

Eugenol-loaded LLDPE films with antioxidant activity by supercritical carbon dioxide impregnation



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ABSTRACT

In this work, linear low density polyethylene (LLDPE) films were impregnated with eugenol using supercritical CO₂ impregnation, as a strategy for obtaining a loaded material to be potentially applied in active food packaging. Eugenol, the main component of clove oil, is well known as a natural antioxidant and antimicrobial agent. Impregnation runs were performed in a lab-scale high pressure stirred cell at 45 °C during 4 h, and the effect of pressure (10, 12 and 15 MPa) and depressurization rate (0.5, 1 and 5 MPa/min) on impregnation yield was evaluated. Yields of 1–6% (w/w) were achieved, with higher values at low depressurization rate. Film samples were characterized by infra-red spectroscopy (FTIR) and differential scanning calorimetry (DSC). FTIR analysis suggested that the distribution of eugenol in the films is not totally homogeneous. DSC revealed a decrease in the crystallinity degree in the impregnated samples as well as in films subjected to pressurization with CO₂ but not loaded with eugenol. Changes in mechanical properties were also investigated in a texturometer. A decrease of LLDPE Young modulus and ductility was observed after high pressure impregnation, although tensile strength was not significantly affected. Finally, the antioxidant activity of the eugenol-loaded films was assessed via the DPPH inhibition test.

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1. Introduction

In the last years, food industry has renewed its interest on active and intelligent packaging, especially in Europe, after the publication of new regulations concerning the use of this kind of materials in food contact applications. At the same time, as consumers demand for minimally processed and preservative-free products is increasing, food industry is putting much attention on antimicrobial (AM) and antioxidant (AO) packaging [1,2].

It is important to remark the difference between passive, active and intelligent packaging systems. On one hand, a *passive packaging* system acts only as a physical barrier between the product and the environment. On the other hand, *active packaging* refers to a material which actively interacts with the packaged product, modifying its conditions. It is designed to deliberately incorporate active components that can either absorb undesired substances

(O₂, CO₂, H₂O, ethylene, off-flavours, etc.) or be released into the inner package atmosphere or the environment around it, acting as AM and/or AO agents, flavouring agents, etc. Finally, *intelligent packaging* systems denote materials which are able to monitor the conditions of packaged food or the environment surrounding it, detecting and/or recording external or internal changes in the product–package–environment system, being able to communicate this information (e.g., humidity, temperature and microbial growth indicators, among others) [2].

Active packaging containing substances with AM and/or AO activity aids to extend the shelf life of food products, contributing to their quality and safety. Furthermore, as naturally derived AM and AO agents – such as plant extracts – are increasingly perceived by consumers as healthier preservatives, their use in food packaging materials is becoming an interesting alternative to synthetic additives [3].

Active substances or additives are incorporated into polymers at industrial scale by several techniques. Some of them, such as hot melt extrusion or casting, require operating above the polymer melting temperature, with some risk of thermal decomposition of sensitive organic compounds. In other conventional processes, such

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as solvent-casting or film soaking into a liquid solution of the active substance, organic solvents are used that must then be removed in order to meet increasingly rigorous regulations, specially concerning materials for pharmaceutical, biomedical and food applications.

In this context, supercritical solvent impregnation (SSI) has been proposed and studied by several authors as an interesting alternative to other conventional techniques due to several advantages [4,5]. Supercritical fluids (SCF) are able to diffuse into polymers and swell them, favouring the incorporation of active substances previously dissolved. In this way, solute loading is not limited to the surface, but it is also retained deeper inside the polymeric matrix.

A typical SSI process consists of three steps. Firstly, the active substance is dissolved by the SCF. This can be done in a separate “saturation” chamber or in the same impregnation cell. Secondly, the loaded supercritical fluid penetrates and swells the polymeric matrix, favouring the internal diffusion of the active substance. Finally, after some contact time, the system is depressurized, with a drastic decrease of the SCF density and the precipitation of the solutes. The loading efficiency depends on several aspects: the polymer properties (density, ramification degree, morphology); the occurrence of specific interactions between the polymer and the active substance; the partition coefficient of the substance between the polymer and the fluid phase (and its dependence on pressure and temperature conditions); the swelling and plasticizing ability of the solvent [4,6]. Depressurization rate is an important operation parameter: a fast depressurization may increase polymer loading, but it can also produce mechanical damages to the material; while a slow pressure decrease preserves the polymeric matrix, but an excessive solute loss in the supercritical fluid can occur, depending on the initial pressure. Therefore, it is a parameter that needs to be optimized in each polymer–solute–solvent system.

In the last years, supercritical CO₂ impregnation has been studied and proposed for several applications, including the development of active packaging materials for food preservation and controlled drug delivery systems. Some of the most recent examples include the impregnation of contact lenses with local action drugs, such as anti-inflammatory agents [7]; wood impregnation with antifungal compounds [8]; incorporation of antibiotics into biopolymer films [9]; impregnation of scaffolds with natural antimicrobial agents [10] and development of active food packaging materials by incorporation of natural bioactive compounds into commercial [11,12] or bio-based polymers [13].

In this study, the incorporation of eugenol into linear low density polyethylene (LLDPE) films by supercritical CO₂ impregnation is proposed in order to develop an active material for food packaging with AO activity. Eugenol is an allylbenzene compound naturally present in many essential oils, specially from clove, nutmeg and cinnamon. It is widely used in food, pharmaceutical, cosmetic and dental care industry as flavouring agent and due to its antibacterial, antifungal and antioxidant properties. Several authors have reported the applicability of eugenol among other plant extracts, as an AO and/or AM agent for improving shelf life and quality of food products, particularly in fruits, vegetables and raw meats. It has been proved [14] that eugenol, as well as thymol and menthol, not only extended shelf life of strawberries, but also maintained better fruit quality with higher levels of sugars, organic acids, phenolics, anthocyanins, flavonoids and oxygen radical absorbance capacity. Moreover, Woranuch and Yoksun [15] suggested the applicability of biofilms containing eugenol-loaded chitosan nanoparticles as an AO active packaging material, while Kechichian et al. [16] reported the AM effect of biofilms with clove and cinnamon powders rich in eugenol in contact with bread slices. Eugenol AO and AM activity has also been observed in combination with modified atmosphere packaging (MAP) in table grapes quality and safety [17]. Furthermore, Fernández-Pan et al. [18] have confirmed the improved AM activity of clove and oregano essential oils incorporated into edible

films compared to the effects of direct addition of the essential oils onto chicken breast surfaces. However, in these cases, eugenol was applied directly to the surfaces or incorporated into the packing material by conventional techniques.

An appropriate knowledge of the system phase behaviour is fundamental for the design and optimization of the supercritical fluid impregnation process. The main relevant questions to be answered are whether the fluid phase is homogeneous or heterogeneous (i.e., if the active substance is completely or partially dissolved in CO₂), and how pressure and temperature conditions affect the CO₂-sorption into the polymer and its swelling and plasticization effect. As most essential oils common constituents, eugenol is readily soluble in supercritical CO₂. This allows impregnation to be performed at mild pressure and temperature conditions, with no need of liquid cosolvents for solubility enhancement, as is usually the case with many polar or high molecular weight compounds such as pharmaceutical drugs. Experimental high pressure liquid-vapour equilibrium data for the eugenol + CO₂ system have been reported by Cheng et al. [19] at 35, 45 and 55 °C and up to 13 MPa. Souza et al. [20] have studied high pressure phase equilibria of clove oil (75% eugenol) + CO₂, reporting a transition from liquid–liquid–vapour behaviour under 35 °C to liquid–vapor behaviour above 40 °C. Solubility in the supercritical phase range between 0.5 and 10 wt.%, according to both studies. In general, it can be concluded that eugenol (or clove oil) solubility increases with pressure and decreases with temperature (at constant pressure) in the range 40–55 °C, due to effect of these variables on CO₂ density. Cheng et al. did not observe significant composition changes in the vapor phase at different temperature or pressure conditions, possibly due to limitations of the experimental setup, but their data clearly show that single phase conditions are achieved at lower pressure when decreasing temperature [19].

On the other hand, to the best of our knowledge, there are no specific studies concerning the interactions between supercritical CO₂ and LLDPE. However, some general behaviour features common to other polymers are expected to occur. For instance, Üzer et al. [21] have observed and measured the expansion of polymethyl-methacrylate (PMMA) rods under CO₂ pressure, as well as the propagation of a boundary between glassy and plasticized parts of the polymer. Volume expansion increased with CO₂ density, reaching a value of almost 25% operating at 15 MPa and temperatures between 35 and 45 °C, which increased up to 80% when naphthalene was loaded. Lei et al. [22] have reported a swelling degree of around 5% and a CO₂ uptake of approximately 0.05 g/g polymer when polypropylene (PP) was swollen at 10 MPa and temperatures of 40 and 60 °C. Moreover, von Schnitzler and Eggers [23] have reported volume expansion values between 1.8% and 2.4% for polyethylene-terephthalate (PET) at 40–50 °C and pressure between 10 and 30 MPa, respectively. More recently, Champeau et al. [24] have investigated, by a novel in situ FTIR spectroscopic technique, the CO₂ sorption and polymer swelling of polyethylene oxide (PEO), poly-L-lactide acid (PLLA), PET and PP samples under high pressure CO₂ (2–15 MPa) at 40 °C, correlating them with the polymers structure. They have observed that both sorption and swelling increase with pressure, and that higher values are reached when specific CO₂/polymer interactions can occur and when the polymeric chains mobility is higher (or the crystallinity is lower). Therefore, the highest values were observed for PEO and PLLA (20–25% CO₂ sorption, 10–35% swelling), and the lowest for PET and PP (5–10% CO₂ sorption, 2–5% swelling). As a general conclusion of these works, it can be observed that swelling and CO₂ uptake are both enhanced by increasing CO₂ density (via pressure increase or temperature decrease). In turn, density improves solute loading, by polymer plasticization and increase of internal diffusion coefficients.

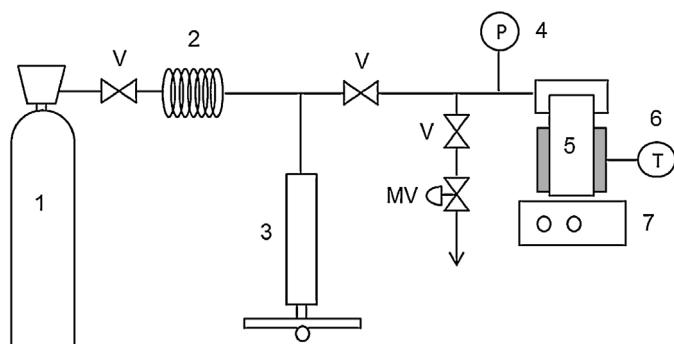


Fig. 1. High pressure impregnation apparatus. 1, CO₂ reservoir; 2, cooling coil; 3, pressure generator; 4, manometer; 5, impregnation cell; 6, temperature controller; 7, magnetic stirrer; V, valves; MV, micrometering valve.

Based on this information, the specific goals of the present work are:

- the experimental study of the supercritical CO₂ impregnation of eugenol into LLDPE films in a lab-scale high pressure system and the analysis of the effects of different operation conditions (namely pressure and depressurization rate) on impregnation yield;
- the characterization of the impregnated films, including an AO activity assessment;
- the evaluation of possible changes in the polymer thermal and mechanical properties due to the high pressure processing and the incorporation of eugenol.

2. Materials and methods

2.1. Materials

Commercial linear low density polyethylene film (LLDPE, M_w : 229,300, M_n : 22,500, MFI: 0.6 g/10 min at 190 °C/2.16 kg, density: 921 kg/m³, thickness: 130 ± 20 μm, Dow-Polisor, Argentina) was used as polymeric matrix in all impregnation tests.

Eugenol (99%, Sigma-Aldrich, USA) was used as active substance and industrial extra-dry carbon dioxide (water content ≤ 10 ppm v/v, Linde, Argentina) as impregnation solvent. 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, USA) was used in the antioxidant activity tests.

Methanol (≥ 99.8%, HPLC grade, Sintorgan, Argentina) was used as solvent in the characterization tests and ethanol (96%, v/v, food grade, Porta, Argentina) was used in food simulant solutions for release studies, as well as for cleaning the experimental apparatus.

2.2. Impregnation equipment and method

Impregnation experiments were performed in a lab-scale high pressure batch apparatus schematically described in Fig. 1. It consists of a stainless steel cell of 50 cm³ of internal volume which can operate up to 25 MPa and 200 °C. This cell is externally heated by an aluminium jacket and a resistance clamp connected to a temperature controller (Instrelec, Argentina). Agitation is provided by a magnetic stirrer (Arcano, Argentina).

First, a certain volume of eugenol – depending on the operation conditions – is loaded into the cell together with a small magnet coated with teflon for stirring purposes. The film samples are then placed into the impregnation cell on a metallic mesh support, which prevent them from sticking during pressurization and keeps them in vertical position. This is important for avoiding deposition of eugenol droplets onto the films surface during depressurization. After closing the cell, CO₂ is liquified in a 2 °C water cooling bath

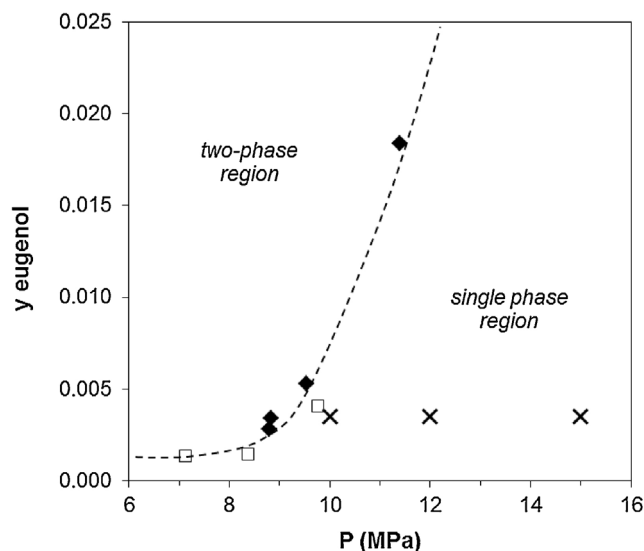


Fig. 2. Eugenol/clove oil solubility in supercritical CO₂ (mole fraction) at $T=45$ °C. Experimental data: (□) eugenol [19], (◆) clove oil [20], (×) Experimental conditions used in this study. Dotted line: phase transition border (trend line).

(Lauda, Germany) and delivered to the cell by means of a hand pump or pressure generator (HiP, USA). The heating resistance is activated and the system is allowed to equilibrate, with all valves closed and under stirring. During this step, minor pressure corrections are performed with the hand pump until stabilizing at the desired value.

The impregnation process proceeds for a certain period of time at constant temperature, pressure and agitation conditions. After this time, the heating and stirring systems are turned off and the cell is depressurized under controlled conditions at constant rate, by means of a micrometering valve (Swagelok, USA). This valve and the outlet line are wrapped with a heating tape in order to avoid the formation of solid particles due to the cooling effect of CO₂ expansion, which may affect the depressurization rate control. After depressurization, the cell is opened and the films are carefully removed and cleaned superficially with tissue paper in order to eliminate eugenol droplets.

The amount of eugenol incorporated into the LLDPE films was measured gravimetrically in a precision balance and impregnation yield (Y%) was calculated according to Eq. (1):

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

where m_0 is the initial mass of the film and m_f is its mass after impregnation. The impregnated samples had to be weighed during some minutes until constant weight, due to residual CO₂ desorption. After this step, each film was placed into a 2 ml vial hermetically closed and stored in a refrigerator at 4 °C protected from light, in order to minimize eugenol evaporation until analysis.

In this work, impregnation runs were performed at three different values of pressure (10, 12 and 15 MPa) and depressurization rate (0.5, 1 and 5 MPa/min) at a constant temperature of 45 °C. In all cases, impregnation was carried out for a period of 4 h before starting depressurization.

The amount of eugenol to be loaded into the cell was determined considering the solubility of this compound in supercritical CO₂ reported in the literature within this experimental range. Fig. 2 shows experimental solubility data [19,20] and the approximate limits of the single-phase region, as well as the operation conditions selected in this work. In all tests the cell was loaded with a constant eugenol/CO₂ mass ratio of 0.013 ± 0.0001 g/g (or a molar ratio of 0.0035), slightly below the solubility at 10 MPa, in order

to ensure total solubilization at all tested pressures. As CO₂ density changes with pressure and the cell volume is fixed, different amounts of eugenol had to be loaded at each pressure level. The polymer/eugenol mass ratio was also maintained constant in all impregnation runs and equal to 0.63 ± 0.05 g/g. Once determined the amount of eugenol required in each run, the mass of LLDPE films to be placed into the cell was calculated in correspondence to it. In each run, three film samples of variable size (5.5–8.5 cm²) were used. In this way, the effect of eugenol concentration during impregnation is not taken into account as a variable, and only the influence of pressure and depressurization rate are analysed. It should be noticed that the eugenol/CO₂ and polymer/eugenol ratios, as well as the impregnation time and temperature, have not been optimized. The influence of these process variables will be subject of a future contribution.

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

Absorbance spectra were obtained in an infrared imaging microscope (Nicolet iN10 Mx, Thermo Fisher Scientific, USA), with a resolution of 4 cm⁻¹, in a wavenumber range of 400–4000 cm⁻¹ with 16 scans, at room temperature. Spectra of pure eugenol, original LLDPE film and eugenol-impregnated film samples were acquired 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.

2.4. Differential scanning calorimetry (DSC)

A calorimetric study was performed in a Perkin Elmer Pyris 1 equipment. Thermograms were obtained directly on film samples heating from 25 to 180 °C and cooling from 180 to 25 °C, both at a rate of 10 °C/min. Analysis was performed on untreated LLDPE films as well as on impregnated samples in order to evaluate the crystallization variation during high-pressure treatment (with and without eugenol). For this reason, as the polymer thermal history should not be removed, only the first heating cycle was considered.

2.5. Mechanical properties

Tensile strength tests were performed on eugenol-impregnated, CO₂-pressurized and untreated LLDPE film samples in a universal testing machine (Instron, USA). The aim of these tests was to determine the impact of the high pressure treatment and the incorporation of eugenol in the mechanical properties of the films. Similar rectangular probes (60 mm × 10 mm) were used in all cases, with an initial grip separation of 30 mm. Tests were conducted under a constant cross-head speed of 100 mm/min until break. Young modulus, yield strength, tensile strength and elongation at break were recorded along each test. Film samples thickness was measured prior to all tests with a precision micrometre (0–25 mm × 0.01 mm, Wembley, China).

2.6. Antioxidant activity via DPPH inhibition

The DPPH inhibition assay was applied to evaluate the free radical scavenging activity of eugenol-impregnated films. DPPH is one of the few stable and commercially available organic nitrogen radical. This compound has a UV–vis absorption maximum at 515 nm, presenting a strong violet colour in methanol solution. In presence of an antioxidant substance, the solution colour fades as the reduction of DPPH proceeds, which allows to record the progress of this reaction by spectrophotometry [25].

For the antioxidant activity determination, the following method was used. A solution of DPPH in methanol was prepared

Table 1
Supercritical CO₂ impregnation yield of LLDPE films (T=45 °C, 4 h).

Pressure (MPa)	CO ₂ density (kg/m ³) ^a	Depressurization rate (MPa/min)	Impregnation yield (%)
10	502.6	0.5	4.51 ± 0.29
		1	4.41 ± 0.69
		5	3.08 ± 0.77
12	659.7	0.5	4.29 ± 0.62
		1	3.54 ± 0.80
		5	3.60 ± 0.71
15	742.2	0.5	5.67 ± 0.09
		1	4.34 ± 0.20
		5	1.31 ± 1.01

^a From NIST.

(0.025 g/l) and placed into dark glass flasks. Impregnated film samples were added into the flasks at time $t=0$ and kept hermetically closed and protected from light for 24 h, without stirring. A control test was performed under the same conditions using a non-impregnated film. After this period, absorbance measurements of the liquid phase were performed in a UV/vis spectrophotometer (Lambda 25, Perkin Elmer, USA). Spectra were obtained in a wavelength range of 400–700 nm and absorbance values at 515 nm were recorded. The percentage of DPPH inhibition (% I) of each sample was calculated according to Eq. (2):

$$\%I = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \quad (2)$$

where $A_{control}$ and A_{sample} are the absorbance values at 515 nm of the control and the impregnated film sample, respectively.

3. Results and discussion

3.1. Supercritical impregnation of eugenol in LLDPE films

As previously mentioned, impregnation was performed at constant temperature (45 °C), three pressure levels (10, 12 and 15 MPa) and three depressurization rates (0.5, 1 and 5 MPa/min), for a period of 4 h before pressure release. Impregnation yield results at each set of operation conditions are shown in Table 1.

It can be seen that yield values ranged approximately between 0.5 and 6 wt.%, with the highest values at 15 MPa and 0.5 MPa/min (5.67%) and the lowest at 15 MPa and 5 MPa/min (1.31%). In general, higher loading values were obtained with slower depressurization rates, although this effect was more pronounced when impregnation was performed at 15 MPa.

At 10–12 MPa, eugenol loading seems to depend weakly on depressurization rate. This can be explained by the fact that at these conditions eugenol is close to its solubility limit, as previously mentioned, and therefore a minor pressure decrease may be enough for producing its partial condensation, reducing its concentration in the fluid phase to the saturation value during most of the depressurization step. Besides, polymer swelling and CO₂ sorption, which increase with solvent density, are lower at these pressure conditions. If the polymer structure is less affected in its morphology, the internal diffusion of eugenol is expected to be lower. On the contrary, at 15 MPa a higher polymer plasticization occurs, and the recovery of its former structure will depend more strongly on the depressurization conditions: if the system is expanded rapidly, the eugenol-loaded CO₂ may diffuse out of the films at a higher rate than the polymer recrystallization. Besides, at 15 MPa the system is initially far from the solubility limit, and therefore eugenol remains dissolved in the fluid phase during a longer part of the depressurization step. Both effects may explain the low impregnation yield obtained at these conditions.

These results are in agreement with the reports of other authors. For example, Yokozaki et al. [26] have observed a similar behaviour in the case of supercritical impregnation of contact lenses with salicylic acid, reporting higher loading values at low depressurization rate (at constant temperature and pressure conditions). Champeau et al. [27] have also observed a higher ketoprofen impregnation yield on PLLA fibres when depressurizing the system at slow rate, as well as a homogeneous distribution along the fibres section area. However, other authors have reported an enhancing effect of depressurization rate on polymer loading, for instance Varona et al. in a study on the impregnation of a modified starch with lavender oil [28]. These opposite trends can be explained in terms of the main loading mechanism acting in each polymeric matrix. In the case of porous or powdered matrices with limited swelling and/or CO₂ sorption capacity, the active substance loading mainly occurs by physical deposition and/or specific interactions onto the surface of the pores or particles, and therefore it may be enhanced by a sudden precipitation induced by a rapid expansion. On the contrary, in the case of impregnation of film-type polymers with higher swelling and sorption capacity (such as polyolefins) the active substance actually dissolves into the swollen polymeric matrix, and the final loading will be determined by the partition coefficient of the solutes between the fluid phase and the polymer and the polymer morphology changes that occur during depressurization.

In addition, fast depressurization resulted in higher yield deviations among films impregnated in the same batch. This is likely to be due to a more difficult control of the conditions inside the cell. Due to the cooling effect of a rapid expansion, temperature gradients may be formed and therefore solubility drop and eugenol condensation might not be homogeneous. This effect was also more pronounced when impregnation was performed at 15 MPa.

In conclusion, the observed yield differences should be explained in terms of the combination of impregnation pressure and depressurization rate. At 15 MPa, the polymer swelling and plasticization degree, as well as the amount of eugenol-loaded CO₂ dissolved into it, are higher. When the system is depressurized at a high rate, eugenol is dragged out with the CO₂ and it condenses mainly outside the films (low yield values); when depressurization is slow, the polymer is allowed to (partially) recover its former structure and crystalline degree and a higher proportion of eugenol is trapped inside when the solubility limit is reached. This can explain that the best impregnation yields were obtained at these conditions (5.67 ± 0.01 wt.%).

In addition, a slow depressurization also resulted in lower differences among films impregnated in the same batch. This is likely to be due to a more difficult control of the conditions inside the cell when it is depressurized fastly. Due to the cooling effect of a rapid expansion, temperature gradients may be formed and therefore solubility drop and eugenol condensation might not be homogeneous. This effect was also more pronounced when impregnation was performed at 15 MPa.

3.2. Infra-red spectrometric analysis

Pure eugenol, the original LLDPE film and four impregnated LLDPE film samples obtained at different conditions ($P = 10\text{--}15$ MPa and depressurization rate = 0.5–5 MPa/min) were analysed by infra-red spectroscopy using a FTIR microscope, as described in Section 2.3. This technique not only allowed to confirm the presence of eugenol in the impregnated films, but also to qualitatively analyse the homogeneity of the impregnation by measurements in different zones of the films. Spectra of pure eugenol, original and impregnated LLDPE films are shown in Fig. 3 as an example.

Eugenol showed a characteristic absorbance peak at approx. 1514 cm⁻¹ (typical for aromatic C=C bonds), while the peak at 725 cm⁻¹ was assigned to LLDPE [29]. Therefore, the relative

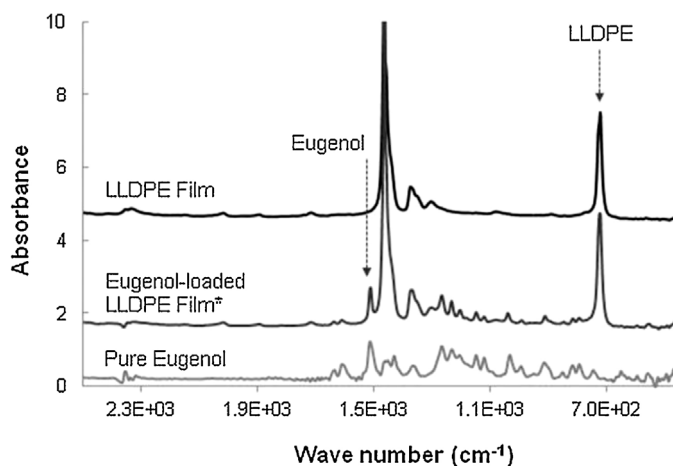


Fig. 3. FT-IR spectra of pure eugenol, LLDPE film and an eugenol-loaded LLDPE film (impregnation conditions: $T = 45^\circ\text{C}$, 4 h, $P = 15$ MPa, depressurization rate = 0.5 MPa/min).

absorbance at 1514 cm⁻¹ in different zones of the films (and among different samples) was taken as a measure of the relative amount of eugenol, useful for comparison purposes. Absorbance was measured at least at three different positions of each film, including points on the edges, and far from the edges (“centre”). Results are reported in Table 2. Eugenol was detected at every analysed point with a global variability up to 20%, suggesting that the impregnation has some degree of heterogeneity. The lowest deviations were obtained for the sample impregnated at 15 MPa and slow depressurization, which supports the hypothesis previously discussed of a more controlled impregnation, CO₂ desorption and recrystallization under these process conditions.

3.3. Thermal properties

The effect of supercritical fluid processing on the thermal properties of the LLDPE films was analysed by DSC, as explained in Section 2.4. As mentioned before, the first heating cycle was taken into account for the discussion in order to evaluate changes of crystallinity during processing. Thermograms of the original LLDPE film, a sample pressurized with pure CO₂ and a sample impregnated with eugenol at selected conditions are compared in Fig. 4. It can be observed the presence of two characteristic peaks, one in the region of 55–65 °C and the most important thermal transition at around 110 °C. The peak at low temperature can be due to the crystallization of low molecular weight chains induced by stretching during film formation. For processed samples, this peak suffers a shifting to higher temperature while the main melting transition does not show important changes. The corresponding melting temperatures (T_{m1} and T_{m2}) are reported in Table 3 for some analysed samples. The mentioned shifting can be explained taking into account the experimental conditions. As the pressurization/impregnation

Table 2

Normalized absorbance (A) at 1514 cm⁻¹ of impregnated LLDPE film samples by FT-IR spectroscopy, measured at different positions in the films.

Sample	1	2	3	4
Pressure (MPa)	10	10	15	15
Depressurization rate (MPa/min)	0.5	5	0.5	5
Position	Normalized absorbance, $A = A_{(1514)}/A_{(725)}$			
Centre A	0.40	0.24	0.33	0.26
Centre B	0.29	–	0.30	–
Edge A	0.25	0.26	0.31	0.26
Edge B	0.35	0.33	–	0.34
Mean value ± SD	0.32 ± 0.07	0.28 ± 0.05	0.31 ± 0.02	0.29 ± 0.05

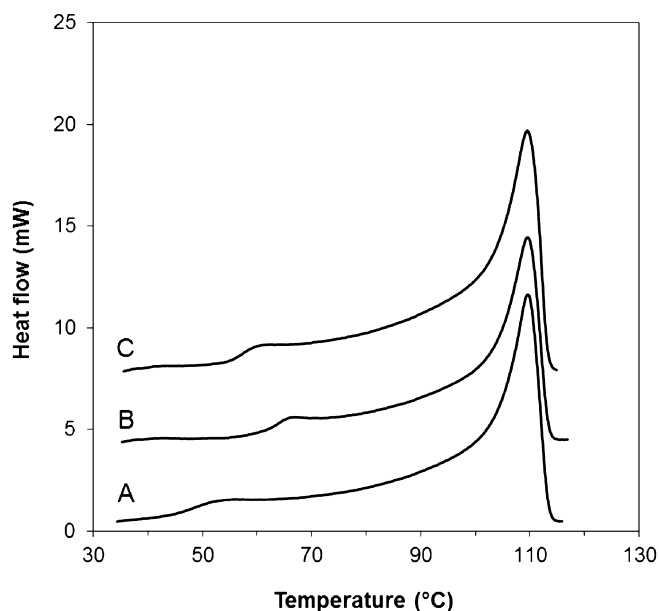


Fig. 4. Thermograms obtained for LLDPE films. A: untreated; B: treated with pure CO₂; C: impregnated with eugenol.

temperature (45 °C) is close to the lower melting temperature of the original LLDPE film, the low molecular weight polymer chains acquire mobility, which is also aided by the plasticizing effect of CO₂. This mobility allows the chains to reorganize in a new crystalline structure with a higher characteristic melting temperature. The observed shifting for the samples impregnated with eugenol was lower than in absence of it, due to the presence of this bioactive compound between polymer chain can interfere in crystal organization, prevailing the plasticizing effect. In the same way, at same depressurization rate, lower T_{m1} values are observed for higher operation pressure, due to the higher CO₂ uptake at higher density conditions, as previously discussed.

Moreover, the crystallinity degree is lower for all samples treated with CO₂ (with or without eugenol), decreasing from 44% for the original LLDPE to 37% after the high pressure processing. The presence of eugenol does not seem to produce further changes. This shows the plasticizing and swelling effect of the supercritical solvent, which may produce deformations in the polymeric matrix, affecting the amount of amorphous and crystalline regions. This behaviour has also been observed by other authors [11]. Therefore, it can be concluded that the high pressure CO₂ processing induced a recrystallization of the low molecular weight chains but decreased the order of longer chains, which are affected only by the presence of the solvent and not by the processing temperature.

Table 3
Thermal properties of original, pressurized and impregnated LLDPE films.

Sample	Experimental conditions P (MPa)/dep. rate (MPa/min)	T_{m1} (°C)	T_{m2} (°C)	Crystallinity degree (%)
Original LLDPE	–	53.58	109.68	44.27
Pressurized films	12/5	68.23	110.02	37.09
	15/1	63.34	110.35	37.18
	10/1	66.73	109.52	37.28
Impregnated films	10/1	61.40	109.52	37.20
	15/5	62.42	110.02	37.86

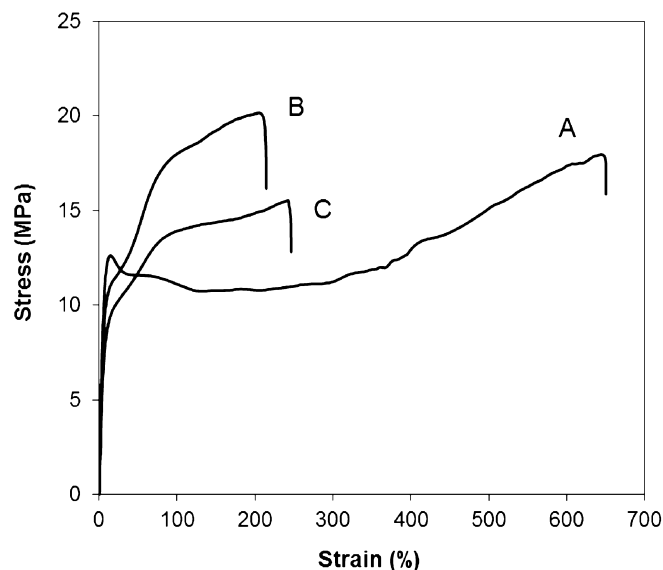


Fig. 5. Stress–strain curves for LLDPE films. A: untreated; B: treated with pure CO₂ (15 MPa/0.5 MPa/min); C: impregnated with eugenol (15 MPa/0.5 MPa/min).

3.4. Mechanical properties

Stress–strain curves for eugenol-impregnated, pure CO₂-treated and untreated LLDPE films are shown in Fig. 5. In the case of impregnated films, only the samples with higher eugenol content (obtained at 15 MPa and 0.5 MPa/min) were tested. Films treated under the same conditions but without eugenol were tested in order to evaluate the effect of the high pressure processing and the incorporation of eugenol separately.

It can be seen that the curve for the untreated films presents the typical behaviour of ductile materials, with a well defined yield peak, while for the samples processed under CO₂ pressure (impregnated or not) the strain hardening begins immediately after yielding, without neck formation during elongation.

The main mechanical properties for each sample are reported in Table 4. A decrease in Young modulus, yield and tensile strength was observed for samples processed with supercritical CO₂, with lower values for the eugenol-impregnated samples. Moreover, a decrease in elongation at break was detected for treated films, although the presence of eugenol produced a slight increase respect to LLDPE films pressurized in absence of this compound. Thus, these results suggest that eugenol has a plasticizing effect on polymer chains, as demonstrated for different essential oils [30,31].

The different stress–strain behaviour of the CO₂-treated samples (with or without eugenol) may be due to their lower crystallinity degree [32], or to a different crystalline morphology obtained for these samples as a result of the cycle of swelling, plasticization and recrystallization produced by the sorption and desorption of the supercritical solvent during the process, and can be correlated to the observed changes in thermal properties. This can also explain the lower Young modulus and yield strength compared with untreated LLDPE films. On the other hand, the lower elongation at break can be understood because of the hardening after yield of the treated samples.

In conclusion, although the high pressure impregnation process decreased the film ductility, it did not affect significantly its flexibility and tensile strength, which are the most important properties for its use as packaging material.

Table 4
Mechanical properties of untreated, pressurized and impregnated LLDPE films.

Sample	Young modulus (MPa)	Yield strength (MPa)	Tensile strength (MPa)	Elongation at break (%)
(A) Untreated	275.11 ± 66.85	12.64 ± 0.26	17.53 ± 0.67	669.06 ± 45.80
(B) CO ₂ -treated	198.36 ± 25.21	10.71 ± 0.43	16.78 ± 2.08	219.62 ± 31.48
(C) CO ₂ -treated with eugenol	167.59 ± 23.07	9.45 ± 0.56	12.84 ± 1.62	249.22 ± 32.82

Table 5
DPPH inhibition percentage (%) of eugenol-impregnated LLDPE films.

Sample	Pressure (MPa)	Depressurization rate (MPa/min)	Eugenol content (%)	24 h		96 h	
				A _(517 nm)	%I	A _(517 nm)	%I
Original LLDPE	–	–	–	0.6244	–	0.5871	–
Impregnated LLDPE	15	0.5	5.19	0.1222	80.43	0.1125	80.84
	15	5	1.31	0.1140	81.74	0.1080	81.60

3.5. Antioxidant activity via DPPH inhibition

Two impregnated LLDPE samples were tested, obtained at a pressure of 15 MPa and different depressurization rates (0.5 and 5 MPa/min), in order to evaluate if the depressurization conditions led to morphological changes that could affect eugenol release or availability. DPPH inhibition results after 24 and 96 h are shown in Table 5. As explained before, they were calculated with respect to a LLDPE film without eugenol as control.

It can be seen that both samples showed approximately 80% inhibition after 24 h, and that this value did not vary after 96 h. This suggests that the impregnated films have a strong antioxidant activity, releasing an amount of eugenol high enough to scavenge DPPH radicals at the tested concentration. The observed limiting inhibition value for both samples may be explained by the partial reversibility of this chemical reaction, as reported by some authors [33].

It is also interesting to notice that the absorbance of the DPPH solution in contact with the control sample slightly decreased between 24 and 96 h. This may be due to some radical scavenging capacity of LLDPE itself, or to a slow diffusion of DPPH from the solution into the film, decreasing its concentration.

4. Conclusions

In this work, the impregnation of LLDPE films with eugenol using supercritical CO₂ as solvent was studied. Loading yields of 0.5–6% were obtained operating at 45 °C, with different combinations of pressure (10, 12 and 15 MPa) and depressurization rate (0.5, 1 and 5 MPa/min), after 4 h impregnation. Best loading results were observed at 15 MPa and slow pressure release, suggesting that at these conditions the polymer swelling and plasticization is higher, allowing a better penetration of eugenol, and afterwards it is allowed to recover its former structure as CO₂ is vented, retaining a higher amount of eugenol. At fast depressurization not only lower yields were obtained, but also a higher deviation between films, due to the difficulty of controlling the conditions inside the cell during a rapid expansion. The presence of eugenol inside the films, as well as its distribution, were studied by FTIR analysis, showing some degree of heterogeneity within a variation range of about 20%.

DSC analysis revealed a decrease of LLDPE films crystallinity after high pressure treatment, observed both in impregnated and non-impregnated samples, suggesting that the main plasticizing effect is due to supercritical CO₂. Mechanical properties were also investigated, and a qualitative modification in the strain-stress behaviour of the material was observed. High pressure treated films showed lower Young modulus and elongation capacity, but the tensile strength was not significantly affected.

Finally, the antioxidant activity of impregnated film samples was assessed via the DPPH inhibition method during 24 and 96 h. A 80% DPPH inhibition was observed, regardless the eugenol loading.

In conclusion, an active LLDPE film could be obtained by means of supercritical CO₂ impregnation as a potential material to be used in food packaging. The influence of some operation variables was explored, while further analysis of the effects of temperature, eugenol concentration and time is envisaged in order to optimize the process and adjust the film characteristics.

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