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Supercritical impregnation of antioxidant mango polyphenols into a multilayer PET/PP food-grade film

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ABSTRACT

Methyl gallate (MG) was used as a model substance to determine the influence of operating conditions on the supercritical impregnation of mango polyphenols into a multilayer polyethylene terephthalate (PET)/polypropylene (PP) food-grade film. The effects of pressure (10–20 MPa), temperature (35–55 °C), impregnation time (3–22 h) and stirring were analysed. The most favourable conditions were obtained under 10 MPa at 45 °C during 22 h in stirring mode. These operating conditions were implemented in the impregnation of a mango leaf extract (MLE). The results show that the MLE-loaded PET/PP films presented a high phenolics load, predominantly in MG (~30 mg MG/100 g film), antioxidant capacity, demonstrated by DPPH assay, and potential application in the preservation of perishable foods such as fruit and vegetables.

1. Introduction

New food packaging technologies are on the market every day as a response to consumer's growing demand for easy to consume, fresh, semi-processed or minimally processed products with a longer shelf-life. Active packaging systems serve this purpose by overcoming some of the limitations of conventional packaging, such as the growth of microorganisms and the oxidation of the product. These active or smart systems present features that reach beyond the mere passive container and physical barrier between the product and the outside. They play an active role in the conservation of food, as they help to extend their shelf-life, make them safer to consume and improve their taste and smell properties while preserving the quality of the product [1].

Active packaging systems should imply positive interaction between the package, the packaged food and the atmosphere. Such interaction is achieved thanks to the inclusion of additives into the packaging material. This should be the most attractive option for consumers since there are no foreign substances in direct contact with the product. Some of the additional benefits that active packaging can provide *versus* traditional packaging, are its antioxidant and antimicrobial properties [2]. Nowadays, synthetic antioxidants are commonly used to maintain the quality of food products; however, consumer's health and safety awareness have driven food industry to seek for other more natural alternatives. Natural antioxidants, and more specifically phenolic antioxidants, are some of the biggest and the most diverse groups of secondary metabolites extracted from plants. They present numerous health benefits [3]. In the case of *Mangifera indica L.* (Mango), part of the *Anacardiaceae* family, its residues (leaves, branches, pulp seeds and bark) can be recycled by other industries as a source of bioactive compounds with a high added value, including polyphenols and carotenoids, among others [4]. Carotenoids present a high value of provitamin A and antioxidant capacity, and they are generally present in the range of 0.9 to 9.2 g/kg in mango pulp [5]. Among mango polyphenols, mangiferin (2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9-xanthone) is the main one, and is in high content in the leaves (36–67 g/Kg dry material) and bark (12–18 g/Kg dry material) [6]. In addition, mango leaves are also rich in other phenolic compounds such as gallic acid (0.4–3.8 g/Kg), methyl gallate (2–20 g/Kg), iriflophenone 3-C-β-D-glucoside (8–118 g/Kg), and quercetin pentosides (1.3–40.9 g/Kg) [6,7]. Amongst all of them, methyl gallate is a phytocompound with antimicrobial and antioxidant properties that has been identified in plants such as *Pelargonium, Cotinuscoggygria, Sapiumsebiferum* and *Mangifera indica L.* [8].

A large number of techniques are used in the industry to incorporate active substances into polymeric matrices. One of the most common techniques is extrusion [9–11]. However, extrusion presents some disadvantages such as the volatilization or degradation of the active agents due to the high temperatures used during the process. Impregnation by immersion is also used in some industrial applications but it requires the consumption and the subsequent evaporation of large amounts of water or toxic organic solvents. This makes the process costly and unhealthy. Naturally, the scientific community has made considerable

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efforts to overcome such drawbacks by developing new impregnation methods [9]. Impregnation by supercritical fluids, also known as Supercritical Solvent Impregnation (SSI) is one of such alternatives. This technique is based on the use of a non-toxic and inert media, commonly CO_2, as solvent to incorporate the active compound into the polymeric matrix. The accessible critical point of carbon dioxide (Pc = 7.38 MPa and Tc = 304.15 K) allows the development of the process at moderate temperature levels which favours the preservation of temperature-sensitive compounds. Moreover, since it is a gas at room temperature and pressure, solvent free products can be obtained. Another advantage of CO_2 is its high diffusivity, that allows CO_2 to penetrate through the amorphous region of the polymeric matrix (CO_2 sorption) causing a temporary increase of the polymer's free volume (swelling), which implies a modification of the polymer's mechanical and physical properties including a decrease in glass transition temperature (T_g) (plasticization). CO_2 acts as a sort of lubricant by augmenting the segmental chain mobility in the polymeric matrix, and thus softening the polymer. This phenomenon dramatically enhances the diffusion of the active compound into the matrix and the impregnation process becomes easier and faster [12]. Due to its multiple advantages, SSI has been applied for several purposes in the pharmaceutical field [13–17], such as producing patches for transdermal drug administration [15], as well as intraocular or contact lenses for the administration of anti-inflammatory or analgesic drugs through impregnated media [2,16,17]

However, this technology has not been so often used in the food industry. Just in the recent years, the research community has intensified the development of food-grade films with functional properties (*i.e.* antibacterial, antifungal, antioxidant, pesticide) by means of supercritical techniques. Current studies have focused on the impregnation of essential oils and some of their components such as thymol [2], cinnamaldehyde [18], 2-nonanone from ruda essential oil [19], eugenol [20] and clove essential oil [21] while more recent studies have also explored the impregnation of pesticides such as terpene ketones in food packages [22,23]. Essential oils have a broad activity spectrum against different spoilage microorganisms and are generally recognized as non-toxic and safe compounds [19]. In addition, they are highly soluble in supercritical carbon dioxide (SC-CO_2), which makes them easy to impregnate into food-grade films by means of the supercritical technique. However, these volatile molecules present some disadvantages in food applications, since they can migrate from the packaging material to the edible product and this could affect the organoleptic quality of the packaged product.

In order to avoid this inconvenience, non-volatile natural molecules with potential antimicrobial and antioxidant activity, such as polyphenols and flavonoids, among others, could be used as additives in functional packaging. However, these molecules have been less studied when used with supercritical impregnation techniques, since they present a scarce solubility in CO_2 because of their complex molecular structure and medium-to-high polarity. Despite this limitation, the solubility of polar molecules could be improved by adding polar solvents to the CO_2 phase, which would aid to their impregnation by supercritical techniques.

In this context, this work focuses on incorporating phenolic substances - methyl gallate and mango leaf extract - into a multilayer film of polyethylene terephthalate (PET) and polypropylene (PP) using supercritical CO_2 as impregnation fluid, evaluating the influence of operating conditions (pressure, temperature, impregnation time, depressurizing rate and stirring mode) through the quantification of the impregnation level, the antioxidant activity and the release kinetic. In addition, a preliminary study on the capacity of the impregnated films for the preservation of perishable food was considered.

Table 1
Polymer properties.

Property	PET	PP
Layer thickness (μm)	12	50
Molecular weight of repeat unit (g/mol)	192.2[a]	42.08[a]
Glass transition temperature (*Tg*) at 1 atm of pressure (°C)	67–80[b]	−20 to −10[c]
Melting temperature (*Tm*) (°C)	247[b]	164[c]

[a] From Ref [46].
[b] From Ref [47].
[c] From Ref [45].

2. Materials and methods

2.1. Materials

Mango leaves, provided by the Institute for Mediterranean and Subtropical Horticulture "La Mayora" (IHSM) of Superior Centre of Scientific Research (CSIC) (Malaga, Spain), were used for producing the antioxidant extract. All the leaves were dried at room temperature until constant weight and kept in the absence of light. Methyl gallate from Sigma-Aldrich Chemie (Steinheim, Germany) and mango leaves extract were used as the active substances for the SSI process. A multilayer thermosealing PET/PP film supplied by Tecnofoodpack S.P.A. (Castelnovetto, Italy) was used for the impregnation tests. The film is formed by a 12 μm polyethylene terephthalate (PET) top layer, a 2 μm adhesive layer and a 50 μm polypropylene (PP) lower layer. It is 64 μm thick and its density is 64.05 g/m^2. The film can withstand thermal treatments up to 100 °C. Some specifications about the properties of PET and PP polymers are displayed in Table 1. Other reagents and solvents used were carbon dioxide (99.995%) supplied by Abello-Linde S.A. (Barcelona, Spain), 2,2-Diphenyl-1-picrylhydrazyl, free radical (DPPH), supplied by Sigma-Aldrich (Steinheim, Germany), and the organic solvents ethanol, acetonitrile, and formic acid, all HPLC gradient grade, were supplied by Panreac (Barcelona, Spain). Ultrapure water (milliQ grade) was used in all the experiments.

2.2. Mango leaf extract production

Mango leaves were extracted by means of a high-pressure equipment supplied by Thar Technology (Pittsburgh, PA, USA, model SF500). The equipment consists of a 500 ml thermostatted stainless extraction vessel, two high-pressure pumps (for carbon dioxide and co-solvent), a back pressure regulator (BPR) and a 500 mL cyclonic separator. It was controlled by Thar Instruments Process Suite Software (Thar Tech. Pittsburgh, PA, USA). 100 g of dried roughly ground leaves (~2.0 mm particle size) were placed inside the extraction vessel. Roughly ground leaves were used because previous studies reported that small particle sizes reduced the efficiency of mango leaf extraction due to the formation of preferential pathways [24]. A mixture of CO_2 + 50% ethanol (w/w) was used as solvent system at a pressure of 20 MPa at 60 °C with a flow rate of 10 g/min for 3 h. Afterwards, the extract was collected from the cyclonic separator and it was kept at 4 °C in absence of light until it was analysed. Finally, the extract was characterized in terms of antioxidant activity and phenolic content. The operating conditions were set according to previous studies published by the authors [4]. Different studies have revealed that this technique, which employs a high concentration of CO_2 (25–50%) with liquid solvents (ethanol or water) at high pressure and temperature, improves the extraction yield of phenolic compounds from mango leaves [4].

2.3. Supercritical impregnation procedure

The impregnation of PET/PP film was performed in a high-pressure equipment supplied by Thar Technology (Pittsburgh, PA, USA, model