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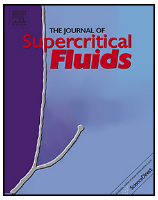

Eugenol-loaded LLDPE films with antioxidant activity by supercritical carbon dioxide impregnation

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ABSTRACT

In this work, linear low density polyethylene (LLDPE) films were impregnated with eugenol using supercritical CO_2 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 CO_2 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.

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 (O_2, CO_2, H_2O, 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 CO_2 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 CO_2 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 CO_2), and how pressure and temperature conditions affect the CO_2-sorption into the polymer and its swelling and plasticization effect. As most essential oils common constituents, eugenol is readily soluble in supercritical CO_2. 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 + CO_2 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) + CO_2, 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 CO_2 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 CO_2 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 CO_2 pressure, as well as the propagation of a boundary between glassy and plasticized parts of the polymer. Volume expansion increased with CO_2 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 CO_2 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 CO_2 sorption and polymer swelling of polyethylene oxide (PEO), poly-L-lactide acid (PLLA), PET and PP samples under high pressure CO_2 (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 CO_2/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% CO_2 sorption, 10–35% swelling), and the lowest for PET and PP (5–10% CO_2 sorption, 2–5% swelling). As a general conclusion of these works, it can be observed that swelling and CO_2 uptake are both enhanced by increasing CO_2 density (via pressure increase or temperature decrease). In turn, density improves solute loading, by polymer plasticization and increase of internal diffusion coefficients.