Food Hydrocolloids 70 (2017) 69–75

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Dissolution kinetics of polyphenol bearing calcium pectate hydrogels in simulated gastric or intestinal media and their anti-carcinogenic capacities

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ARTICLE INFO

Article history:
Received 30 October 2016
Received in revised form
18 February 2017
Accepted 21 March 2017
Available online 21 March 2017

Keywords:
Cherry laurel
Sunflower pectin gels
Polyphenol encapsulation
SEM
Dissolution kinetics
MCT 116 cell culture
Anti-proliferative activity

ABSTRACT

Cherry laurel is an underutilized agricultural resource with a significant content of polyphenolic compounds that potentially may demonstrate antioxidant and anti-carcinogenic activities. In this study, the preparation of calcium pectate gels bearing cherry laurel polyphenols was aimed and their dissolution behaviors in simulated gastric and intestinal media were tested. In addition, the released fractions were collected and tested in cell culture media in order to determine the anti-proliferative characteristics. Cherry laurel polyphenol extracts were mixed with pectin dispersions (1:1) and calcium pectate gels were prepared by the extrusion dripping method. The freeze dried gel samples were analyzed for morphological characteristics (SEM) and dissolution kinetics in simulated gastrointestinal media. The rate of release in gastric medium was substantial (≥94.9%) in all cases and dissolved fractions demonstrated significant anti-proliferative activity (approx. 85% inhibition for 55K06 and 61K04 extracts) against MCT 116 colon carcinoma cells even after 20× dilution. The findings were discussed with emphasis on their relevance to pectin-polyphenol interactions in dissolution behavior of the gels in simulated gastrointestinal media, and also to the preparation of functional lyophilizates that can be utilized in functional foods and pharmaceuticals.

1. Introduction

Pectin is a major heteropolysaccharide found in plant cell walls, mainly composed of linearly linked galacturonic acid residues. In industrial applications, the degree of methyl esterification determines the gelation characteristics of pectin products. In the case of high methoxyl pectin (HMP) products, elevated solid material (i.e., sugar) content and low pH are necessary for gelation. Negatively charged carboxyl groups of low methoxyl pectin form cross-links with calcium ions which induces the ionotropic gelation of pectin (Sriamornsak, Thirawong, & Puttipipatkhachorn, 2005). LMPs can be gelled in media with lesser amounts of solids (%), consequently their utilization in calorie reduction is quite practical. LMPs are generally manufactured from HMPs based on acid, alkali or enzymatic treatments, whereas genuine sources of LMPs are relatively limited (Iglesias & Lozano, 2004). Sunflower heads are the major by-products of sunflower oil processing industry. Shi, Chang, Schwarz, Wiesenborn, and Shih (1996) developed an optimized methodology to isolate low methoxy pectin (LMP) from de-seeded sunflower heads. This product can safely be utilized as a fat replacer in food formulations and de-seeded heads contain 15–25% pectin by weight (Shi et al., 1996).

Pectin products have been widely utilized as drug carriers, ingredients in various controlled and sustained drug release formulations (Kyomugasho et al., 2016; Wong, Colombo, & Sonvico, 2011). Since pectins are generally resistant to digestion by human gastrointestinal enzymes but degraded by colonic microflora (Liu, Fishman, Kost, & Hicks, 2003), pectin based delivery systems have the potential to protect the bioactive compounds during the GI transit, while providing controlled release at targeted tissues. Tablets coated with a pectin film as well as pectin based pellets or microparticles are commonly used to deliver drugs to the colon (Wakerly, Fell, Attwood, & Parkins, 1996; Wong et al., 2011). Similarly, pectin products are being utilized in functional food formulations, for example, in the encapsulation of bioactive ingredients in order to improve the encapsulation efficiency and protect the bioactive materials (Drusch, 2007; Sansone et al., 2011).

In the intact plant cells, phenolic compounds are located

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http://dx.doi.org/10.1016/j.foodhyd.2017.03.026
0268-005X/

primarily within the vacuoles which prevent their interactions with cell wall components (i.e., cellulose, pectin, hemicellulose etc). However, during the process of eating, rupturing of cell walls enables the interactions between the two moieties (i.e., phenolics and cell wall components) (Phan et al., 2015). The strength of interactions could affect the bioavailability of phenolics due to the varying rates of release in the gastrointestinal tract. According to Phan et al. (2015), for example, significant amounts of polyphenols can be bound to cellulose (0.4–1.4 g/g cellulose). Consequently, the behavior of polyphenol-carbohydrate complexes in the gastrointestinal tract needs to be clarified. As reviewed by Bordenave Hamaker & Ferruzzi (2014), rheological characteristics and digestibility of carbohydrate dispersions may be affected by the presence of phenolic compounds, while extractability of phenolics and their bioavailability are sensitive to the interactions with macromolecules including pectins. Furthermore, coexistence of apple phenolics and pectin were demonstrated to enhance the biological activity of the fruit (Aprikian et al., 2003). The adsorption characteristics of phenolic compounds to cell walls materials were dependent on the size of pores in cell walls and phenolic compounds and also their affinity to the cell wall materials (Bindon, Madani, Pendleton, Smith, & Kennedy, 2014). The coexistence of multiple phenolics in plant materials could affect both the extent of adsorption on cell walls and the extractability of the adsorbed materials (Bindon et al., 2014). This also could imply that absorption of phenolics in the human body may be clearly affected by noncovalent forces. According to Renard, Baron, Guyot, and Drilleau (2001), adsorption of polyphenols to apple cell wall carbohydrates was mostly governed by weak bonds such as H-bonds and possibly hydrophobic interactions, whereas Yuksel, Avci, and Erdem (2010) demonstrated that non-covalent hydrophobic interactions occurred between polyphenols and other food ingredients. Since polyphenols are small molecules, they might also be trapped into the porous network of larger molecules (Jakobek, 2015). Polymerized polyphenols could be encapsulated in hydrophobic pockets of macromolecules (Le Bourvellec & Renard, 2012). Pectin-rich fractions of blueberry could both protect anthocyanins in the body and promote colonic health (Lin, Fischer, & Wicker, 2016). These findings imply the utilization of pectin based matrices in the effective delivery of phenolic compounds. Due to the presence of an inert porous network, calcium pectate beads were shown to lead to the diffusion controlled release of active materials (Sriamornsak and Nunthanid, 1998; Aydin & Akbuğa, 1996).

Cherry laurel (*Laurocerasus officinalis* Roem, syn: *Prunus laurocerasus* L.) is a member of Rosaceae family, which is native to western Asia and also grown over the Balkans. Due to its edible fruits, it is commonly cultivated in northern Turkey (i.e., Black Sea region) as a native fruit crop (Islam, 2002) and also in Bulgaria, Serbia, Iran and some of the Mediterranean countries (Kolayli, Küçük, Duran, Candan & Dinçer et al., 2003). It can be classified to be an evergreen shrub or small tree (Sulusoglu, 2011; Çalışır & Aydın, 2004). The phenolic compound profile of cherry laurel has been partly elucidated and anti-oxidative properties of cherry laurel phenolics have been investigated (Alasalvar, Al-Farsi, & Shahidi, 2005; Karabegovic et al., 2014, 2013; Karahalil & Şahin, 2013; Kolayli et al., 2003). Recently, 14 different phenolic compounds were identified in cherry laurel, about half of which were demonstrated to exist in this fruit for the first time (Bayrambaş, 2016).

In this study, we have manufactured lyophilized forms of cherry laurel polyphenol bearing calcium pectate gel beads. The morphological characteristics of lyophilized beads as well as their dissolution kinetics in simulated gastrointestinal fluids were determined. The dissolved fractions were collected and their antiproliferative capacities against HCT 116 cells were studied. We discuss the findings in relevance to the utilization of functional lyophilizates in food formulations.

2. Materials and methods

2.1. Materials

All chemicals including simulated gastric and intestinal fluids were obtained from Sigma-Aldrich Corp and used without further purification.

2.2. Preparation of sunflower head pectin

Sunflower (*Helianthus annuus* L.) heads were collected from a local farm in Tekirdağ, Turkey. Pectin isolation was based on the optimized protocols described in Shi et al. (1996). Sunflower heads (SFH) were vacuum dried down to a moisture level of <8%. Immediately afterwards, dried SFH was ground to approx. 12 mm using a laboratory grinder. After grinding, powdered SFH was treated with an alkali solution (pH 7.5, 16 °C) for 25 min. The solvent to solute ratio was 28:1. Afterwards, the mixture was filtered and the filter cake was diluted with a sodium hexametaphosphate solution (1:25, 75 °C, pH 3.5, 1 h), while the medium pH was adjusted with 1 M phosphoric acid. The mixture was precipitated using 1 M nitric acid (5 °C, 1:5 acid solution:filtrate ratio) while the mixture was kept stirred (1 h). Once again, the precipitate was collected and mixed with 0.1 M nitric acid solution at a precipitate:solvent ratio of 1:2 for 20 min. This operation was repeated twice. Thus formed pectin gel was washed with an ethanolic solution (700 ml ethanol L^{-1}) 6 times at a gel to solvent ratio of 1:2. This process lowered the mineral content of pectin, enhanced the color attributes and removed acid which in turn increased the sample pH. After washing, the gel was dried in vacuum oven (55 °C, 16 h). Finally, dried products were ground using a laboratory grinder and sifted through 2 mm screens (Shi et al., 1996). Pectin samples were kept frozen at −20 °C until further use. In order to ensure the absence of proteins in the pectin samples, Kjeldahl and Lowry analyses were carried out for 10 different pectin samples, and proteins were not detected.

2.3. Fruit materials and the extraction of cherry laurel polyphenols

The fruit materials were harvested from three different cherry laurel varieties (55K06, 61K04 and 61K05) grown at Black Sea Region of Turkey, by the Black Sea Agricultural Research Institute Directorate of Samsun. 55K06 was harvested at an altitude of 30 m at Samsun province, Turkey. 61K04 and 61K05 were picked at Trabzon province near Araklı Yalıboyu, at an altitude of 10 and 40 m, respectively. Fruits were sampled when they reached full ripeness in September 2014. Approximately 20 kg of cherry laurel fruits were picked and kept at −20 °C immediately after the harvest and until further laboratory use.

40 g cherry laurel samples were mixed with 400 ml methanol and incubated for 15 min in an ultrasonic water bath kept at 65 °C (Karabegovic et al., 2014). Methanol insoluble materials were separated by filtration (i.e., 0.45 μm PTFE membrane filter). Using a rotary evaporator system (40 °C), methanol soluble phenolics were concentrated and methanol was removed from the samples. Concentrated polyphenol samples were transferred to amber glass containers and kept refrigerated (4 °C) until further use.

2.4. Sample preparation

Cherry laurel polyphenol bearing calcium pectate gels were prepared by the extrusion dripping method. 1% sunflower head