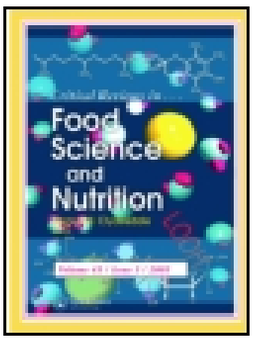

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REVIEW

Supercritical impregnation for food applications: a review of the effect of the operational variables on the active compound loading

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ABSTRACT

The $scCO_2$-assisted impregnation process has arisen as an effective method to impregnate solid materials. Its multiple advantages include high diffusion, it allows to obtain free-solvent materials and to operate under low temperatures, which permits to process thermolabile solutes. These characteristics have allowed its application at industrial scale for the impregnation of wood with fungicides and in the last years for textile dyeing. Meanwhile, other numerous applications are still being studied at laboratory scale. One potential field of application corresponds to the food-related industry, which includes the use of $scCO_2$-assisted impregnation process to develop active materials for food packaging and to generate food-grade materials loaded with nutraceuticals for functional food applications. In this framework, this article summarizes the advantages and the main drawbacks with the $scCO_2$-assisted impregnation process. The effect of the processing variables of the $scCO_2$-assisted impregnation process is discussed in terms of the incorporation of active compounds within polymer structures. Including the principles and description of the process and a review of the investigated systems for a better understanding.

KEYWORDS

$scCO_2$-assisted impregnation process; supercritical carbon dioxide; active packaging; functional foods

Introduction

The $scCO_2$-assisted impregnation process has emerged as an effective method to incorporate active compounds into polymers for different applications. In this process, a substance is dissolved in the supercritical fluid (dissolution stage) and the high diffusivity and low surface tension of the supercritical fluid allow the polymer to swell and deposit or promote absorption of a compound within a polymeric matrix (sorption stage). The deposition or absorption of the active compound could be enhanced or not during the subsequent depressurization stage (Champeau et al. 2015c). Carbon dioxide (CO_2) is the most used fluid among the different impregnation applications because it is relatively inert towards reactive substances, non-flammable, tasteless, odorless, has a low cost and has relatively low critical pressure and low critical temperature values ($P > Pc = 7.38\,MPa$ and $T > Tc = 304.15\,K$) (Clifford and Williams 2000). Therefore, organic compounds dissolved in supercritical CO_2 ($scCO_2$) are not susceptible to thermal degradation. Another advantage of $scCO_2$ is its high diffusivity with its adjustable solvent power in terms of temperature, pressure and the use of co-solvents for compounds with low solubility in $scCO_2$. Furthermore, CO_2 is a gas at ambient conditions of temperature and pressure, which allows to obtain solvent-free materials (Hrnčič et al. 2018). Due to its several advantages, the $scCO_2$-assisted impregnation process has been used extensively for pharmaceutical purposes as the production of patches for transdermal drug administration (Dias et al. 2011) as well as contact lenses for the delivery of anti-inflammatory or analgesic drugs (Costa et al. 2010). It is currently used at industrial scale for the impregnation of wood with fungicides and in the last years has found its place in textile dyeing applications (Weidner 2018), due to the several advantages that offers over the traditional water dyeing process; there is no need to evaporate water from the dyed material, no wastewater production and therefore no wastewater treatment is necessary. Furthermore, short batched cycles allow to obtain dyes uptakes above 98%, allowing to reduce energetic requirements (up to 40%) and process costs compared with the traditional dyeing process (DyeCoo 2018). Through this example, it is possible to observe the great potential that has $scCO_2$-assisted impregnation process to be used at industrial scale in other applications of great interest that currently are being studied at laboratory scale as those related to food.

In the food industry field, the process has been used for the impregnation of nutraceutical compounds into food-grade materials for the development of functional food (Pantić et al. 2016a), arising as an alternative technique to conventional methods to encapsulate active compounds into a polymer matrix in order to protect them against degradation (Smirnova et al. 2004) and for the development of active packaging using natural compounds (Belizón et al. 2018), emerging as an alternative method to overcome the

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disadvantages of the conventional processes used to incorporate active compounds into polymers as the extrusion and coating processes.

In this framework, this article summarizes the advantages and associated problems of the $scCO_2$-assisted impregnation process. The effects of the processing variables of the $scCO_2$-assisted impregnation process (pressure, temperature, depressurization rate and time) are discussed in terms of the incorporation of active compounds into polymer structures. This analysis represents a fundamental step in the understanding of this technique for the design of systems used in active food packaging as well as in functional foods. This paper also includes the principles and description of the process and a review of the investigated systems of the application of the $scCO_2$-assisted impregnation process for food-related applications.

$scCO_2$-assisted impregnation process

In $scCO_2$-assisted impregnation process the solvent power, high diffusivity and low surface tension of the supercritical fluid are the key properties that allow the incorporation of an active compound into a polymer matrix. In this process, three stages can be distinguished: (1) dissolution, (2) sorption of the mixture and (3) depressurization of the system.

The first stage of the $scCO_2$-assisted impregnation process involves the dissolution of the pure substance or mixture of compounds to be impregnated in the $scCO_2$. The following stage of the $scCO_2$-assisted impregnation process considers the sorption of the mixture (active compound and $scCO_2$) in the polymer matrix. First, in this stage the molecular diffusion of an active compound in the polymer structure is improved due to the polymer swelling and plasticization effect caused by the high-pressure CO_2, whose extent depends mainly on the chemical nature of the polymer and CO_2 conditions (temperature and pressure) (Guney and Akgerman 2002; Shen et al. 2008). The last fact makes the main difference and advantage with respect to the impregnation with liquids (soaking). Furthermore, the $scCO_2$-assisted impregnation process allows to incorporate active compounds with high or low affinity towards a polymer. In the first case, the retention of the active compound is mainly achieved in this stage by means of its chemical absorption in the polymer phase, through an incorporation mechanism known as molecular dispersion. Meanwhile in the second case, the entrapment of the compound in the polymer structure is achieved mainly during the depressurization of the system (next stage) by means of its physical entrapment in the polymer structure (see "Mechanisms of impregnation using $scCO_2$"). This mechanism of incorporation, known as deposition, is specific for supercritical fluids, which means that it depends on polymer swelling and on the solubility of the active compound in the CO_2-phase.

The final stage of the $scCO_2$-assisted impregnation process corresponds to the depressurization of the system. As mentioned above, this stage is related with an incorporation mechanism specific for supercritical fluids. For active compounds with low affinity towards a polymer, a fast depressurization rate could increase the amount of active compounds incorporated due to the rapid decrease of the solvent power of CO_2. Nevertheless, a very fast depressurization could cause unwanted structural changes inside the polymer structure as foaming, cracking or could even destroy the material due to the drastic increase in CO_2 volume (Di Maio and Kiran 2018). Thus, the depressurization rate corresponds to a parameter that must be carefully analyzed for each case.

The $scCO_2$-assisted impregnation process has been carried out in three different modes: (1) Static, (2) Dynamic and (3) Semi-dynamic. Nevertheless, the most used by far is the static mode (see Table 1). Figure 2 shows a typical outline of the experimental setup for a supercritical batch impregnation process. In this configuration, determined amounts of active compound (in excess or not) and polymer are placed in the same impregnation reactor, but physically separated by a metal or paper filter in order to avoid direct contact between them. Then, pre-heated CO_2 is introduced into the thermostated high-pressure cell and the system is pressurized up to the working pressure. In this configuration, the dissolution of the active compound and the sorption of the mixture (active compound and $scCO_2$) are carried out simultaneously (see Figure 1). Meanwhile, for a dynamic process (Comin et al. 2012; Díez-Municio et al. 2011) the dissolution of the active compound can be done either in the same impregnation reactor or in a previous dissolution reactor. In the last option, the sorption of the mixture (active compound and $scCO_2$) in the polymer matrix begins only when the saturated mixture (active compound and $scCO_2$) is allowed to enter in the impregnation cell with a constant CO_2 flow. In some studies a semi-dynamic mode has been used (Ubeyitogullari and Ciftci 2016, 2017), in this method the system works in a static or dynamic way by time intervals. In the static mode period, the compound is dissolved in the CO_2-phase. Meanwhile in the following dynamic mode period, the dissolved compound diffuses into the polymer matrix.

A recent trend corresponds to the impregnation of active compounds previously obtained by $scCO_2$-assisted extraction. For example, Belizón et al. (2018) reported the supercritical extraction of polyphenols from mango leaves and their subsequent impregnation in PET/PP films. In another work, Cejudo Bastante et al. (2017) reported the obtainment of an olive leaf extract and its further impregnation in PET/PP films. Thus, in the cases where the active compound to be impregnated is a supercritical extract, it will be highly convenient to perform the impregnation process through an operation mode that integrates the $scCO_2$-assisted extraction and impregnation processes in order to achieve considerable savings in energy, time and raw materials. Fanovich et al. (2013) have proposed this integrated operation mode for the development of scaffolds loaded with an antimicrobial compound.

After a determined impregnation time, the system must be controlled depressurized (third stage). Previous to the depressurization of the system an alternative cooling stage has been used in some cases with the objective to prevent the loss of the active compound through decreasing the

Table 1. Review of the published articles.

Compound	Method	Utilized amount of active compound	Material	Pressure (MPa)	Temperature (°C)	Depressurization rate (MPa min^{-1})	Time (h)	Highest incorporation percentage (wt/wt)	References
Cinnamaldehyde	Static	Not in excess	PLA electrospun mats	12	40	1.0	3	3.29	López de Dicastillo et al. (2018)
Methyl gallate	Static	In excess	PET/PP films	10 and 20	35, 45 and 55	0.2	3, 15 and 22	0.004	Belizón et al. (2018)
Thymol	Static	In excess and Not in excess	LDPE/Cloisite 20A nanocomposite films	9, 12 and 15	40	10	0.5 to 5	1.62	Rojas et al. (2018)
Thymoquinone and R-(+)-pulegone	Static	Not in excess	LDPE films	10 and 15	45	0.5 and 2	2 and 4	5.59	Goñi et al. (2017b) Herrera et al. (2017)
Thymoquinone and R-(+)-pulegone	Static	Not in excess	LDPE/sepiolite nanocomposite films	9 and 13	45	0.5 and 2	2 and 4	8.60	Goñi et al. (2017a)
Thymol	Static	Not in excess	LDPE/C20A	12	40	1 and 10	0.25 to 5	1.19	Rojas et al. (2017)
Cinamaldehyde	Static	In excess	PLA films	9 and 12	40	0.1 to 10	3	13	Villegas et al. (2017)
Thymol	Static	In excess and Not in excess	PLA films	9 and 12	40	0.1 to 1.0	3	20.5	Torres et al. (2017)
Thymol	Static	Not in excess	PLA/nanofibers	12	40	1	3	24	Alvarado et al. (2018)
Olive leaf extract and Caffeic acid	Static	In excess	PET/PP films	10, 20, 30 and 40	35 and 55	0.1 and 10	22	—	Cejudo Bastante et al. (2017)
Clove essential oil	Static	Not in excess	LLDPE films	12 and 20	25, 35 and 45	—	0 to 4	4.02	Medeiros et al. (2017)
Phytosterols	Semi-dynamic	In excess	Nanoporous starch aerogels	45	70, 90 and 120	1 L min^{-1} (atmosferic conditions)	3	9.9	Ubeyitogullari and Ciftci (2017)
Eugenol	Static	Not in excess	LLDPE films	10, 12 and 15	45	0.5, 1 and 1.5	4	6	Goñi et al. (2016)
Phytol	Static	In excess	Silica and alginate aerogels	200	40	0.2	24	30.1	Mustapa et al. (2016)
Thymol	Static	In excess	Cellulose acetate films	10	35	—	2, 5, 28 and 32	63.84	Milovanovic et al. (2016)
Phytosterols	Semi-static	In excess	Nanoporous starch aerogels	45	70	1 L min^{-1} (atmosferic conditions)	3	5.5	Ubeyitogullari and Ciftci (2016)
Vitamins K3 and D3	Static	Not in excess	Alginate aerogel spheres	15 and 20	40	0.3	1 to 24	~12	Pantić et al. (2016a)
Vitamin D3	Static	Not in excess	Alginate aerogel spheres	8	5, 15, 25 and 35	—	1 to 24	~12	Pantić et al. (2016b)
Thymol	Static	In excess	Starch gels	15.5	35	0.3	24	4.02	Milovanovic et al. (2015a)
Thymol	Static	In excess	Cellulose acetate films	10,15 and 20	35 and 50	0.3	2 to 32	72.26	Milovanovic et al. (2015b)
2-nonanone	Static	Not in excess	LLDPE films	12, 17 and 22	40	1 and 10	3	0.34	Rojas et al. (2015)
Thymol	Static	In excess	LLDPE films	7, 9 and 12	40	—	4	1.30	Torres et al. (2014)
Cinamaldehyde	Static	In excess	Cassava starch films	15 and 25	35	1 and 10	3 and 15	0.25	de Souza et al. (2014)
Oregano Essential Oil	Static	In excess and Not in excesss	Starch microspheres	8–15	40–50	0.083 to 0.11	3, 6 and 24	—	Almeida et al. (2013)
Natamycin	Static	Not in excess	Alginate films	20	40	—	2.5, 4 and 14	16.29	Bierhalz et al. (2013)
Flax Oil	Static and dynamic	In excess	b-glucan aerogels	15 and 30	40 and 60	—	2,6 and 8	65.39	Comin et al. (2012)
Lavandin oil	Static	Not in excess	Modified starch powder	10, 11 and 12	40–50	0.07–0.15	2	15	Varona et al. (2011)
Lactulose	Static and dynamic	In excess	Chitosan scaffolds and microspheres	10	100	0.6, 1 and 3.3	1 and 3	8.6	Díez-Municio et al. (2011)
Lecithin and erythorbate	Static	—	HDPE, high-density polyethylene films	20.7	65	—	0.75	—	Cardona et al. (2012)

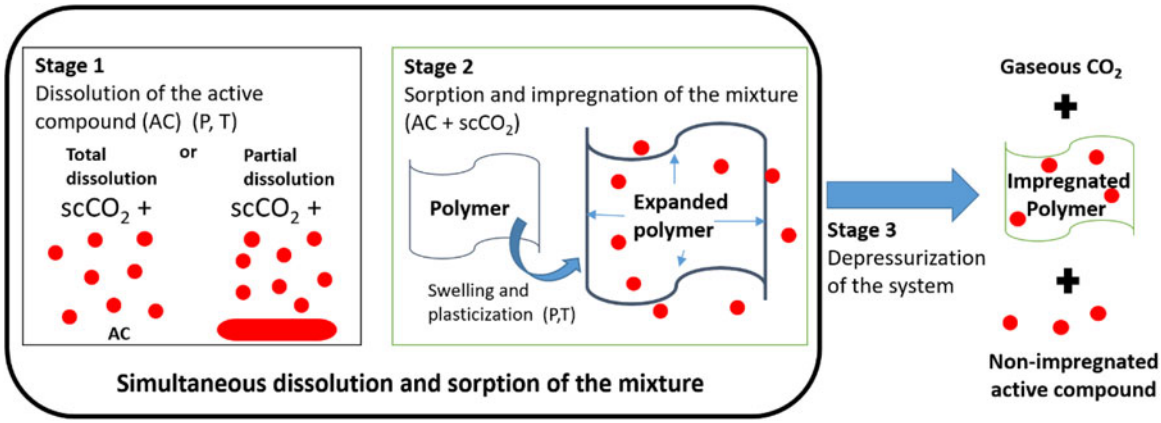

Figure 1. Schematic representation of the three steps of the impregnation process.

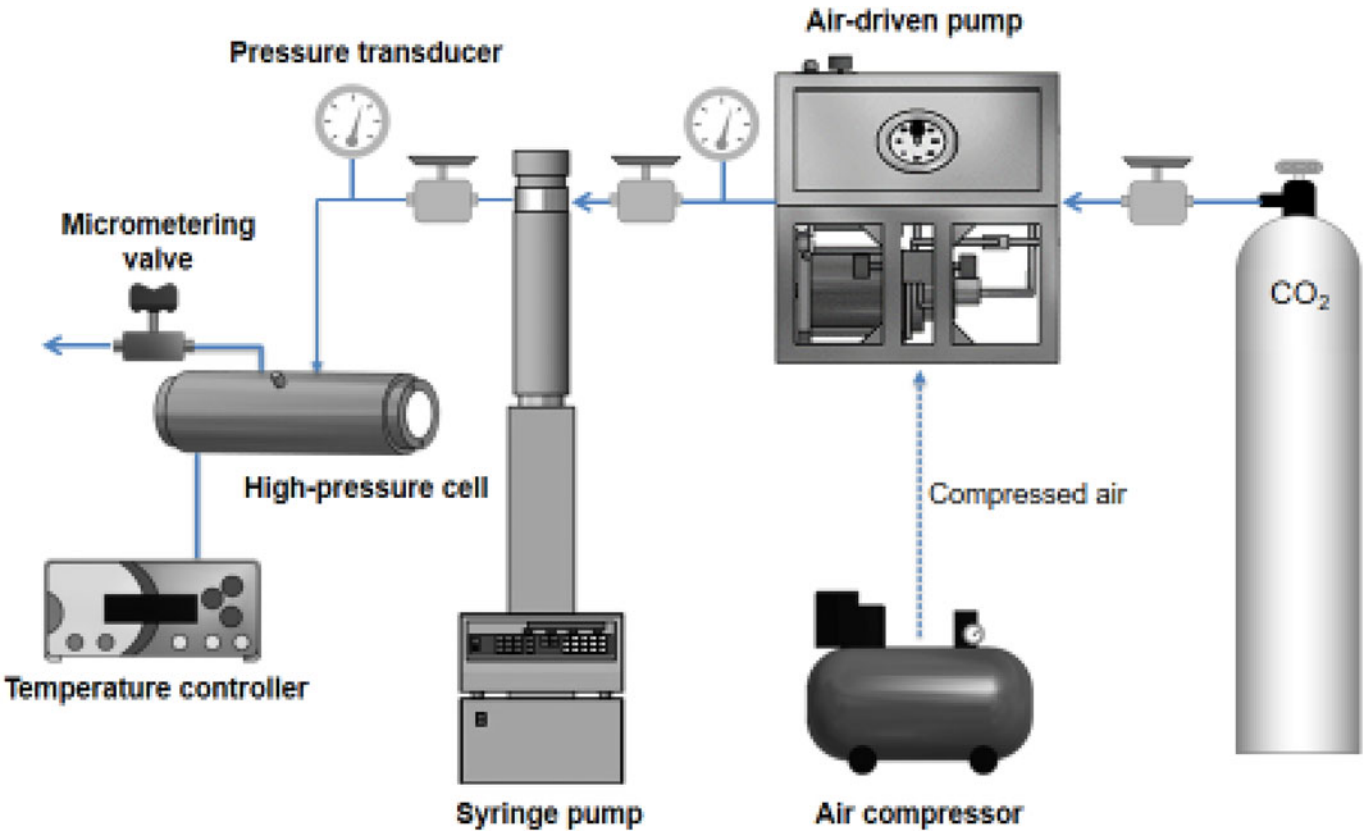

Figure 2. Common outline of the experimental setup used for a supercritical batch impregnation process.

active compound solubility in the CO_2-phase (Almeida et al. 2013; Ubeyitogullari and Ciftci 2016, 2017).

$scCO_2$-assisted impregnation process for food related applications

For food related applications, the $scCO_2$-assisted impregnation process has been used mainly with two purposes, both linked to the development of sustained release materials: (1) to develop active food packaging materials and (2) to impregnate food-grade materials with nutraceuticals for functional food applications. Table 1 shows the review of the published articles that have investigated the use of the $scCO_2$-assisted impregnation process for applications in the food industry. Among the studies that investigate the process, most of them analyze the effect of the operational variables on the active compound incorporation, evaluate if the impregnated materials can exert the required activity (i.e. antimicrobial, antioxidant or other) and if this activity can be maintained during long periods of time through release or dissolution test analyses, which are complemented by a physical-chemical characterization in order to explain the transport properties of the impregnated materials.

Development of active food packaging

In this area, special emphasis has been given to the development of antimicrobial and antioxidant release systems

(emitters) (Alvarado et al. 2018; Bierhalz et al. 2013; Cejudo Bastante et al. 2017; de Souza et al. 2014; Goñi et al. 2016; Medeiros et al. 2017; Milovanovic et al. 2016; Milovanovic et al. 2015b; Rojas et al. 2015; Torres et al. 2017; Torres et al. 2014; Villegas et al. 2017). The antimicrobial materials have potential application in the packaging of fresh and processed meat, fresh and smoked fish, fresh and processed fruits and vegetables, bakery products and ready-to-eat meals. Meanwhile, antioxidant materials have potential application in the packaging of fresh fatty fish and meat, fat-containing instant powders, seeds, nuts, oils and fried products (Yildirim et al. 2018). Although it is also possible to find some researches focused on the development of insecticidal (Goñi et al. 2017a, 2017b; Herrera et al. 2017) or oxygen scavenger (Cardona et al. 2012) food packaging materials (see Table 1). The development of these materials has been carried out considering the use of natural compounds, most of them GRAS (Generally Recognized as Safe), being one of the current trends on the $scCO_2$-assisted impregnation field the use of natural active compounds obtained by $scCO_2$-assisted extraction process (Belizón et al. 2018; Cejudo Bastante et al. 2017). Another current tendency for the development of active food packaging corresponds to the preferential use of biopolymers rather than commonly used polyolefins (see Table 1).

The effectiveness of an active material will depend of: (1) obtaining an impregnated material with an active compound concentration that allows to exert the required activity and (2) of its ability to provide a sustained supply of active compounds at suitable rates for food protection (Yam and Zhu 2012). The last characteristic can be evaluated through laboratory-level release tests using liquid solutions known as food simulants. Belizón et al. (2018) reported that, when employing this type of release test and using ethanol 100% as food simulant at 40 °C, impregnated Polyethylene terephthalate/polypropylene (PET/PP) films with methyl gallate or with a mixture of mango polyphenols were capable of delivering these compounds in a sustained manner, reaching the total release of these compounds at 13 days and 26 days, respectively. In another work, and using water as food simulant at 35 °C, Milovanovic et al. (2016) reported that cellulose acetate films impregnated with thymol were capable of delivering this compound during long periods of time, reporting equilibrium times between 2 and 22 days. Moreover, the high thymol concentration in the cellulose acetate films allowed the samples to show a strong antibacterial activity against several microorganisms as *E. coli* and *S. aureus*. The periods of time reported by Milovanovic et al. (2015b) were longer than those reported by Torres et al. (2017) (10 to 20 hours) for thymol released from PLA films to an ethanol 10% vol/vol solution at 40 °C. Nevertheless, this result is not totally comparable with those reported by Milovanovic because of the different simulants and temperatures employed for the release assays, but it is comparable with the results reported by Torres et al. (2014) for the release of thymol from linear low-density polyethylene (LLDPE) and with the results reported by Rojas et al. (2017) for the release of thymol from low-density polyethylene (LDPE) films. From these results, it can be seen that the thymol equilibrium concentration in an ethanol 10% (vol/vol) solution at 40 °C was established at times near to 5 hours and 3 hours for thymol released from LLDPE and LDPE films, respectively. These studies also report the diffusion coefficients of thymol in the materials, estimated between 7.5×10^{-13} and 1.8×10^{-12} m^2/s, and between 1.47 and 1.50×10^{-12} m^2/s, respectively. These values are higher than those reported by Torres et al. (2017) for thymol in PLA films ($0.8–2.3 \times 10^{-13}$ m^2/s), which can be explained by the lower affinity of thymol towards polyethylene materials, which involves a faster release of thymol from the polymer structure. In another work the fast release of 2-nonanone has been reported from LLDPE films (Rojas et al. 2015). From the results showed in this study, it can be seen that the 2-nonanone concentration equilibrium in an ethanol 10% (vol/vol) solution was reached between 2 and 4 hours and the diffusion coefficient took values between 3.0 and 6.8×10^{-12} m^2/s.

The sustained-release characteristic of a material can be improved through the modification of its transport properties using nanofillers, being one of the last tendencies for the development of active food packaging. In this framework, Alvarado et al. (2018) reported that PLA films containing 4.7% (wt/wt) of Poly (vinyl alcohol) (PVA) nanofibers containing cellulose nanocrystals (CNC) showed a thymol release rate significantly lower than the rate established in nanofibers-free PLA. They reported in PLA based nanocomposites diffusion coefficient values (5.5×10^{-14} m^2/s) four times lower than those reported in the same study for thymol in nanofibers-free PLA (2.5×10^{-13} m^2/s). In another work, Rojas showed that the intercalated structure of a nanocomposite based on LDPE with 2.5% (wt/wt) of organo-modified montmorillonite (OMMT) nanoclays decreased the diffusion coefficient of thymol ($7.0–9.0 \times 10^{-14}$) thirteen times compared with its diffusion coefficient in LDPE ($1.2–1.3 \times 10^{-12}$) without nanoclays (Rojas et al. 2018).

Development of carrier materials loaded with nutraceutical compounds

In the last years, $scCO_2$-assisted impregnation process is receiving increasing attention as a green technique for the development of carrier materials loaded with nutraceutical compounds for its application in food systems (functional food). It emerges as an alternative technique to the conventional methods to encapsulate active compounds in a polymer matrix in order to protect them against degradation, maintaining its bioactivity, improving its bioaccessibility and consequently its bioavailability (Smirnova et al. 2004). In this process the carrier matrix must be generated before the impregnation process and must fulfill the pharmaceutical demands for nutraceuticals carriers; it needs to be biocompatible (soluble in body) and biodegradable (suitable for enzymatic decomposition in the body) (García-González et al. 2011). Moreover, the material employed must have a matrix structure that allows suitable release kinetics characteristics. Under these requirements, the search for carrier

materials has turned its gaze mainly towards polysaccharides as starch and alginates and especially towards aerogels based on these polysaccharides obtained through $scCO_2$ drying (Lovskaya et al. 2015). Due to its outstanding properties as low density, large open pores and high inner surface areas, aerogels have been proposed as matrices and carriers for active substances (Bierhalz et al. 2013; Comin et al. 2012; Díez-Municio et al. 2011; Mustapa et al. 2016; Pantić et al. 2016a, 2016b; Ubeyitogullari and Ciftci 2016, 2017).

Starch is a promising low-cost, renewable, abundant, and food grade polysaccharide used as solid matrix for encapsulation of nutraceutical compounds through different methods, including the $scCO_2$-assisted impregnation process. Almeida et al. used microspheres of sorghum starch to microencapsulate oregano essential oil by means of $scCO_2$-assisted impregnation process. They obtained formulations, with antioxidant activity between 319 and 946 as Trolox equivalent antioxidant capacity per g of particles (μmol TEAC/g), that ensured a better stability of the essential oil (Almeida et al. 2013). Meanwhile, Varona et al. (2011) used modified starch to microencapsulate lavandin oil, obtaining impregnation loads (2–14% (wt/wt)) in the range of essential oil loads obtained by other processes like Particles from Gas Saturated Solutions (PGSS) or PGSS-drying. Starch has also been used for aerogels formation through $scCO_2$ drying and further impregnation through the $scCO_2$-assisted impregnation process. Milovanovic reported the incorporation of thymol up to 4.02% (wt/wt) into the porous structure of corn and tapioca starch aerogels (Milovanovic et al. 2015a). In another work, the nanoporous structure of wheat aerogel was exploited by Ubeyitogullari et al. for the formation of phytosterols nanoparticles using $scCO_2$-assisted impregnation process aiming to decrease the size and crystallinity of the phytosterols in order to enhance their solubility in water. This last characteristic is one of the most important parameters limiting the enrichment of food with bioactive lipid compounds. The proposed impregnation method allowed to prepare starch aerogels containing 9.9% (wt/wt) of phytosterols. The impregnated phytosterols had an average size between 59 and 87 nm, with a crystallinity degree reduced due to their supercritical processing, which improved their water solubility (Ubeyitogullari and Ciftci 2016, 2017).

Alginate is another natural food-grade polysaccharide widely used as matrix for encapsulation of nutraceuticals. This material was used by Pantić et al. (2016a, 2016b) for the generation of an aerogel loaded with vitamin D3, following as main objectives to change its aggregation state to an amorphous state during the impregnation in order to increase the solubility of vitamin D3 in water, obtaining as main results the improvement of dissolution of vitamin D3 by twenty times compared with its crystalline form and an impregnation concentration of vitamin D3 of up to 12% (wt/wt).

Mechanisms of impregnation using $scCO_2$

The affinity between the polymeric material and the active compound is the main criterion that will define the mechanism of incorporation of an active compound and its molecular state in the material. For active compounds that present good affinity to the polymer, important chemical interactions are the driving force of the process that leads to high active compounds incorporation and its better molecular dispersion in the polymer. This is the case of cinnamaldehyde with polylactic acid (PLA) films where the formation of hydrogen bonds between the hydrogen of the hydroxyl group of PLA and the oxygen of the aldehyde belonging to the cinnamaldehyde allows cinnamaldehyde incorporations of up to 13% (wt/wt) (Villegas et al. 2017), is also the case of lactulose in chitosan scaffolds where the possible interaction established between the carbonyl group of lactulose and the amine groups of chitosan allows a lactulose incorporation of up to 8.6% (wt/wt) (Díez-Municio et al. 2011) and of thymol with cellulose acetate and PLA films. In the first case, the strong chemical affinity between the hydroxyl group of thymol and the hydrogen bonds of cellulose acetate chains leads to high thymol incorporation of up to 71.21% (wt/wt) (Milovanovic et al. 2016). Meanwhile, a secondary interaction between the phenolic group of thymol and the free carboxylic groups of PLA has been reported as the responsible for the high thymol incorporation (up to 24% (wt/wt)) in the polymeric matrix (Alvarado et al. 2018; Torres et al. 2017). In fact, a possible partial occupation of carboxylic groups available in PLA films due to their interaction with vinyl acetate groups of nanofibers of cellulose could explain the lower amount of thymol incorporated into PLA-based nanocomposites, which shows the importance of the presence of free carboxylic groups on the greater affinity between thymol and PLA (Alvarado et al. 2018). In all of these cases the incorporation has probably occurred following the second mechanism of impregnation using supercritical fluids reported by Kazarian which is not specific for supercritical fluids (Kazarian 2000), meaning that it does not depend on polymer swelling, involving chemical interactions between active compound and polymer, favoring the preferential partitioning of the solute within the polymer phase.

FTIR-ATR spectroscopy was used by Ubeyitogullari and Ciftci (2016, 2017) to investigate the intermolecular interactions between phytosterols and nanoporous starch aerogels, showing that there was no chemical interaction between the nanoporous starch aerogel matrix and the impregnated phytosterols. There was also no chemical interaction reported between alginate and silica aerogels impregnated with phytol (Mustapa et al. 2016). In both cases, deposition is the main mechanism of incorporation of the active compounds in the aerogels matrix. Rojas et al. (2017) investigated the intermolecular interactions between thymol and LDPE based nanocomposites, showing that the band associated to the vibration of the aromatic ring of thymol appears as a single band in the impregnated nanocomposite, exposing the great chemical affinity between thymol and organo-modified nanoclays. Meanwhile, in the LDPE film this band consists of a gathering of smaller bands, which would indicate that thymol could be interacting with LDPE through weak interactions. In general, thymol and other natural active compounds have low affinity towards polyolefins. For example,

in LLDPE the reported maximum amount of thymol (Torres et al. 2014), 2-nonanone (Rojas et al. 2015), eugenol (Goñi et al. 2016) and clove essential oil (Medeiros et al. 2017) impregnated in this material using $scCO_2$ resulted equal to 1.30, 0.34, 6 and 4.02% (wt/wt), respectively. In all of these cases, the incorporation probably has occurred by means of physical entrapment of the active compound in the polymer matrix during the CO_2 depressurization step, an impregnation mechanism known as deposition.

Effect of the experimental conditions on the active compound loading

Effect of pressure

From the analysis of the data available from published articles that have investigated the use of the $scCO_2$-assisted impregnation process for applications in the food industry, it can be established that the effect that could result from increasing CO_2 pressure under isothermal conditions on the incorporation of an active compound inside a material depends mainly on the concentration gradient of the active compound established between the CO_2-phase and the polymeric matrix. The concentration gradient of active compound established between the CO_2-phase and the polymeric matrix depends on the saturation of the CO_2-phase with the active compound and on the maintenance of this saturation condition during the impregnation assay. Thus, if the CO_2-phase is maintained saturated with the active compound (due to an excessive mass of compound placed in the impregnation cell), the increase in CO_2 pressure (or density) will lead to an increase of the amount of active compound dissolved in the supercritical CO_2-phase. Therefore, the active compound will be more concentrated in the CO_2-phase while pressure increases as a result of the well-known increment of the solubility of an active compound as CO_2 pressure increases. Generating a greater concentration gradient of the active compound between the CO_2-phase and the polymeric matrix as pressure increases will promote the active compound impregnation in the material. Moreover, the swelling phenomenon of the polymer increases with CO_2 pressure, which could be positive for the active compound incorporation since its diffusion inside the polymer is facilitated. This positive effect of pressure on the active compound loading has been reported in several publications. The increase in the incorporation of flax oil in β-glucan aerogels when pressure increased from 8 to 15 MPa under isothermal conditions was reported by Comin et al. (2012). These authors explained this effect mainly due to the great increment of flax oil solubility, from 1.50 to 7.21 g L^{-1} when temperature is held at 40 °C. The great increase in CO_2 solvent power as density increases was also part of the explanation given by de Souza et al. (2014) for the increment in cynamaldyde incorporation in cassava starch films when CO_2 pressure was increased from 15 to 25 MPa at 35 °C and by Cejudo Bastante et al. (2017) to explain the increase in the incorporation of caffeic acid in PET/PP films as pressure increased from 10 to 40 MPa. The same effect of increased CO_2 pressure has been reported for the incorporation of thymol into different materials, independent of the affinity between them and thymol. Torres et al. (2014) reported the increase in thymol incorporation into LLDPE films (low affinity towards thymol) as pressure increased from 7 to 12 MPa at 40 °C. Meanwhile, Milovanovic reported the increase in thymol incorporation in cellulose acetate films (high affinity towards thymol) as pressure increased from 10 to 15 MPa, while a further increase of pressure to 20 MPa did not significantly influence the impregnation yield. This is due to the fact that the maximal loading capacity of the cellulose acetate was almost reached after 24 h at 15 MPa (Milovanovic et al. 2013). Thus, the possitive effect of CO_2 pressure is limited by the saturation of the material with the active compound; if the material has been saturated with an active compound, an increase in pressure and the concomitant increase in the amount of active compound dissolved in the CO_2 phase does not involve an increase in the active compound loaded in the polymer. This effect can also be seen in a research published by Torres et al. (2017) for thymol incorporation into PLA films and in the research developed by Villegas et al. (2017) for cinnamaldehyde incorporation into PLA films. Both studies reported that increasing CO_2 pressure from 9 to 12 MPa at 40 °C does not produce an increment in the active compound incorporation degree. These results could be explained by the saturation of PLA films in both cases, due to the high affinity between PLA and these type of compounds.

On another hand, an opposite effect to the one explained in the previous paragraph could be derived from the increasing of the CO_2 pressure under isothermal conditions using a non-saturated CO_2-phase with the active compound (the mass of the active compound is not in excess, being completely dissolved in the CO_2-phase). An increase in pressure from an initial CO_2-phase not saturated with the active compound will result in a dilution of the active compound due to the increment of the CO_2 mass fraction as pressure increases because of the increment of CO_2 density meanwhile the mass of active compound dissolved in the CO_2-phase remains constant. Thus, a lower concentration gradient of the active compound established between the CO_2-phase and the polymer matrix is generated as pressure increases, which results negative for the incorporation of the active compound in the polymer structure. Almeida et al. (2013) reported the decrease of the oregano essential oil incorporation into a starch-based material when pressure increased above 10 MPa because, for all the evaluated CO_2 pressures, the amount of dissolved oregano essential oil was the same. The same effect of increasing pressure on the active compound incorporation was observed by Rojas et al. (2015) for 2-nonanone incorporation into LLDPE films when presure increased from 12 to 22 MPa at 40 °C and for thymol incorporation in LDPE-based nanocomposites when pressure ranged between 12 and 15 MPa at 40 °C, by Milovanovic for thymol incorporation into cellulose acetate films when a non-saturated with thymol CO_2-phase was used and pressure took values between 10 and 30 MPa (Milovanovic et al. 2013), by Medeiros et al. (2017) for clove

essential oil in LLDPE films when pressure took values between 15 and 25 MPa, and for the incorporation of lavandin into a starch-based material when CO_2 pressure increased from 10 to 12 MPa (Varona et al. 2011). Varona et al. related this effect mainly to two factors. First, to the higher dilution of the lavandin in the CO_2-phase as pressure increased and second, to the weakening of the chemical interactions between lavandin and the polymer given by the intensification of interactions between CO_2 and the polymer that involves the increase in polymer swelling as pressure increases.

The relevance of the concentration gradient of the active compound established between the CO_2-phase and the polymeric matrix over the effect that pressure could have on the active compound incorporation in the polymer structure is even clearer with the results reported by Goñi et al. (2016), who assessed the effect of pressure (10, 12 and 15 MPa) at 40 °C on eugenol incorporation into LLDPE films as well as the effect of pressure (10 and 15 MPa) at 45 °C on the incorporation of a ketones mixture (thymoquinone and R-(+)-pulegone) in LDPE films (Goñi et al. 2017b). In both studies, the assessment of the CO_2 pressure was carried out using a constant active compound concentration in the CO_2-phase, that involved the same initial active compound gradient concentration between the CO_2-phase and the polymeric matrix for all tested pressures. From the study on the impregnation of eugenol, it can be seen that the eugenol incorporation degree was very similar for 10 and 12 MPa and weakly dependent on the depressurization rate used. The same result was obtained for the ketone mixture incorporation when pressure increased from 10 to 15 MPa (Goñi et al. 2017b) (Goñi et al. 2016).

Effect of temperature

Temperature is another important parameter that could affect the incorporation of active compounds in a polymer structure in the $scCO_2$-assisted impregnation process and its possible effect depends mainly on the process conditions, (1) if operating above the critical mixture point is selected or not and (2) if the range of pressure used is under or above the crossover pressure. The crossover pressure can be defined as the point over which the solubility of one solute increases, while below this point solubility decrease with temperature at constant pressure (Chimowitz et al. 1988).

If the process conditions are selected in order to operate under complete miscibility (the mass of the active compound is not in excess, being completely dissolved in the CO_2-phase) between the active compound and CO_2-phase and the range of pressure used is below the crossover pressure, the solubility of the active compound in the CO_2-phase will decrease as temperature increases under isobaric conditions. Thus, the affinity between the active compound and CO_2-phase will decrease, which is positive for the incorporation of the active compound in the polymer structure (Duarte et al. 2009). Furthermore, the CO_2 mass fraction will decrease as temperature increases due to the decrease in density and therefore the active compound will be more concentrated in the CO_2-phase. Finally, the increase in temperature could change the structural properties of the polymer and therefore its transport properties. Above the glass transition temperature, the chain mobility of the polymer could allow a better diffusion of the supercritical solution ($scCO_2$ + active compound) and more CO_2 can be sorbed in the polymer, while the swelling of the polymer could be enhanced. These phenomena have been mentioned to explain the positive effect of increasing temperature from 40 to 50 °C on the incorporation of lavandin essential oil in modified starch powder using pressure values between 10 and 12 MPa (Varona et al. 2011), of clove essential oil in LLDPE films when temperature increased from 25 to 45 °C using 15 and 25 MPa (Medeiros et al. 2017) and of oregano essential oil in starch microparticles when temperature increased from 40 to 50 °C at 10 MPa (Almeida et al. 2013).

On another hand, if the process conditions are selected with the aim of not operating under complete miscibility (due to an excessive mass of compound placed in the high-pressure cell) between the active compound and the CO_2-phase, the effect of temperature will depend mainly on the solubility of the active compound in the CO_2-phase. Thus, for impregnation assays that use an active compound in excess and values of pressure below the crossover pressure, the increase in temperature under isobaric conditions could have a negative effect on the incorporation of the active compound in the polymer matrix due to the decrease in the solvent power of CO_2 and therefore in the amount of dissolved active compound. This phenomenon has been mentioned to explain the negative effect of increasing temperature on the incorporation of caffeic acid in PET/PP films when pressure increased from 35 to 55 °C using pressure values between 10 and 40 MPa and of flax oil in β-glucan aerogels when temperature increased from 40 to 60 °C at 15 MPa (Comin et al. 2012). Meanwhile, if pressures above the crossover pressure are used, generally the increase in temperature under isobaric conditions could have a positive effect due to the increase in the amount of active compound dissolved in the CO_2 phase, because of the increment of its dissolvent power as temperature increases, and therefore its availability to be impregnated in a material. These arguments were exposed by Belizón et al. (2018) to explain the increase in the amount of methyl gallate impregnated in PET/PP films when temperature increased from 35 to 55 °C using a pressure value equal to 20 MPa. In a special case, the incorporation of thymol in cellulose acetate films (Milovanovic et al. 2015b) was negatively affected when temperature increased from 35 to 50 °C using pressure values (from 10 to 20 MPa) above crossover pressure (6.5 MPa). This can be explained because thymol shows a reverse trend; its solubility decreases with temperature for pressures beyond the crossover pressure (Kapadia 2001).

However, the positive effect of pressure is limited by the saturation of the material with the active compound. This fact could be one of the main factors that could explain why the amount of phytosterols incorporated in nanoporous starch aerogels did not increase significantly when temperature increased from 70 to 120 °C (Ubeyitogullari and Ciftci

2017). The authors of this work mentioned that another limiting factor could be the diffusion into the nanoporous starch aerogels, nevertheless the diffusion coefficient of CO_2 in a polymer tends to be higher as temperature increases, therefore over a CO_2 diffusion capacity point the increase in temperature should be always positive to the active compound incorporation.

Effect of the depressurization rate

The final step of the $scCO_2$-assisted impregnation process involves the depressurization of the system, which is crucial for the final active compound loading concentration because during this stage the depressurization rate employed, generally taking values between 0.05 and 10 MPa min^{-1}, could improve the strength of physical-chemical interactions between polymer and active compound. The establishment of a slow depressurization rate could facilitate the absorption or deposition of an active compound into a polymer only if the active compound remains dissolved in the supercritical phase (the active compound is far away from its solubility limit) during most of the depressurization stage, and the affinity between the active compound and polymer is greater than the established between the active compound and CO_2. Under these conditions, the positive effect of using a slow depressurization rate has been reported for the incorporation of 2-nonanone in LLDPE films (Rojas et al. 2015), thymol in LDPE-based nanocomposites (Rojas et al. 2017) and in PLA films (Torres et al. 2017), eugenol in LLDPE films (Goñi et al. 2016), Oregano Essential Oil in starch microspheres (Almeida et al. 2013) and for lactulose in chitosan scaffolds and microspheres (Díez-Municio et al. 2011).

If the active compound is close to its solubility limit in the CO_2-phase, the active compound incorporation will probably be weakly dependent on the used depressurization rate. In this regard, Goñi et al. reported that the incorporation of eugenol in LLDPE films at pressures between 10 and 12 MPa seems to depend weakly on the depressurization rate. This is because in this range of pressures eugenol is close to its solubility limit and therefore a minor pressure decrease may be enough to produce its partial condensation, reducing its concentration in the fluid phase to the saturation value during most of the depressurization step. Other studies reported that the active compound incorporation was independent of the depressurization rate employed, as for cinamaldehyde in PLA films and for terpene ketones (thymoquinone and R-(+)-pulegone) in LDPE/sepiolite nanocomposites (Goñi et al. 2017a) and LDPE films (Goñi et al. 2017b).

On another hand, for active compounds with a higher affinity to the CO_2-phase than to the polymer, the establishment of a slow depressurization rate has generally a negative impact on the active compound loading because during the depressurization stage the active compound prefers to stay dissolved in the supercritical phase instead of in the polymer and even part of the active compound could be re-solubilized in the CO_2-phase and escape with it decreasing the loading degree. This fact has been mentioned to explain the decrease of the caffeic acid incorporated in PP/PET films (Cejudo Bastante et al. 2017) and the decrease in the amount of cinamaldehyde incorporated in starch films (de Souza et al. 2014), when a slow depressurization rate was used. In both cases the active compound incorporation was increased when a fast depressurization rate was used. Thus, for compounds with higher affinity to the CO_2-phase than to the polymer, a high depressurization rate could favor the entrapment of the active compound within the polymer.

Effect of the impregnation time and diffusive process

The $scCO_2$-assisted impregnation process of active compounds in polymers corresponds to a process governed both by thermodynamics and by the kinetic process. The equilibrium conditions depend on thermodynamics; this means on the active compound concentration gradient and on the physical-chemical properties of both polymer and fluid phase (chemical affinity). Meanwhile, the time required to reach the equilibrium conditions depends on the impregnation kinetics process. The study of the kinetics of the $scCO_2$-assisted impregnation process of an active compound into a polymer can be approached in two ways: (1) in situ, i.e. through measurements made during the $scCO_2$-assisted impregnation process and (2) ex situ, i.e. through measurements made after the $scCO_2$-assisted impregnation process. The in situ study can be done by measuring changes occurring in the polymer directly using, for example, a quartz crystal microbalance (QCM) (Hussain and Grant 2012) or ATR-IR spectroscopy (Champeau et al. 2015b; Kazarian et al. 1997; Kazarian and Martirosyan 2002), or indirectly through measurements done in the CO_2-phase using, for example, UV/Vis spectroscopy (Ngo et al. 2003) or quantitative gas chromatography (GC-FID) (Díez-Municio et al. 2011). Through in situ methods, a low amount of impregnation assays is needed to determine the incorporation kinetics at determined pressure and temperature values. Furthermore, they avoid any error introduced by releasing the CO_2 in ex situ studies. These correspond to the main advantages of in situ over ex situ methods. Nevertheless, the ex situ methods need less sophisticated analytical methodologies than the in situ methods. Thus, generally the study of the effect of time on the active compound incorporation has been carried out using ex situ methods like gravimetric methods (weighing the sample after and before the $scCO_2$-assisted impregnation process) or quantifying the amount of active compound incorporated into the polymer structure by means of an analytic method (Almeida et al. 2013; Bierhalz et al. 2013; Cejudo Bastante et al. 2017; Comin et al. 2012; de Souza et al. 2014; Goñi et al. 2017a; Medeiros et al. 2017; Milovanovic et al. 2015a; Pantić et al. 2016a).

The kinetics of the active compound transfer depends mainly on the diffusion phenomenon in the amorphous zones of a polymer structure, polymer zones where the active compound could be absorbed or deposited depending on the affinity between the active compound and the polymer, phenomenon characterized by the diffusion coefficient of the mixture (active compound and $scCO_2$) (Champeau et al. 2015c; Guney and Akgerman 2002; Shen et al. 2008).

The diffusion coefficient of the mixture in the polymer will be greater compared to the diffusion coefficient of the pure compound in the polymer which allows to reach the equilibrium condition at a shorter time compared to the required using conventional impregnation with liquids (soaking). Comin et al. (2012) reported that the amount of flax oil incorporated into b-glucan aerogels increased over time under static conditions using a CO_2-phase saturated with flax oil at 15 MPa and 313 K, reaching the equilibrium condition at residence times under 4 hours. In another work, Almeida et al. reported that the antioxidant activity of starch spherules impregnated with oregano essential oil at 10 MPa and 313 K, which is related to the antioxidant compound incorporation degree, increased over time reaching the maximum impregnation yield at 6 hours. This work reported that the antioxidant activity of the materials decreased when a longer impregnation time was applied (24 hours) due to the thermal degradation of the compound (Almeida et al. 2013). The thermal degradation phenomenon during the supercritical impregnation process has been reported in other studies. Pantić et al. (2016a) reported that the concentration of vitamins D3 and K3 in alginate aerogel spheres decreased between the 1st and the 24th hour due to exposure to CO_2 at 313 K and 15 MPa.

This improvement of the solute mass transfer can be explained due to physical changes in the amorphous and crystalline structures of the polymer matrix caused by the swelling and plasticization effect, which depends mainly on the temperature and pressure of $scCO_2$ (Medeiros et al. 2017; Rojas et al. 2017). If temperature is above the glass transition temperature, a better diffusion of the supercritical mixture (active compound and $scCO_2$) could be established due to the greater chain mobility of the polymer (Champeau et al. 2015a). Furthermore, the swelling and plasticization of a polymer tends to increase with pressure, which is positive for the diffusion of the supercritical mixture into the polymer. Rojas et al. observed the positive effect of increasing pressure on the diffusion coefficient of thymol in LDPE nanocomposites. In this work, the increase in the diffusion coefficient of thymol in LDPE nanocomposites from 2.39×10^{-12} m^2 s^{-1} to 2.44×10^{-11} m^2 s^{-1} when pressure was increased from 12 to 15 MPa allowed to reduce the time necessary to reach the equilibrium concentration in the polymer from 180 minutes to 60 minutes (Rojas et al. 2018). Milovanovic et al. observed the same effect of pressure on the time necessary to reach the equilibrium concentration of thymol in cellulose acetate films. In this work, the impregnation time necessary to saturate cellulose acetate films with thymol decreased by half when pressure increased from 15 to 20 MPa (Milovanovic et al. 2015b).

Conclusions

The CO_2-assisted impregnation process has proven to be an efficient method to incorporate active compounds into different food-grade materials and polymers used for active food packaging. This technique allows to incorporate active compounds with high or low affinity towards a polymer. In the first case, through an incorporation mechanism known as molecular dispersion and in the second case through a mechanism known as deposition, which is specific for supercritical fluids, this means it depends on the polymer swelling and plasticization effect caused by the $scCO_2$ and on the solubility of the active compound in the CO_2-phase.

An analysis of the effect of the operational conditions on the active compound incorporation was made using the results reported by several researches. From this analysis, it was possible to observe a tendency for pressure and temperature on the active compound loading. The increase in pressure (i.e. solubility) tends to have a positive effect on the active compound loading under isothermal conditions as long as the CO_2-phase is maintained saturated with the active compound during the impregnation assay. Meanwhile, the increase in temperature tends to have also a positive effect on the active compound loading if the range of pressures used is below the crossover pressure for systems operating under complete miscibility between the active compound and CO_2 or if the utilized pressures are above the crossover pressure for systems that do not operate under full miscibility. From these results, it can be seen that the effect of pressure and temperature can be predicted mainly from the knowledge of the phase behavior between an active compound and $scCO_2$. In fact, the high knowledge about binary systems (CO_2 + pure substance) has motivated the study of the $scCO_2$-assisted impregnation process for the development of active materials to be carried out mostly using a pure substance. Nevertheless, an active material could be more efficient containing a mixture instead of a pure substance. Thus, the study of complex multiphase mixtures becomes essential in order to support future work in several applications of the $scCO_2$-assisted impregnation process.

On the contrary, the effect of the depressurization rate on the active loading cannot be revealed only through the knowledge of the phase behavior between an active compound and $scCO_2$ because its effect depends on the affinity between the components of the system (CO_2 + active compounds + polymer) during the depressurization stage. Therefore, further research on ternary systems comprising CO_2 + active compounds + solid substrates is required to predict the effect of the depressurization rate on the active compound loading.

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