

Near critical and supercritical clove essential oil impregnation in LLDPE films: stability and kinetic study

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ABSTRACT

Near critical and supercritical carbon dioxide (CO₂) impregnation of active agents in polymer matrices is an innovative technology for developing active packaging materials. Its major advantage is the processing of thermosensitive compounds at low temperatures. Clove essential oil (CEO), a multicomponent active agent, was incorporated in linear low-density polyethylene (LLDPE) films by conventional immersion method and by high-pressure CO₂ impregnation. For conventional kinetic assays, the LLDPE film was placed in a Petri dish containing CEO at 25 °C and atmospheric pressure. For kinetic assays at high-pressure conditions, the pressure was kept constant at 150 bar while the temperature (25, 35 and 45 °C), CEO:CO₂ mass ratio (2 and 10%) and time (intervals up to 8 h) were screened in a variable-volume high-pressure cell. Stability of high-pressure CEO impregnation was determined by keeping LLDPE samples stored during 1 year in a domestic freezer at -18 °C. The maximum amount of CEO incorporated by high-pressure impregnation at 150 bar, 45 °C and 10% CEO:CO₂ mass ratio was 8 times higher than the impregnated amount by the conventional immersion method. CEO impregnation reached its maximum after 2 hours of processing. Differences between initial and final CEO mass after 1 year of storage varied from 0.3 to 3.2 mg CEO g⁻¹ LLDPE, suggesting that high-pressure impregnation is stable and CEO remains incorporated in LLDPE films when stored under such conditions. CO₂ at high-pressure conditions is a suitable medium to incorporate thermosensitive active compounds in polymer matrices for active packaging films.

Keywords: eugenol, carbon dioxide, polymer, active packaging.

1. INTRODUCTION

The principle of incorporating an active agent into a polymer matrix is related to the concept of active packaging: a technological material that interacts with food products preventing or slowing their deterioration. Apart from acting as a selective barrier for moisture and gases, these packages include carbon dioxide (CO₂) scavengers or emitters, moisture absorbents, antioxidant and antimicrobial migrating systems [1,2].

Active agents from natural sources are perceived as compounds with low health risk. The clove essential oil (CEO) is among the most prominent naturally derived active agent due to its antimicrobial, antioxidant, antifungal, antiviral, and insecticidal properties [3]. Clove essential oil is naturally rich in eugenol (around 85-95% of total oil composition), besides presenting β -caryophyllene, α -humulene, and eugenyl acetate in different proportions, depending on raw material and extraction conditions [4]. The advantage of using multicomponent active agents, as the CEO, in impregnation assays instead of pure and chemically synthesized ones is a potential synergistic effect of all the components in antimicrobial and antioxidant activities, besides avoiding isolation and purification steps that imply in increased processing costs [5,6].

Several manufacturing processes have been developed to incorporate active agents in packaging films, such as solvent casting, surface coating, thermocompression, extrusion and

by immersion of films into a solution containing the active agent. However, these processes generally require the use of organic solvents that, depending on the application, should not be present in final product, or are performed at high temperatures that may damage thermosensitive compounds. The impregnation assisted by high-pressure CO₂ (near critical or supercritical) overcomes most problems related to these conventional techniques. This method is advantageous because the formation of carrier material and the active compound incorporation occur in separated steps, avoiding the exposure of thermosensitive compounds to high temperatures [7]. At the final step of processing, the CO₂ is completely removed through depressurization with no degradation of active compounds and polymer matrix [8].

In this sense, the main goal of this work was to incorporate CEO in linear low-density polyethylene (LLDPE) films by conventional immersion method and by using near critical and supercritical CO₂ as impregnation fluid by means of kinetic assays. It also aims to evaluate the influence of operational conditions (pressure, temperature, and CEO:CO₂ mass ratio) on the amount of CEO incorporated in LLDPE, as well as the stability of the high-pressure processing after storing the active films at controlled conditions of temperature for 1 year.

2. MATERIALS AND METHODS

2.1 Materials

Pellets of LLDPE (MFI: 0.95 g/10 min at 190 °C/2.16 kg, density: 919 kg m⁻³, Dowlex TG 2085B) were kindly supplied by Videplast (Videira, SC, Brazil). Clove essential oil (*Eugenia caryophyllus* leaves, 99%, CAS Number 8015-97-2) obtained by steam distillation was purchased from Ferquima (Brazil). Carbon dioxide (99.9%, White Martins, Brazil) was used as impregnation medium.

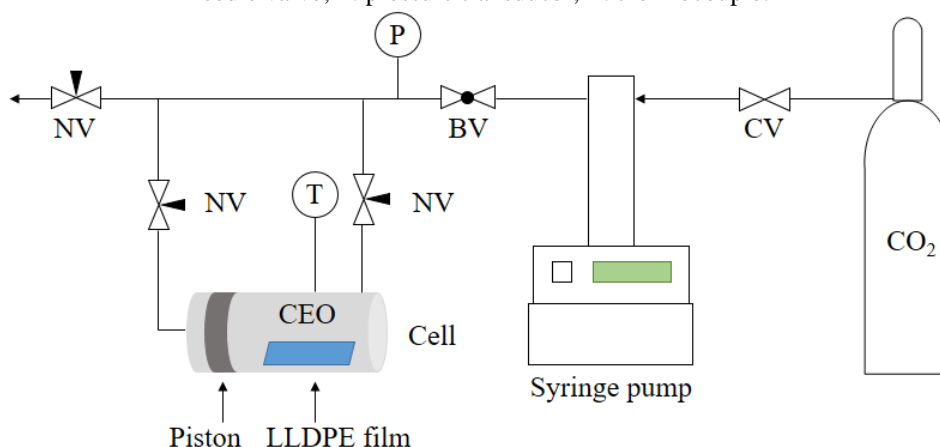
2.2 Film preparation

The LLDPE films were produced by thermo-compressing the pellets in a hydraulic press (PHS 15t, Ico Comercial, Brazil) at 130 °C with a 250 μm polyester film as frame. First, 4 g of LLDPE pellets were placed at the center of frame without applying pressure to ensure a uniform heat flow throughout the material until melting. Then, pellets were gradually pressed to produce transparent, flexible and homogeneous films with average thickness of 300 μm. The film thickness was measured with a digital micrometer (Mitutoyo, Japan, ±0.001 mm accuracy) at three random positions.

2.3 CEO impregnation in LLDPE films

The CEO impregnation in LLDPE films was conducted in a variable-volume high-pressure cell schematically presented in Figure 1. The high-pressure cell contains two sapphire windows (Swiss Jewel, USA) for visualization and a movable piston for inner pressure modulation. The pressure was controlled by a syringe pump (260HP, Teledyne Isco, USA), wherein the CO₂ is the pneumatic fluid of pressurization, and measured by a pressure transducer (LD301, Smar, Brazil). The cell was connected to a thermostatic water bath (MQBTC99-20, Microquímica, Brazil) and a thermocouple J type (Salcas, Brazil). A magnetic stirrer (753A, Fisatom, Brazil) and a Teflon-coated stirring bar maintained the continuous agitation of the cell content.

Figure 1. Schematic diagram of experimental apparatus for impregnation. CV: check valve; BV: ball valve; NV: needle valve; P: pressure transducer; T: thermocouple.



Before impregnation, one sample of LLDPE film (7.2 x 1.2 cm, 300 mg) was placed inside the high-pressure cell. A precise amount of CEO was weighed in analytical balance (AUY 220, Shimadzu, Philippines, ± 0.0001 g accuracy), and loaded in the cell. A known amount of CO₂ in liquid state (7 °C, 100 bar, density of 0.9374 g cm⁻³ [9]) was loaded using the syringe pump until a desired mass ratio of CEO to CO₂ was achieved.

Impregnation assays were performed under the subsequent conditions of near-critical and supercritical CO₂: CEO:CO₂ mass ratios (2 and 10%), pressures (150 and 250 bar), and temperatures (25, 35 and 45 °C) [10]. After finishing the impregnation time of 4 hours, the system was depressurized and the LLDPE film was recovered from the cell. The non-impregnated CEO remaining on film surface was removed with a paper towel. The LLDPE films were washed by dipping in methanol at room temperature (25 °C) to ensure no residual CEO on film surface, and weighed in analytical balance (AUY 220, Shimadzu, Philippines, ± 0.0001 g accuracy) to determine the mass of CEO impregnated in LLDPE films (expressed as mg of CEO per g of film).

2.4 Kinetic study

The kinetic of conventional CEO incorporation was performed by immersing LLDPE films in CEO without pressure. For this assay, the LLDPE film was placed in a Petri dish with 5 mL of CEO at 25 °C during 4 hours. At each pre-established time, the film was removed from the dish, carefully cleaned with paper towel, washed in methanol, weighed in analytical balance (AUY 220, Shimadzu, Philippines, ± 0.0001 g accuracy) and placed back in contact with CEO until next measurements.

The kinetic assays at high-pressure conditions were performed under the conditions: constant pressure (150 bar), CEO:CO₂ mass ratios (2 and 10%) and temperatures (25, 35 and 45 °C), while varying the impregnation time in intervals up to 4 h. After kinetic assays, LLDPE films were cleaned with paper towel, washed in methanol, and weighed in analytical balance (AUY 220, Shimadzu, Philippines, ± 0.0001 g accuracy). In this case, each kinetic point represented a different assay (destructive experiments).

2.5 Stability at controlled temperature

The stability of high-pressure CEO impregnation was determined by keeping LLDPE samples, protected by aluminum foil, stored during 1 year in a domestic freezer at -18 °C. After the pre-determined period, the mass of impregnated films was measured in analytical

balance (AUY 220, Shimadzu, Philippines, ± 0.0001 g accuracy) in order to determine the mass of CEO retained in LLDPE films.

3. RESULTS

The CEO, completely soluble in CO₂ under the studied conditions of CEO:CO₂ mass ratios (2 and 10%), pressures (150 and 250 bar) and temperatures (25, 35 and 45 °C), was visualized through the sapphire windows evidencing the single-phase region of the system. The results of impregnated CEO mass, expressed as mg CEO g⁻¹ LLDPE film, as function of processing conditions are presented in Table 1.

Table 1. Impregnated mass of CEO in LLDPE films, initial and after 1 year of storage, under different operational conditions of P, T and CEO:CO₂ mass ratios.

P (bar)	T (°C)	Mass ratio (%)	Mass of CEO (mg g ⁻¹)	Mass of CEO after 1 year (mg g ⁻¹)
150	25	2	12.9	12.6
250			9.9	9.2
150	35		20.0	18.5
250			12.8	12.2
150	45		32.8	30.0
250			26.0	24.2
150	25	10	19.0	18.3
250			16.8	15.8
150	35		29.5	26.6
250			24.5	24.2
150	45		41.4	39.6
250			38.2	35.0

The mass of impregnated CEO ranged from 9.9 to 41.4 mg of CEO per g of LLDPE, with the highest amount incorporated at 150 bar, 45 °C and 10% CEO:CO₂ mass ratio and the lowest at 250 bar, 25 °C with 2% CEO:CO₂ mass ratio. Thus, the best impregnation conditions in this study were achieved with increasing temperature and CEO:CO₂ mass ratio while reducing the pressure, also reported for similar systems consisted of essential oils impregnated in polymer films [11,12].

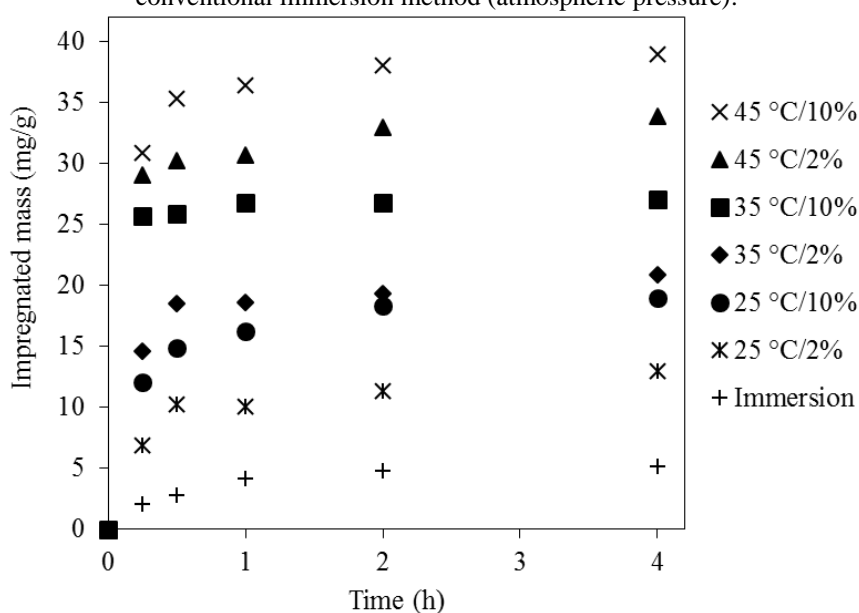
An increased active compound incorporation was observed when increasing the CEO:CO₂ mass ratio. In this case, this result can be attributed to the higher concentration gradient, the driving force for impregnation, besides an increased affinity of CEO to the polymer matrix. When increasing the temperature from 25 to 45 °C, the diffusion rates of CEO and CO₂ are increased into the polymer matrix due to a reduction in CO₂ density coupled with an increased polymer chain mobility. The pressure exerted almost no influence on CEO incorporation when it was augmented from 150 to 250 bar. This observation is related to the CEO solubility in high-pressure CO₂: with increasing pressure and CO₂ density, as consequence, the solubility of the active agent in CO₂ phase is enhanced due to the high solubilization power of CO₂ [13]. For this reason, it would be expected an enhancement of the active compound impregnation. However, the opposite effect was experimentally observed. Once the system is rapidly depressurized, the CEO is dragged out from the LLDPE phase because of its high affinity and partition to the CO₂ phase, especially at 250 bar, thus reducing the amount of CEO remained in the polymer film.

The variation between initial and final CEO mass impregnated in LLDPE films after 1 year of storage ranged from 0.3 to 3.2 mg CEO g⁻¹ LLDPE (Table 1). This result suggests that the high-pressure CO₂ impregnation is a stable process and the active agent remains

incorporated in LLDPE films. Further studies will be conducted with different temperatures and conditions of storage.

The kinetic assays, presented in Figure 2, indicate that the highest amount of CEO was incorporated in LLDPE films at high-pressure conditions under all impregnation times. The best impregnation condition was observed at 150 bar, 45 °C with 10% CEO:CO₂ mass ratio, which agrees with previous results of CEO impregnation as function of operational parameters. The kinetic experiments at 150 bar, 10% CEO:CO₂ mass ratio with two temperatures, 35 and 45 °C, were conducted up to 8 hours, but samples showed no additional impregnation. Therefore, considering all the evaluated temperatures, CEO:CO₂ mass ratios and processing time, the amount of CEO incorporated at 2 and at 4 hours was very similar, suggesting that the active compound impregnation in LLDPE films can be performed in 2 hours or less, which is advantageous for reducing operational costs and processing time.

Figure 2. Kinetic assays of CEO incorporation in LLDPE films by high-pressure impregnation (150 bar) and conventional immersion method (atmospheric pressure).



The maximum amount of CEO incorporated at high-pressure conditions (150 bar, 45 °C, 10% CEO:CO₂ mass ratio) was 8 times higher than the incorporated amount by the conventional method (immersion without pressure). This result reinforces the potential of using CO₂ as impregnation medium due to an increased CEO incorporation in polymer films. The differential of this work is related to the employment of high-pressure CO₂ to impregnate active agents in polymer films. This process is advantageous due to the solubilization power and high diffusivity of CO₂ at high-pressure conditions that solubilize and carry nonpolar active agents through the polymer network, making the process faster than that employing the pure diffusive mechanism, as the immersion method performed in absence of pressure.

4. CONCLUSIONS

The CEO incorporation in LLDPE films was successfully performed at high-pressure conditions compared with the conventional immersion method. The highest amount of CEO is impregnated at the highest temperature (45 °C), the highest CEO:CO₂ mass ratio (10%), and the lowest pressure (150 bar). The active agent remains incorporated in LLDPE film after 1 year of storage. The CEO impregnation in LLDPE films reaches its maximum at 2 h of processing, which is advantageous for reducing time and operational costs. The high-pressure CO₂ impregnation is an emerging and innovative technology to incorporate multicomponent

active agents, especially thermosensitive ones, in polymer matrices to develop active food packaging materials with potential antioxidant and antimicrobial activities.

5. ACKNOWLEDGEMENTS

The authors acknowledge Videplast (Brazil) for supplying the LLDPE pellets, and the Brazilian governmental agencies: National Council for Scientific and Technological Development (CNPq), Foundation to Support the Research and Innovation in Santa Catarina State (FAPESQ), and Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support and G. R. Medeiros scholarship.

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