Food Hydrocolloids 83 (2018) 229–238

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Pectic polysaccharides with different structural characteristics as inhibitors of pancreatic lipase

Eliana-Yissel Aguilera-Angel [a], Mauricio Espinal-Ruiz [b], Carlos-Eduardo Narváez-Cuenca [a, *]

[a] Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá DC, Colombia
[b] Departamento de Nutrición y Bioquímica, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá DC, Colombia

ARTICLE INFO

Article history:
Received 20 February 2018
Received in revised form
5 May 2018
Accepted 6 May 2018
Available online 9 May 2018

Keywords:
Pectic polysaccharides
Pancreatic lipase
Digestion
Molecular weight
Methoxylation degree

ABSTRACT

The effect of molecular weight (MW) and methoxylation degree (MD) of apple (A) and citrus (C1-C5) pectic polysaccharides (PPs) on both the digestion of emulsified lipids and the pancreatic lipase activity was evaluated by using a static *in vitro* digestion system and a simplified system, respectively. The first system consisted in the simulated digestion [composed of an initial (before digestion), oral, gastric, and intestinal phases] of a soybean oil-in-water emulsion containing PPs at initial concentrations of 0 (control) and 0.1% (*w/w*) of PPs with pancreatic lipase. The microstructure and particle size distribution of the soybean oil-in-water emulsions were characterized before (initial phase) and after (intestinal phase) the *in vitro* digestion process. During the intestinal phase the free fatty acid released were measured as well. The second system consisted of an acidic (H_2SO_4) back titration of Tris-HCl buffer after the hydrolysis of triacetin with pancreatic lipase, in the presence of PPs at concentrations of 0 (control) and 0.01% (*w/v*). Among tested PPs A [MW, 209 kDa; and MD, 73.3% (*mol/mol*)] was the most effective one in inhibiting the activity of pancreatic lipase in both the static *in vitro* digestion model (63% activity as compared to the control) and the simplified model (61% activity as compared to the control). From the contour plot, after mathematical modelling, MD of PPs had a greater influence as compared to MW on the inhibition of the activity of pancreatic lipase, with higher inhibition of enzymatic activity as the MD was increased.

1. Introduction

Overweight and obesity are pathological conditions characterized by an excessive accumulation of body fat (Phillips, 2013). These pathological conditions are related to the risk of suffering several chronic non-communicable diseases such as cardiovascular disease (Farooqi, 2011), dyslipidemia (Klop, Elte, & Cabezas, 2013; Nguyen, Magno, Lane, Hinojosa, & Lane, 2008), diabetes (Lunagariya, Patel, Jagtap, & Bhutani, 2014), metabolic syndrome (Nguyen et al., 2008), hypertension (Nguyen et al., 2008), and cancer (Farooqi, 2011). According to World Health Organization (2018), in 2016 more than 1900 million adults (18 years and older) were classified as overweight, whereas 650 million of them (corresponding to 34%) were classified as obese.

The high incidence of both overweight and obesity prevalence worldwide requires the development of novel treatments to mitigate their adverse effects on human health. The development of overweight and obesity treatments mainly focuses on the restriction of energy intake by controlling the consumption of energetic nutrients such as fat, protein, and carbohydrates (Guyenet & Schwartz, 2012). Because fats have a higher caloric content (9 kcal/g) as compared to those for proteins (4 kcal/g) and carbohydrates (4 kcal/g), the control of fat calories consumption might be an effective strategy to modulate the overall calorie intake (Mendoza, Garcıa, Casas, & Selgas, 2001). It has been proposed that the control of fat calories can be carried out by restricting both fat intake (Bray & Popkin, 1998) and fat absorption (Lunagariya et al., 2014). On the one hand, the restriction of fat intake in both overweight and obese people may exhibit several problems such as the modification of the feeding behavior of people which is often a difficult and a non-controllable practice (Swinburn, Caterson, Seidell, & James, 2004), the preference for high-energy over low-energy foods (Blundell et al., 2005), the decrease of the satiety feeling (Wisén & Hellström, 1995), and the increase of the hunger feeling (Guyenet & Schwartz, 2012). On the other hand, the

* Corresponding author.
E-mail address: cenarvaezc@unal.edu.co (C.-E. Narváez-Cuenca).

https://doi.org/10.1016/j.foodhyd.2018.05.009
0268-005X/

restriction of fat absorption has demonstrated to be an effective strategy for controlling fat calorie intake (Lunagariya et al., 2014). The restriction of fat absorption can be accomplished through the consumption of prescription drugs such as Lipstatin (Hochuli et al., 1987) and Orlistat (Guerciolini, 1997); phytochemicals such as saponins (Cheeke, 1971), polyphenols (Nakai et al., 2005), and caffeine (Bray & Tartaglia, 2000); and soluble dietary fibers such as hemicellulose (Papathanasopoulos & Camilleri, 2010), alginate (Dettmar, Strugala, & Craig Richardson, 2011), carrageenan (Shipe, Senyk, & Boor, 1982), and pectic polysaccharides (PPs) (Espinal-Ruiz, Restrepo-Sánchez, & Narváez-Cuenca, 2016a). Interestingly, soluble dietary fibers control fat digestion by multiple effects on the gastrointestinal tract, including gastrointestinal transit time, increased digesta viscosity, and control of digestive enzymes (Edwards, 1995; Grundy et al., 2016; Gunness & Gidley, 2010; Lairon, Play, & Jourdheuil-Rahmani, 2007).

Esterification degree of the carboxyl group of galacturonic acid (GalA) moieties with either methanol or acetic acid [hereinafter referred as methoxylation (MD) and acetylation (AD) degrees, respectively] as well as the molecular weight (MW), are structural parameters defining the physicochemical and functional properties of PPs (Padayachee, Day, Howell, & Gidley, 2017; Willats, Knox, & Mikkelsen, 2006; Willats et al., 2001). It has been suggested that PPs are capable to restrict fat digestion by several mechanisms, such as inhibition of the pancreatic lipase activity (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, & Narváez-Cuenca, 2014a), modification of the rheological properties of gastrointestinal fluids (Espinal-Ruiz, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2016b; Zhang, Zhang, Zhang, Decker, & McClements, 2015), binding of key gastrointestinal components (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narvá;ez-Cuenca, & McClements, 2014b), and alteration of the fat aggregation state (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014c; Espinal-Ruiz et al., 2016b). According to Espinal-Ruiz et al. (2014a), PPs are capable of inhibiting the activity of pancreatic lipase through a non-competitive mechanism (interaction of PP with pancreatic lipase in a different site than the catalytic pocket), reducing both the rate and extent of the lipid digestion process. The non-competitive inhibitory effect of pectin on lipase activity was previously demonstrated by using the interfacial Michaelis-Menten model (Espinal-Ruiz et al., 2014a), demonstrating that pectin is capable to bind to pancreatic lipase in a different position to the active site. The magnitude of this inhibition process was found to depend on both the concentration and the structural characteristics of PPs such as MW and MD (Espinal-Ruiz et al., 2014c), being the magnitude of this inhibition increased with both the increase of PP concentration and the simultaneous increase of MW and MD (Espinal-Ruiz et al., 2016b). By means of an *in vitro* digestion system, Espinal-Ruiz et al. (2014c) found an inhibitory effect caused by PPs towards the pancreatic lipase activity related to the flocculation process of lipids, which may restrict the fraction of lipids which can be hydrolyzed. The flocculation process was also found to be stimulated by both the concentration (Espinal-Ruiz et al., 2014c) and the simultaneous increasing of both MW and MD of PPs (Espinal-Ruiz et al., 2016b). So far, however, it has not been possible to establish the relative contribution of each parameter separately to the overall capacity of PPs to inhibit the fat digestion process (Espinal-Ruiz et al., 2016b). The aim of this research was, therefore, to determine the relative contribution of both MD and MW of PPs to the overall inhibitory effect of the pancreatic lipase activity and therefore to find the levels of such structural characteristics that make PPs more suitable for controlling fat digestion.

2. Materials and methods

2.1. Chemicals

Ox bile extract with a cholic acid content of 55% (*w/w*) was purchased from MP Biomedicals Inc. (Solon, OH, United States). Triacetin 99% (*w/w*) was purchased from Pfaltz & Bauer Inc. (Stanford, CT, United States). D-(+)-galacturonic acid monohydrate (purity $\geq$ 97.0% *w/w*), mucin from porcine stomach (type II, bound sialic acid $\leq$ 1.2% *w/w*), pepsin A from porcine gastric mucose (E.C. 3.4.23.1, activity $\geq$250 units/mg solid), lipase from porcine pancreas (E.C. 3.1.1.3, type II), bovine serum albumin (BSA), pullulan analytical standards for gel permeation chromatography (MW ranging from 342 Da to 366 kDa), and pectin from apple fruit (denominated as A) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, United States). The manufacturer reported that one unit of pepsin A activity would produce a ΔA_{280} of 0.001 per min at pH 2.0 and 37 °C using hemoglobin as substrate, and that the pancreatic lipase activity is either 100–400 units per mg protein (using olive oil as substrate) or 30–90 units per mg protein (using triacetin as substrate) when incubating during 30 min [one unit of lipase activity being defined as the amount of enzyme required to release 1 μeq of free fatty acids from either triacetin (pH 7.4) or olive oil (pH 7.7) in 1 h at 37 °C]. Citric commercial PP denominated as C1 was purchased from CIMPA SAS (Bogotá DC, Colombia), whereas citric commercial PPs denominated as C2 and C3 were purchased from Quimilíz Ltda. (Bogotá DC, Colombia). Citric commercial PPs denominated as C4 and C5 were kindly donated by CP Kelco Inc. (Lille Skensved, Denmark) and TIC Gums Inc. (Belcamp, MD, United States), respectively. Soybean oil was purchased from a commercial food supplier (Éxito SA, Bogotá DC, Colombia) and stored at 4 °C in darkness until use. The manufacturer reported that the soybean oil contained approximately 17, 55, and 28% (*w/w*) of saturated, monounsaturated, and polyunsaturated fatty acids, respectively. All other chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, United States) and Merck KGaA (Darmstadt, Germany).

2.2. Characterization of pectic polysaccharides (PPs)

PPs (Denominated as A and C1-C5) were characterized by their total ash content by calcination at 450 °C (According to AOAC method 942.05), total nitrogen content by the Kjeldahl method (According to AOAC 970.22), total uronic acid content (Section 2.2.1), MD and AD (Section 2.2.2), and MW relative to pullulans (Section 2.2.3), as follows.

2.2.1. Total uronic acid content

The total uronic acid content was obtained through the spectrophotometric method reported by van den Hoogen et al. (1998). A 120-μg/mL aqueous solution of each PP was prepared. An aliquot of 200 μL of each PP solution was mixed with 1200 μL of 12.5 mM sodium tetraborate prepared in 98% (*w/w*) sulfuric acid. The mixture was vortexed during 1 min and incubated at 100 °C during 5 min. After cooling in an ice-water bath, the solution was mixed with 20 μL of 0.15% (*w/v*) *m*-hydroxydiphenyl reagent prepared in 0.5% (*w/v*) NaOH. After 5 min of incubation at room temperature, the absorbance of the pink-colored mixture was measured at 520 nm (total absorbance of the sample) on a spectrophotometer (V-1200, Shanghai Mapada Instruments Co., Shanghai, China). Two different blanks were measured at 520 nm for each reaction mixture: A blank with no addition of *m*-hydroxydiphenyl reagent (replacing it with 0.5% *w/v* NaOH) and a blank with no addition of PP sample (replacing it with distilled water). The absorbance values obtained for blanks were subtracted from the total absorbance of the sample. A calibration line was obtained by using standard