keywords	1-octen-3-ol, LDPE films, supercritical CO2-assisted impregnation, F. verticillioides, S. zeamais.

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Bioactive LDPE films loaded with natural pesticides for stored grain protection

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Abstract

The objective of this work was to incorporate the biopesticide 1-octen-3-ol in polyethylene (LDPE) films using supercritical CO_2–assisted impregnation with the aim to obtain bioactive films for stored grain protection and food preservation. The influence of different pressures and depressurization rates on the amount of the volatile organic compound impregnated was evaluated. Results reveal that the best impregnation yield of biopesticide in films (*Y*%) is associated with low depressurization rate (0.5 MPa·min^{-1}) and mild pressure conditions (~9 MPa). The release kinetics of 1-octen-3-ol from the impregnated films was also investigated. In addition, the biocidal activity of impregnated films obtained under the influence of the best operational conditions was tested towards the main pests of stored grains. Films showed insecticidal activity against *Sitophilus zeamais* and also produced a decrease of the fungal growth rate of *Fusarium verticillioides*.

Keywords: 1-octen-3-ol, LDPE films, supercritical CO_2-assisted impregnation, *F. verticillioides, S. zeamais.*

1. Introduction

Hermetic storage systems such as silo bags are considered an effective tool to prevent damaging pest infestations during food storage. However, a number of biological interactions, such as grain-insect-fungi can arise when good agricultural practices are not taken into account, causing grain deterioration and losses reaching up to 10% (Casini, Rodriguez, & Bartosik, 2009). Among the main pests of economic relevance related to yield reduction or production unfit for sale, causing substantial annual losses of stored grain such as wheat, rice and maize (Casini & Santajulia, 2008), the weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionoidae) and the fungal pathogen *Fusarium verticilliodes*, can be mentioned.

In Argentina, synthetic pesticides as methyl bromide and phosphine are used to reverse this problem (Bartosik, Cardoso, & Manetti, 2016), but these substances have been included since 2012 in the category of prohibited and restricted, respectively, by the National Ministry of Health (Ministerio de Salud de la Nación, 2012), because of the extensive use of these agents is related to resistance development, human diseases and contamination of food and environment (Cordeiro, Corrêa, Rosi-Denadai, Tomé, & Guedes, 2017; Who, 2002). Then, an alternative is the use of biopesticides, active agents considered of minimum risk, for controlling pests of stored grains. Thus, biopesticides are considered as novel eco-friendly tools with increasing demand (EPA, 2017). Due to the fact that these natural compounds are highly volatile, an adequate implementation in the application sites should seek mechanisms for their retention and protection against environmental agents (Nerio, Olivero-Verbel, & Stashenko, 2010). Thus, the development of active packaging incorporating natural products is an innovative technology for food preservation during storage (Rojas et al., 2015). 1-octen-3-ol (Fig. 1) is a fungal volatile organic compound (VOC) which mediates interspecies interaction, being a semiochemical for insects

(Usseglio et al., 2017). Numerous studies have shown the potential antifungal, insecticidal, repellent and herbicidal activities of this compound (Zhao, Yang, Lix, Mu, & Liu, 2011). In particular, a study carried out by our research group has shown the biological activities of 1-octen-3-ol against the above mentioned maize grain pests: the insect *S. zeamais* and the fungus *F. verticillioides* and its mycotoxin, fumonisin B_1 (Herrera, Pizzolitto, Zunino, Dambolena, & Zygadlo, 2015) .

Supercritical CO_2-assisted impregnation is a process that allows the incorporation of natural active compounds into polymeric materials (Kikic & Vecchione, 2003). Currently, this technique, considered environmentally friendly, is used in the preparation of polymeric systems for controlled delivery of drugs and bioactive compounds with high volatility (Kiran, 2016; Rojas et al., 2015; Torres, Romero, Macan, Guarda, & Galotto, 2014). The use of CO_2 as solvent for incorporate bioactive agents has the advantage of being chemically inert, non-toxic and when it is in supercritical conditions ($P>Pc$ = 7.38 MPa and $T>Tc$ = 31°C) can dissolve a wide range of organic compounds. In addition, its low critical temperature allows preservation of thermolabile compounds during processing (Cocero, Martín, Mattea, & Varona, 2009).

Low-density polyethylene (LDPE) is a commercial and economic material used commonly for active packaging due to their excellent thermal stability and mechanical resistance (Goñi, Gañán, Strumia, & Martini, 2016; Teymouri, 2011). Also, this material is used for the confection of hermetic bags for postharvest grain preservation (INTA, 2009).

In this study, the main objective was to incorporate 1-octen-3-ol in LDEP films by supercritical CO_2-assisted impregnation, evaluating different process conditions and their influence in the amount of volatile organic compound impregnated. Also, insecticidal and antifungal activities of impregnated films obtained under the best operational conditions against *F. verticillioides* and *S. zeamias* were tested.

2. Materials and methods

2.1. Materials

1-octen-3-ol (purity 98%, MW: 128.21 g·mol^{-1}, bp: 84°C) was purchased from Sigma-Aldrich (Steinheim, Germany).

Commercial low density polyethylene film (LDPE, M_w: 229300, M_n: 22500, MFI: 0.6 g/10 min at 190°C/2.16 kg, density: 921 kg·m^{-3}, thickness: 130 ± 20 μm, Dow-Polisur, Argentina) was used as polymeric matrix in the impregnation tests.

Industrial extra-dry carbon dioxide (water content ≤ 10 ppm v/v) used as impregnation solvent was purchased from Linde (Argentina).

2.2. Insects

The *S. zeamais* adults used in the laboratory assays were obtained from Manfredi Agricultural Experimental Station (Córdoba, Argentina). The colony was maintained in laboratory for one year without exposure to pesticides. The weevils were reared at 28°C, a photoperiod of 12:12 h (light: dark), and 70% relative humidity in breeding chamber, according to Food and Agriculture Organization of the United Nations protocols (FAO, 1974). Insects were fed a diet of maize grains free of insecticides.

2.3. Fungal strain

The fungal strain *F. verticillioides* M3125, provided by Dr. Robert Proctor (United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL, USA), was used for this study.

2.4. Supercritical CO_2-assisted impregnation of LDPE films

Supercritical CO_2-assisted impregnation of LDPE films with 1-octen-3-ol was carried out in a 50 ml high-pressure cell with magnetic stirring and temperature control, using the apparatus schematically shown in Fig. 2 and following the methodology described by Goñi et al. (2016). The initial mass of 1-octen-3-ol to be loaded into the cell was determined according to preliminary solubility studies, in order to ensure complete solubilisation of this compound in the fluid phase, and maintain a constant alcohol mole fraction ($y = 2\times10^{-3}$) for

all tested conditions. This approach allows to evaluate the effect of pressure, and therefore CO_2 density, independently from the alcohol concentration in the fluid phase, which is the driving force for the mass transport phenomenon. Thus, the corresponding amount of 1-octen-3-ol was added into the cell and films with a mean surface area of 32.75 ± 1.85 cm^2 were incorporated in a metal support with the purpose of prevent the contact between the films, ensuring a homogeneous impregnation through both sides of the films, and also maintaining the films in a vertical position in order to avoid the deposition of solute on the film surface during depressurization. Impregnation was performed at four different pressure values (7.5-14.5 MPa), corresponding to different fluid densities (200-750 $kg \cdot m^{-3}$), and two depressurization rates (0.5 and 5 $MPa \cdot min^{-1}$), controlled with a micrometering valve (Swagelok, USA). All experiments were carried out at constant temperature (45°C), for 4 h, under agitation. In Table 1 shows experimental conditions for all runs.

The mass of 1-octen-3-ol incorporated into the films was determined gravimetrically following the methodology described by Goñi et al. (2017), by measuring the film mass increase in a precision balance after gently cleaning the film surface with tissue paper, in order to remove residual amounts of deposited compound. Then, impregnation yields (*Y%*) were defined as follows (eq. 1):

$$Y\% = \frac{m_f - m_0}{m_0} \times 100 \quad (1)$$

where m_o and m_f are the original and the final mass of the film, before and after impregnation, respectively.

Despite their volatility, the evaporation of the alcohol impregnated into the films was not fast enough to affect the measurement. The possible effect of moisture adsorption or desorption was checked using a control sample (neat LDPE film), which was weighed with the samples before and after the impregnation process. Preliminary runs were also performed by subjecting film samples to high pressure CO_2 (without compound) in order to check that the film weight was not affected by the pressurization-depressurization cycle itself, and that the CO_2 desorption was complete within few minutes before weighing.

2.5. Fourier transformed infra-red spectroscopic analysis (FTIR)

FTIR analysis of original and impregnated LDPE films as well as pure 1-octen-3-ol was performed in a Nicolet iN10 Mx equipment (Thermo Fisher Scientific, USA), in order to confirm the incorporation of the alcohol in the impregnated films. Absorbance spectra were obtained in transmission mode with a resolution of 4 cm^{-1}, in a wavenumber range of 400–4000 cm^{-1} with 16 scans, at room temperature, and normalized in order to identify characteristic absorbance peaks and quantify their relative absorbance values. Background spectra were acquired before each test for air humidity and carbon dioxide correction. Furthermore, absorbance spectra were measured at 3 different positions of the films (centre and sides), in order to determine the homogeneity of the impregnation in the polymeric matrix. For that purpose, the ratio between the absorbances of LDPE and 1-octen-3-ol characteristic peaks was calculated.

2.6. Release kinetics and diffusion coefficient estimation

Release kinetics of 1-octen-3-ol from impregnated LDPE films to air was assessed in order to investigate a possible effect of impregnation conditions (i.e. pressure and depressurization rate) on the mass transport properties of the films, which could lead to a difference in release rates. For that purpose, 8 cm^2 film samples were placed in Petri dishes (50 mm diameter) in vertical position, allowing release from both sides of the films. Release profiles were determined gravimetrically, by registering the weight variation of the films along time, using a precision balance (±0.0001 g). The experiment was carried out in a laboratory at constant temperature (19°C) and relative humidity (approx. 70%). Non-impregnated films were used as control in order to check possible variations due to water vapor adsorption or desorption.

Diffusion coefficient values of 1-octen-3-ol (D) in LDPE films impregnated at different process conditions were estimated using a mathematical model based on the second Fick's law for unidimensional non-steady state diffusion (eq. 2) (Siepmann, Siegel, & Siepmann, 2012):

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial x^2}\right) \quad (2)$$

where C is the diffusive species concentration and D its diffusion coefficient. The solution of this equation depends on the geometry and characteristics of each particular system (initial and boundary conditions). In this case, the following conditions were assumed: (a) the diffusion process can be described by second Fick's law; (b) during desorption process, the diffusion coefficient D remains constant (i.e., concentration-independent, a valid assumption at low solute concentrations); (c) the diffusion is unidimensional (i.e., mass transfer occurs only in the film thickness direction, and edge effects are negligible; (d) mass transfer resistance is only located in the polymer side (perfect sink conditions in the air can be assumed); (e) the initial 1-octen-3-ol concentration in the film is uniform.

In this work, an analytical solution of the second Fick's law proposed by Crank (1975) was applied. This solution is valid for diffusion in thin slabs when the previous conditions (*a*-*e*) can be assumed, in which the cumulative mass of solute released as a function of time is given by eq. (3):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}exp\left[-\frac{(2n+1)^2\pi^2}{L^2}Dt\right] \quad (3)$$

where M_t is the mass of solute released after time t, M_∞ is the total released mass (or the initial volatile alcohol content in the film), L is the film thickness and D is the diffusion coefficient of the solute in the film, considered concentration-independent. M_t and M_∞ for compound was calculated from gravimetric measurements.

2.7. Bioactivity assays

2.7.1. Antifungal assay. Antifungal activity of impregnated films against *F. verticillioides* was evaluated according to Pizzolitto et al. (2015), with some modifications. The

experiments were performed using Potato Dextrose Agar (PDA) in Petri dishes (90 mm diameter). The culture medium was autoclaved at 120°C for 15 min before cooling at 45°C. The inoculum was prepared by growing *F. verticillioides* M 3125 on PDA for 7 days at 25°C to obtain heavily sporulating cultures. A conidial suspension was placed in aqueous solution and after homogenizing, the suspension was counted using a Neubauer chamber and adjusted to 106 conidia·mL^{-1}. PDA plates were inoculated centrally with 10 µL of the spore suspension. Finally, impregnated films with a mean surface area of 20 ± 1 cm^2 were placed into the screw of the Petri dishes on a sterilized aluminum foil tray. Neat films were used as control samples, using the same procedure as for the impregnated films. All the experiments were repeated twice. The antifungal activity was measured as a growth assessment. Two perpendicular diameters of the growing colonies were measured daily (mm) until the colony reached the edge of the plate (approx. 7 days) and the mycelial inhibition percentage was calculated as follows (eq. 4):

$$\%\ \text{Inhibition} = \left(1 - \frac{A_t}{A_c}\right) \times 100 \quad (4)$$

where A_t refers to the area of fungal growth under treated films, and A_c denote the area of fungal growth under untreated films (control).

2.7.2. Insecticidal assay. The insecticidal activity of impregnated films against *S. zeamais* was assayed according to the methodology described by Herrera, Goñi, Gañan, & Zygadlo (2017). Briefly, ten adult insects (of mixed sexes and ages), some maize grains (1.5 ± 0.04 g) and impregnated films were placed in 30 ml glass vials, acting by fumigant and contact modes, then, the vials were sealed with screw cap. The films used in this experiment presented a surface mean area of 32.75 ± 1.85 cm^2. Controls were kept under the same conditions using non-impregnated films. Thus, the mortality percentage of weevils was calculated after 24 h of exposure, by counting the dead insects, and then the films were placed in new vials with new insects and maize grain, in order to evaluate their residual toxicity. This procedure was repeated until the mortality percentage was lower than 50%. Experiments were performed by triplicate.

In addition, impregnated films with the same dimensions as described above were placed in glass vials without insects nor maize grains, in order to quantify the amount of 1-octen-3-ol in the flasks during the period of time assayed. The compound concentration in the flask at every stage was calculated by measuring the weight loss of the impregnated films over time.

3. Results and discussion

3.1. Supercritical CO_2–assisted impregnation of LDPE films with 1-octen-3-ol

As previously mentioned, impregnation of LDPE films with 1-octen-3-ol was performed at four CO_2 pressure values, corresponding to a wide range of fluid densities, and two depressurization rates, maintaining constant the other process conditions (i.e., temperature, alcohol mole fraction, stirring rate and contact time). CO_2 density, which is controlled by setting the pressure, is a key parameter in the impregnation process as it influences the solubility of the active compound, the sorption and swelling of the polymer, and consequently the mass transfer behavior of the system (Goñi, Gañan, Martini, & Strumia, 2017).

The incorporation of 1-octen-3-ol into LDPE films was confirmed by FTIR analysis and the spectra of pure alcohol, neat and impregnated LDPE films are shown in Fig. 3. It can be seen that both treated and untreated LDPE films presented the characteristic absorbance peak at 725 cm^{-1} which is associated with $–CH_2–$ bonds in the paraffinic structure (Krehula, Katančić, Siročić, & Hrnjak-Murgić, 2014), while the absorption band at ~900 cm^{-1} corresponding to vinyl $CH=CH_2$ group from 1-octen-3-ol is only present in impregnated film spectra (Socrates, 2004). Furthermore, 1-octen-3-ol was always detected when measuring in different positions of the films (edges and center) although its distribution showed a global variability of approx. 30%. Goñi et al. (2016) have observed a similar variability in LDPE films impregnated with eugenol using supercritical CO_2.

Impregnation yield (*Y%*) results at several CO_2 density conditions (corresponding to the different pressure tested) and two depressurization rates are shown in Fig. 4. As can be seen, the incorporation of 1-octen-3-ol ranged between 1.5 and 3.5% approx. An effect of

both density and depressurization rate on *Y%* can be observed. On one hand, it can be seen that for slow depressurization (0.5 $MPa \cdot min^{-1}$), *Y%* increases with CO_2 density until approx. 400 $kg \cdot m^{-3}$ and then it starts to decrease as density increases, while at fast depressurization (5 $MPa \cdot min^{-1}$) this effect is always negative. On the other hand, results show that at intermediate CO_2 density conditions, depressurization conditions have a significant effect on the impregnation yield, obtaining higher values when slow depressurization is used (0.5 $MPa \cdot min^{-1}$), while at low (200 $kg \cdot m^{-3}$) and high (750 $kg \cdot m^{-3}$) density values there is no significant difference on *Y%*. The influence of depressurization rate may be explained by a higher removal of solute from the polymer matrix by a "dragging" effect due to the rapid desorption of CO_2 (Goñi et al., 2016).

The negative effect of CO_2 density on solute loading has been reported and explained by several authors in terms of the solute partition coefficient (Champeau, Thomassin, Tassaing, & Jérôme, 2015; Hussain & Grant, 2012). At higher density, the solvent power of CO_2 increases, and the solute partition coefficient (defined as the ratio of solute concentration between the fluid phase and the polymer) shifts towards the fluid, thus decreasing the polymer loading. Other authors have observed a maximum in impregnation yield at intermediate density (or pressure) values, explained as a result of two opposite effects: at high density, the effect on the partition coefficient prevails, while at low density conditions the plasticizing effect of CO_2 absorption by the polymer –which favors solute penetration– has a predominant effect (Li & Han, 2000; Shen et al., 2008). In our case, the first behavior was observed at fast depressurization, and the second phenomenon prevailed at slow depressurization.

In conclusion, the highest impregnation yields of biopesticide into the films were associated with lower depressurization rates (0.5 $MPa \cdot min^{-1}$) and intermediate density values (corresponding to operation pressures of 9-10 MPa).

3.2. 1-octen-3-ol release and diffusion coefficient estimation

Release experiments into air under ambient conditions (19°C and 70% relative humidity), were conducted in order to estimate the diffusion coefficient of 1-octen-3-ol in the matrix polymer, by fitting the experimental data to the mathematical model presented in Eq. 3 with

D as adjustable parameter. Two typical experimental curves, as well as the model fitting, are presented in Fig. 5. The estimated values of *D* are shown in Fig. 6. As can be seen, *D* values in the range of 1–4 × 10^{-13} $m^2 \cdot s^{-1}$ were obtained. To the best of our knowledge there are no specific data of *D* for 1-octen-3-ol in LDPE in the literature. However, the values are comparable to other oxygenated linear C8 compounds in LDPE, for example: *n*-octanol (4.0–4.7 × 10^{-13} $m^2 \cdot s^{-1}$) (Koszinowski & Piringer, 1990), *n*-octanal (4.0–5.4 × 10^{-13} $m^2 \cdot s^{-1}$) (Johanson & Leufven, 1994; Sadler & Braddock, 1991; Shimoda, Matsui, & Osajima, 1987) and 3-octen-2-one (7.3 × 10^{-13} $m^2 \cdot s^{-1}$) (Shimoda et al., 1987), all measured at 23°C. The somewhat lower values obtained in this study may be attributed to the lower testing temperature (19°C).

Although the differences are small, *D* values seem to decrease with CO_2 density and with depressurization rate. The diffusion rate of a molecule in a polymeric matrix mainly depends on the tortuosity of the structure, which is affected by the length and crosslinking degree of polymer chains, as well as the crystallinity degree, the size and distribution of crystalline domains. It has been pointed out that polymer recrystallization may be enhanced by CO_2 sorption under high pressure conditions, as it promotes chain mobility (plasticization), in an analogous way as thermal annealing (Sun, Cooke, Bates, & Wynne, 2005). As CO_2 sorption increases with density, operating at higher pressure conditions may increase crystallinity in some degree, reducing the diffusion coefficient values. Nevertheless, in order to confirm this hypothesis, thermal analysis of the impregnated samples should be performed.

3.3. Biological activity of impregnated films

The biological activity of LDPE films impregnated under the best operational conditions was evaluated against two of the most important maize pests: *F. verticillioides* and *S. zeamais*. As mentioned before, under the tested process conditions, only slight differences were observed on the release profiles of 1-octen-3-ol and its diffusion coefficients, and therefore the best operational conditions were selected based on the highest impregnation yield obtained. Consequently, LDPE films were impregnated at *P* = 9.5 MPa (CO_2 density

= 415 kg·m^{-3}), depressurization rate = 0.5 MPa·min^{-1}, T = 45ºC and t = 4 h, in excess of 1-octen-3-ol, and these samples were used for the activity tests.

3.3.1. Antifungal activity.

Treated films with *Y%* = 3.82 ± 0.41% were tested as fumigant devices against *F. verticilliodes*. Fig. 7 shows the fungal growth curves in presence of impregnated films and neat LDPE (control) along time. A decrease of fungal growth rate in presence of the impregnated films can be observed, compared to the control samples, corresponding to an inhibition percentage of 55.44%. Besides, the lag phase of fungal growth, quantified from the abscissa of the curves, was extended in comparison to the control sample (72 and 39 h, respectively).

3.3.2. Insecticidal activity.

The insecticidal toxicity of films treated under the best impregnation conditions is shown in Fig. 8. It can be seen that in the first three stages the treated films showed a high mortality (100%) and afterwards it starts to decrease. These results can be related to the 1-octen-3-ol concentration in the impregnated films over time. Thus, in the first three stages the concentration of biopesticide is high, with values of 0.022 to 0.017 g·l^{-1}, respectively, causing mortality in all insects (100%); then, in the fourth stage the concentration of 1-octen-3-ol decrease to 0.011 g·l^{-1} producing an insect mortality of about 80%. Finally, in the next stages (5, 6, 7) the mortality declines to 70, 60 and 30% with a concentration of biopesticide of 0.008, 0.006 and 0.004 g·l^{-1}, respectively. On the other hand, the untreated films did not show toxicity against insects during the time range evaluated. Some studies (Herrera et al., 2015; Zhao et al., 2011) showed the insecticidal activity in fumigant assays of pure 1-octen-3-ol against *Tribolium castaneum* and *S. zeamais* with a lethal dose 50 (LD $_{50}$) of 0.0168 and 0.0277 g·l^{-1} air, respectively, after 24 h of exposure. In this work, it has been observed that in all stages the concentration of 1-octen-3-ol in the vials was below the LD $_{50}$, obtaining mortality levels from 36 to 100%. This indicates the ability of the films to act through fumigant and contact modes. In agreement with these results, previous reports carried out by our research group revealed a good toxicity of impregnated films with terpene ketones against *S. zeamais* (Herrera et al., 2017).

4. Conclusions

The incorporation of agents with biological activity, such as biopesticides, in polymeric matrix is a new technology for development of active materials for packaging and preservation food. In the present study, films of LDPE with 1-octen-3-ol obtained under the best conditions of supercritical impregnation (lower depressurization rates and intermediate pressure) were evaluated against *F. verticillioides* and its vector insect *S. zeamais*, showing a good control toward both pests of the stored grains. This activity can be conserved during different stages, associated with the gradual release of the biopesticide. Thus, this study could contribute to the development of an active material with applicability in food preservation.

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Table 1. Experimental conditions and yield (*Y*%) for supercritical CO_2-assisted impregnation of LDPE films with 1-octen-3-ol. T = 45ºC and time = 4 h for all runs. Impregnation yields are shown as mean values ± standard error.

Run no.	Pressure (MPa)	CO_2 density ($kg \cdot m^{-3}$)	Depressurization rate ($MPa \cdot min^{-1}$)	*Y%*
1	7.5	209.28	0.5	2.84 ± 0.28
2	9.5	414.56	0.5	3.39 ± 0.27
3	9.8	466.43	0.5	3.32 ± 0.24
4	11.0	603.15	0.5	2.35 ± 0.21
5	14.5	731.74	0.5	2.13 ± 0.16
6	7.5	209.28	5.0	2.75 ± 0.24
7	9.2	365.70	5.0	2.40 ± 0.21
8	10.2	526.25	5.0	2.50 ± 0.11
9	11.0	603.15	5.0	1.63 ± 0.22
10	14.5	731.74	5.0	1.73 ± 0.30

Figure captions

Figure 1. Chemical structure of 1-octen-3-ol

Figure 2. High-pressure impregnation system. 1: CO_2 reservoir; 2: cooling coil; 3: pressure generator; 4: manometer; 5: impregnation cell; 6: temperature controller; 7: magnetic stirrer; V: valves; MV: micrometering valve.

Figure 3. FT-IR spectra for original and impregnated LDPE film and pure 1-octen-3-ol. Impregnation conditions: CO_2 density = 410 kg·m^{-3}, depressurization rate = 0.5 MPa·min^{-1}, T = 45 °C and t = 4 h.

Figure 4. Effect of CO_2 density on the impregnation yield (*Y%*), at different depressurization rates (dp). Impregnation conditions: T = 45ºC; t = 4h; dp = 0.5 MPa·min^{-1} (■), and 5 MPa/min (○). Vertical bars represent standard error. Lines are only visual guidance.

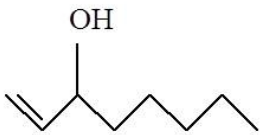
Figure 5. Release profiles of 1-octen-3-ol from LDPE films impregnated at P = 9.5 MPa (■) and 11 MPa (○), with depressurization rate = 0.5 MPa·min^{-1}, T = 45ºC and t = 4 h. Dots: experimental data; lines: model fitting.

Figure 6. Diffusion coefficients for 1-octen-3-ol in LDPE films at ambient conditions (T=19ºC, RH=60%). Impregnation conditions: T = 45ºC; t = 4h; dp = 0.5 MPa/min (■) and 5 MPa/min (○).

Figure 7. Antifungal effect of untreated (■) and impregnated (○) LDPE films on *F. verticilliodes* along time of exposure. Vertical bars represent standard error. Lines are only for visual guidance.

Figure 8. Insecticidal effect of treated LDPE films against *S. zeamais* and its relationship with the concentration of 1-octen-3-ol in the fumigation chamber. Bars represent the mortality percentage and the concentration of 1-octen-3-ol in the fumigation chamber is represented by the square points. Data are shown as means values with standard error.

Figure 1. Chemical structure of volatile organic alcohol



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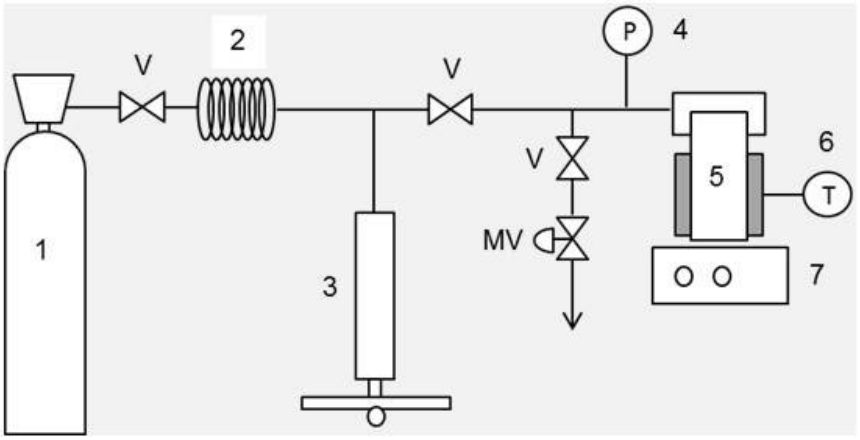

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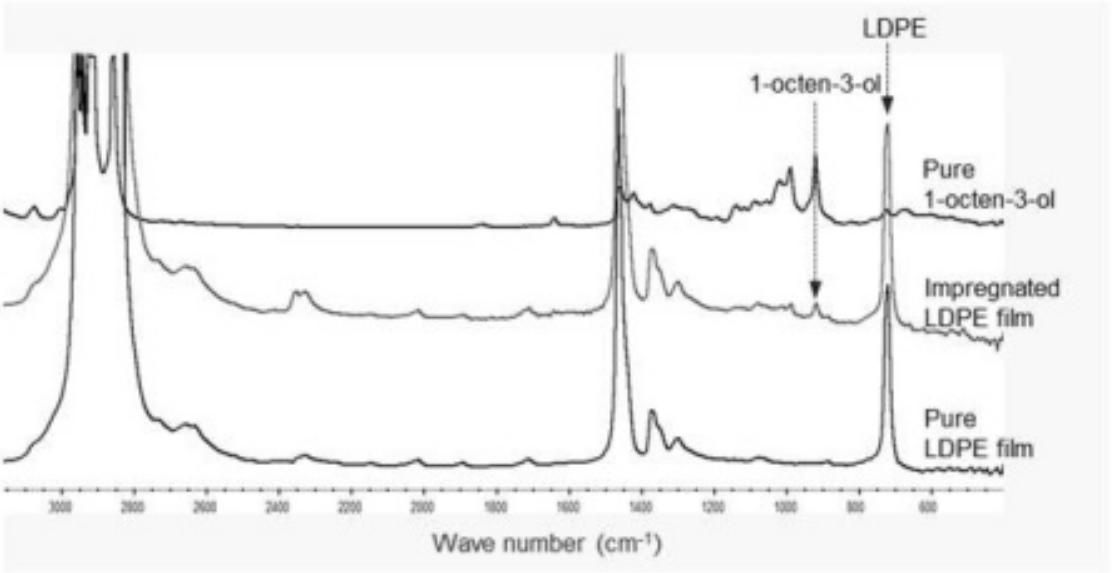

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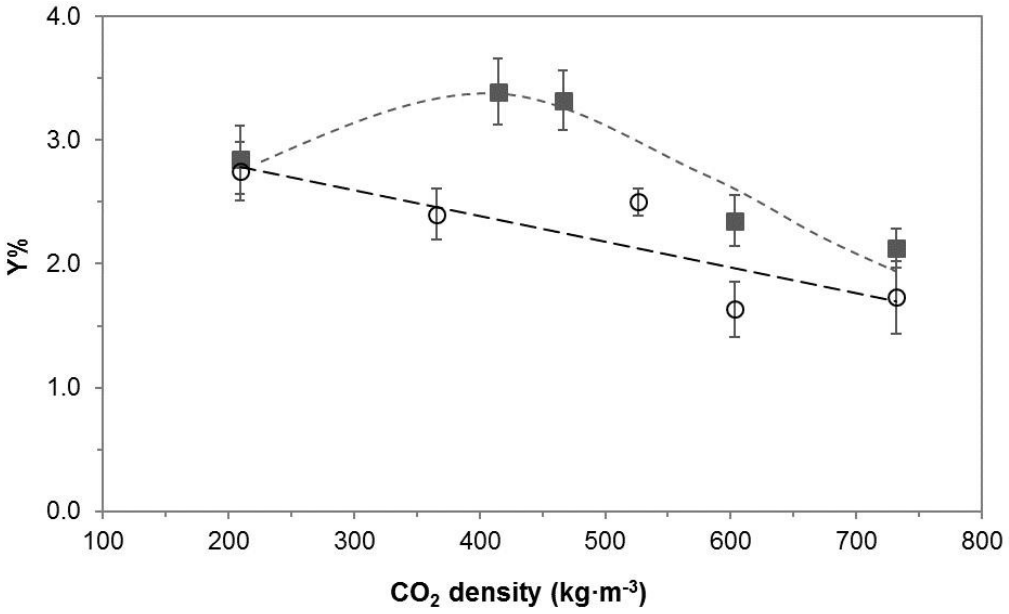

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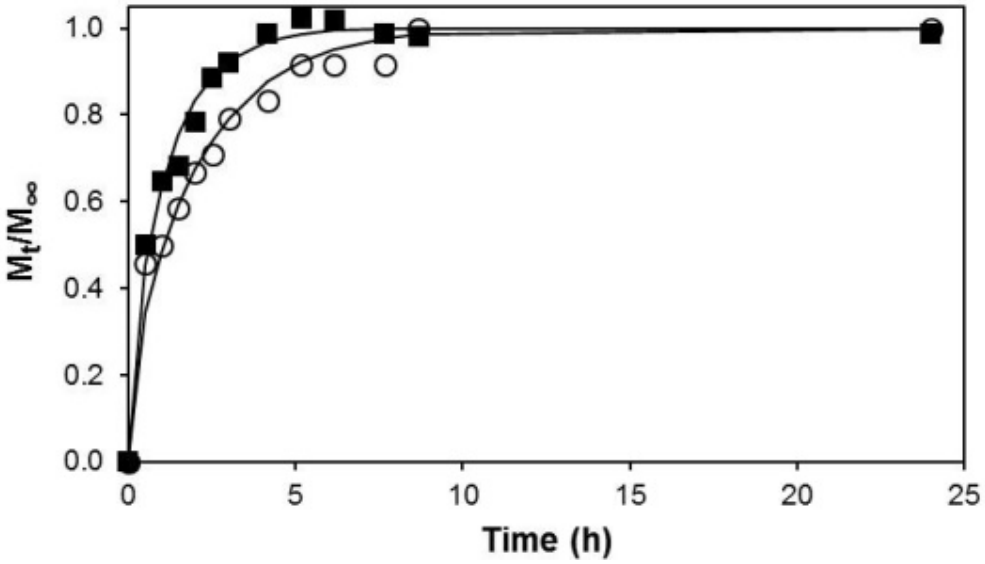

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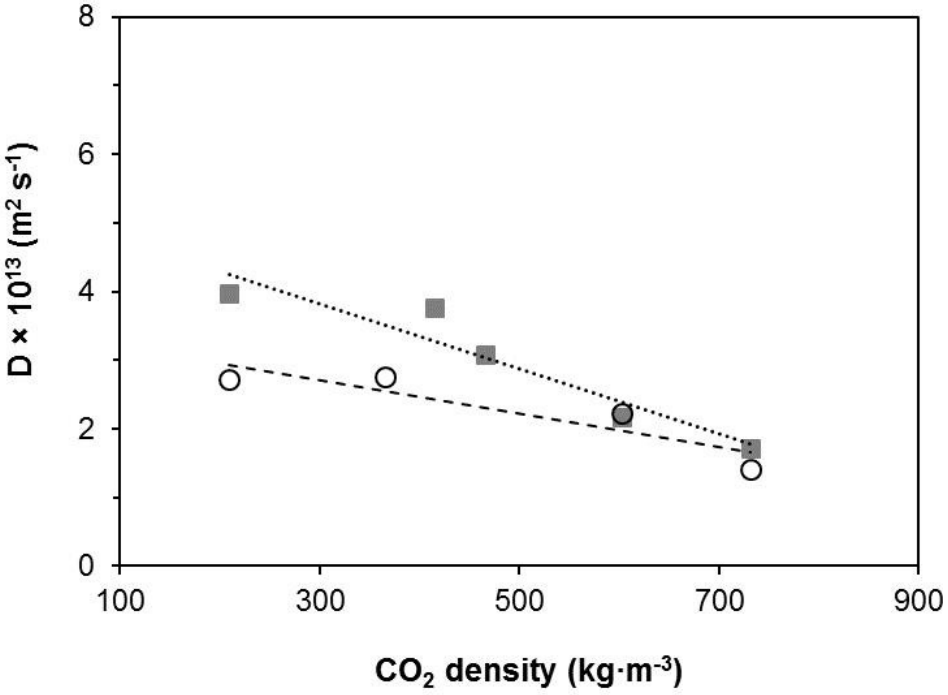

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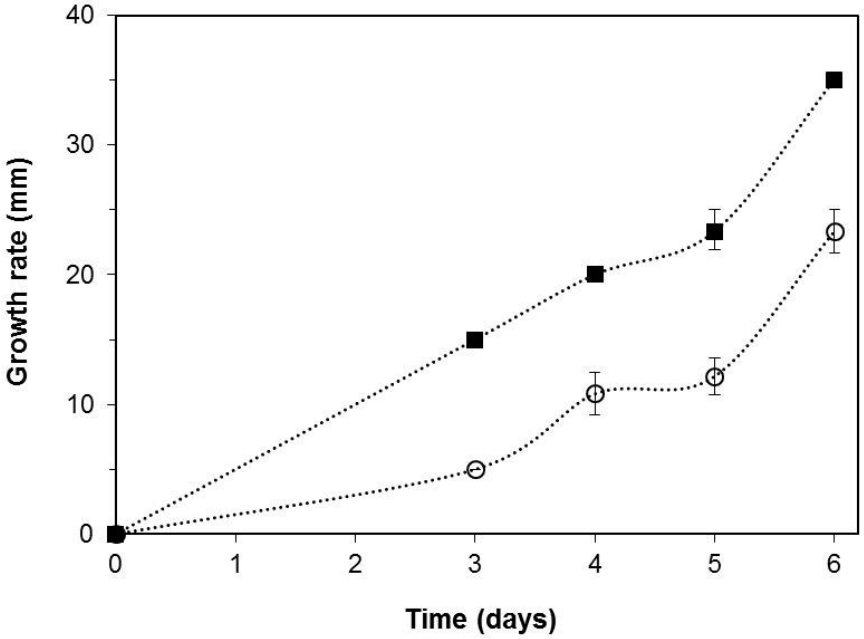

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