Food Chemistry 196 (2016) 518–525

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Solubility, photostability and antifungal activity of phenylpropanoids encapsulated in cyclodextrins

Miriana Kfoury [a,b], Anissa Lounès-Hadj Sahraoui [b], Natacha Bourdon [b], Frédéric Laruelle [b], Joël Fontaine [b], Lizette Auezova [a], Hélène Greige-Gerges [a], Sophie Fourmentin [b,*]

[a] Bioactive Molecules Research Group, Doctoral School of Science and Technology, Department of Chemistry and Biochemistry, Faculty of Sciences-2, Lebanese University, Lebanon
[b] Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV), Université du Littoral Côte d'Opale (ULCO), F-59140 Dunkerque, France

ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 14 September 2015
Accepted 22 September 2015
Available online 25 September 2015

Keywords:
Cyclodextrin
Encapsulation efficiency
Phenylpropanoids
Photostability
Phytopathogens
Solubility

ABSTRACT

Effects of the encapsulation in cyclodextrins (CDs) on the solubility, photostability and antifungal activities of some phenylpropanoids (PPs) were investigated. Solubility experiments were carried out to evaluate the effect of CDs on PPs aqueous solubility. Loading capacities and encapsulation efficiencies of freeze-dried inclusion complexes were determined. Moreover, photostability assays for both inclusion complexes in solution and solid state were performed. Finally, two of the most widespread phytopathogenic fungi, *Fusarium oxysporum* and *Botrytis cinerea*, were chosen to examine the antifungal activity of free and encapsulated PPs. Results showed that encapsulation in CDs significantly increased the solubility and photostability of studied PPs (by 2 to 17-fold and 2 to 44-fold, respectively). Free PPs revealed remarkable antifungal properties with isoeugenol showing the lowest half-maximal inhibitory concentration (IC_{50}) values of mycelium growth and spore germination inhibition. Encapsulated PPs, despite their reduced antifungal activity, could be helpful to solve drawbacks such as solubility and stability.

1. Introduction

Plant fungi diseases are one of the main reasons of great economic yield losses in crop management and postharvest storage. They are also the main cause for alterations in the appearance, flavor and reduction of nutritional values of food products (Magro, Carolino, Bastos, & Mexia, 2008). Synthetic fungicides and preservatives usually employed to treat phytopathogenic fungi or to inhibit food spoilage are toxic and harmful to environment and health. Indeed, many problems could result from their use such as development of resistance in fungi, destruction of beneficial species, and accumulation of chemical waste in the environment (Jacometti, Wratten, & Walter, 2010). Chemical residues could also remain within the plant tissues or fruits. The 1986 report from the US National Academic of Sciences indicated that chemical residues cause serious health risks (reprotoxicity, carcinogenicity, neurotoxicity) (National Agricultural Chemicals Association, 1986). Therefore, consumers and farmers are becoming more interested in less toxic formulations. It is also important to take in consideration legal issues that might arise regarding the use of some fungicides or preservatives. Consequently, it is becoming an ultimate need to find safe alternatives to synthetic agrochemicals and minimize health and environmental damages. Many secondary plant metabolites are safe natural sources of antifungal substances and are classified as GRAS (Generally Recognized As Safe) by the Food and Drug Administration (FDA) (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gandara, 2009). Recent studies suggested that these metabolites could be used to develop ecocompatible and safe fungicides and preservatives (Amorati, Foti, & Valgimigli, 2013) or as raw materials to synthesize more active agents (Bhatti et al., 2014). Indeed, phenylpropanoids (PPs) family is recognized to have potent activities against a wide range of bacteria and fungi (Dambolena, Lopez, Meriles, Rubinstein, & Zygadlo, 2012; Khan et al., 2010; Zabka & Pavela, 2013). Furthermore, it has been shown that some PPs could show synergistic effects on the activities of antifungal agents (Fujita, Fujita, & Kubo, 2007). However, they have generally low aqueous solubility and are susceptible to degradation promoted by heat, metals, oxygen, light and free radicals (Turek & Stintzing, 2013). Encapsulation in cyclodextrins (CDs) can enhance their aqueous solubility and stability and maintain their bioactivity (Ciobanu et al., 2013; Hadaruga, Hadaruga, Costescu, David, & Gruia, 2014; Hadaruga et al., 2006; Kfoury, Auezova, Greige-Gerges, Ruellan, & Fourmentin, 2014; Kfoury,

* Corresponding author.
E-mail address: lamotte@univ-littoral.fr (S. Fourmentin).

http://dx.doi.org/10.1016/j.foodchem.2015.09.078
0308-8146/

Landy, Auezova, Greige-Gerges, & Fourmentin, 2014). Nevertheless the effect of encapsulation on the physiochemical properties and bioactivity of these metabolites is far to be fully investigated.

CDs are cyclic oligosaccharides with a hydrophilic surface and hydrophobic cavity that allow them to encapsulate guests and form inclusion complexes (Szejtli, 1998) either in solution or solid state (Kfoury, Auezova, Fourmentin, & Greige-Gerges, 2014). CDs are derived from starch and are non-toxic and biodegradable under favorable conditions (Fenyvesi et al., 2005). They are widely used for soil remediation and food formulation (Astray, Mejuto, Morales, Rial-Otero, & Simal-Gándara, 2010; Landy, Mallard, Ponchel, Monflier, & Fourmentin, 2012). The most common native CDs are composed of 6, 7 and 8 glucosyl units and called α-, β- and γ-CD, respectively. β-CD has limited aqueous solubility, thus, derivatives like 2-hydroxypropylted-β-CD (HP-β-CD), randomly methylated β-CD (RAMEB) and a low methylated β-CD (CRYSMEB) with improved safety and enhanced water solubility were synthesized (Kurkov & Loftsson, 2013) and showed a good ability to solubilize PPs (Kfoury, Landy, et al., 2014).

Our study aimed to evaluate the effects of encapsulation in two native CDs (α-CD and β-CD) and three β-CD derivatives (HP-β-CD, RAMEB and CRYSMEB) on some physicochemical and biological properties of seven PPs: *trans*-anethole, estragole, eugenol, isoeugenol (phenylpropenes) and caffeic acid, *p*-coumaric acid and ferulic acid (hydroxycinnamic acids) (Table 1). Inclusion complexes were prepared both in solution and in solid state. Previous differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) studies gave supporting evidences for the inclusion of PPs in CDs in the solid state (Kfoury, Auezova, Greige-Gerges, Ruellan, & Fourmentin, 2015; Kfoury, Auezova, Greige-Gerges, et al., 2014). In the present study, loading capacities as well as encapsulation efficiencies (EE%) were determined and solubility, photostability and antifungal assays against *Fusarium oxysporum* and *Botrytis cinerea* were carried out. *Fusarium* species are toxigenic and harmful fungi responsible of vascular diseases in wide variety of plants, such as watermelon, cucumber, tomato, pepper, bean and cotton. *B. cinerea* causes Botrytis Blight or gray mold disease in a variety of plants such as strawberry, grape, asparagus, bean, beet, carrot and rhubarb. Many species of these two fungi are resistant to common fungicides (Leroch, Kretschmer, & Hahn, 2011). Therefore, new alternatives to these chemicals are needed.

2. Materials and methods

2.1. Chemicals and fungal strains

Estragole was provided by Fluka Chemicals and *trans*-anethole (99%) was purchased from Aldrich. Eugenol (99%), isoeugenol (99%), caffeic acid (>99%), *p*-coumaric acid (98%) and ferulic acid (99%) were purchased from Acros Organics. CRYSMEB (>98%, DS = 4.9) was provided from RoquetteFrères (Lestrem, France), α-CD (>98%), β-CD (>97%), HP-β-CD (>98%; DS = 5.6) and RAMEB (>98%, DS = 12.6) were purchased from Wacker-Chemie (Lyon, France). CDs were dried under vacuum before use. Distilled deionized water and absolute ethanol were used throughout this work. Fungal strains *F. oxysporum* and *B. cinerea* were kindly provided by Jérôme Muchembled from ISA (Institut Supérieur d'Agriculture, Lille, France).

2.2. Solubility experiments

Excess amount of PP was added to pure water or to a 10 mM CD solutions. Mixtures were shaken overnight at 25 °C and then filtered through 0.45 μm cellulose filters prior to analysis. Aliquots of the aqueous filtrates were diluted in ethanol. The concentration of PP in the filtrates was determined spectrophotometrically at the maximum wavelength of each (*trans*-anethole: 258 nm; estragole: 225 nm; eugenol: 280 nm; isoeugenol: 261 nm; caffeic acid: 327 nm; *p*-coumaric acid: 311 nm and ferulic acid: 325 nm). UV–Visible measurements were carried out using a UV–Visible dual-beam spectrophotometer (Perkin Elmer Lambda 2S) with a 1 cm thick quartz cuvette. For all PPs, standard curves were prepared by dissolving them in ethanol followed by serial dilutions. Linear regression was used to calculate their solubility in water or in CD solutions.

2.3. Preparation of solid inclusion complexes and physical mixtures

2.3.1. Freeze-drying

A freeze-drying method was used to prepare CD/PP solid inclusion complexes. Suspensions were prepared by mixing CDs and PPs in water in a 1:1 M ratio at a concentration of 10 mM. The mixtures were agitated using a laboratory shaker at 300 rpm for 24 h at 25 °C. Suspensions were then filtered, frozen and lyophilized at 85 °C and 0 Pa in a Christ Alpha 2–4 LD Freeze dryer until all moisture had been sublimated.

2.3.2. Physical mixtures

The same amounts of CDs and PPs used to prepare solid inclusion complexes were mixed together by a spatula until a homogeneous mixture was obtained.

2.4. Loading capacity and encapsulation efficiency

For each PP, three batches of freeze-dried CD/PP inclusion complex were prepared. Ten milligrams of each complex were dissolved in 10 mL ethanol leading to the release of encapsulated PP from the CD cavity to the ethanolic solution. Then, samples were diluted and the concentration of PP was determined by UV–Visible absorbance measurements at the maximum wavelength corresponding to each PP (see Section 2.2). All preparations and measurements were done in triplicate.

The loading capacity of solid inclusion complexes (mg PP/g inclusion complex) was determined as follows:

$$\text{Loading capacity} = \frac{\text{PP}_{\text{exp}}\ (\text{mg})}{\text{Inclusion complex}\ (\text{g})} \quad (1)$$

The EE% was calculated using the following equation:

$$\text{EE}\% = \frac{\text{PP}_{\text{exp}}\ (\text{mg})}{\text{PP}_{\text{T}}\ (\text{mg})} \times 100 \quad (2)$$

PP_{exp} stands for the experimental PP content which is the quantified amount of PP in the solid inclusion complex. PP_T is the theoretical PP content (the amount of PP initially used to prepare the inclusion complex).

2.5. Photodegradation experiments

During photodegradation experiments, CD/PP inclusion complexes in solution and in solid state were submitted to UVC irradiation using 10 UVC lamps (254 nm, 15 W each) by using a Multirays apparatus (Heliosquartz). Inclusion complexes in solution were transferred in a quartz reactor whereas freeze dried complexes and physical mixtures were spread on watch glass prior to irradiation. PPs in water and physical mixtures were used as references. At each time interval (5, 10, 20, 40 and 60 min), aliquots of irradiated inclusion complexes in solution or in solid state were withdrawn and diluted or dissolved in water, respectively. The concentration of PP was determined by Static Headspace-Gas