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In vitro evaluation of the anti-digestion and antioxidant effects of grape seed procyanidins according to their degrees of polymerization

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ABSTRACT

Few studies have evaluated the anti-digestion effects of grape seed procyanidins (GSPs) compared to their strong anti-oxidant activities according to different degrees of polymerization (DPs). Additionally, the effects of GSPs with different DPs on the binding sites of digestive enzymes are unexplored. In this work, the anti-digestive and anti-oxidant activity of GSPs with different DPs were verified and compared. Anti-digestive activity assays were conducted on key digestive enzymes. Although all procyanidins fractions possessed antioxidant activity as expected, there was no significant difference in the antioxidant activity among procyanidins with different DPs. In contrast, the anti-digestive activity of the fractions increased significantly as the DP increased. Meanwhile, molecular docking provided a putative mechanism of anti-digestion, and GSPs can block the enzyme sites by hydrogen interactions, hydrophobic interactions and electrostatic interactions. These results indicated that the higher-molecular-weight procyanidins had equal or lower antioxidant activity and a greater anti-digestion effect than lower-molecular-weight procyanidins.

1. Introduction

Proanthocyanidins (PAs) are secondary plant metabolites that are widely found in a wide variety of edible plants (*e.g.* berries, grapes, and nuts). Therefore, daily intake of PAs may occur through the consumption of plants and plant-based foods. In recent years, because of their beneficial health effects, the demand for PAs has increased. According to reports, grape seed is an abundant natural source of these compounds and composes the majority of phenolic products found in the market (Fernandez, Vega, & Aspe, 2015; Luo et al., 2016). In grape seeds, PAs are composed of oligomeric procyanidins (OPCs) and polymeric procyanidins (PPCs) that consist of flavan-3-ol monomer units, *i.e.* (−)-epicatechin, (+)-catechin, and (−)-epicatechin-3-O-gallate, linked through C4-C8 or C4-C6. Plant PAs may also contain either A- or B-type linkages (Gu et al., 2003), but in grape seeds, only B-type linkages (*i.e.* procyanidins) were found (Choy, Jaggers, Oteiza, & Waterhouse, 2013).

Many studies have been conducted over the last two decades concerning the benefits of procyanidins. Procyanidins play crucial roles in the prevention of various diseases and are beneficial for human health. Furthermore, procyanidins have a variety of biological properties, including antioxidant, antimicrobial, antiviral, and anti-inflammatory activities (Bashir, Manoharan, & Miltonprabu, 2016; Weber et al., 2007). Procyanidins are internationally recognized natural antioxidants that act against free radicals and have attracted worldwide interest because of the anti-aging effects of procyanidins-rich diets and cosmetics (Jiao, Wei, Chen, Chen, & Zhang, 2017). Additionally, previous studies indicated that the anti-inflammatory properties of procyanidins may contribute to their cardiovascular benefits (Terra et al., 2009). As expected, procyanidins-rich diets have been associated with a reduced risk of chronic cardiovascular diseases, including hypertension and dyslipidemia. (Nunes, Pimentel, Costa, Alves, & Oliveira, 2016). Among the beneficial effects of procyanidins, their most attractive property is

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their potent inhibitory effect on some cancers, including skin cancer, prostate cancer, lung cancer, and stomach cancer (Akhtar, Meeran, Katiyar, & Katiyar, 2009; Katiyar, 2016; Rossi et al., 2010; Schmidt, Erdman, & Lila, 2006). Other beneficial aspects of procyanidins include preventing radiation damage, mutation, and vision degeneration, and improving skin disorders (Jing, Zhang, & Yan, 2015).

However, knowledge of the negative effects of these polyphenols is lacking. Several studies have reported that polyphenols inhibit enzymes, including α-amylase, α-glucosidase, pepsin, lipase, and trypsin (Gu, Hurst, Stuart, & Lambert, 2011; He, Lv, & Yao, 2007; Li et al., 2015), the activities of which are considered beneficial only for those who are obese (Salvado, Casanova, Fernandez-Iglesias, Arola, & Blade, 2015), or have diabetes (Sui, Zhang, & Zhou, 2016) or gastro-esophageal reflux disease (GERD) (Strugala, Kennington, Campbell, Skjak-Braek, & Dettmar, 2005). However, for most healthy individuals and those who are deficient in digestive enzymes, the anti-digestive activity may be harmful to health. Generally, the trace mount of PAs in food does not cause obvious symptoms of indigestion. Grape seed procyanidins (GSPs) possess broad pharmaceutical activities and are used in pharmaceuticals and the food industry. According to published results and the data on file with the manufacturer of these products, digestive side-effects may occur in some patients, even at the normal dosage. Furthermore, enzymes have specificities. Pancreatic α-amylase can hydrolyze the α-1,4-glucoside bond in the molecular chain of starch, then cut the chain into short-chains made of dextrin, oligosaccharides, and a small amount of maltose and glucose, so that the viscosity of starch decreases rapidly to “liquefaction”. Pepsin is a digestive protease secreted by the gastric chief cell in the stomach that breaks down the proteins in food into small peptide fragments. Pancreatic lipase is a key enzyme that digests dietary triglycerides to glycerol and fatty acids in the small intestine. Kusano et al. (2011) demonstrated that proanthocyanidins from bark extract of Acacia mearnsii exhibited strong inhibitory activities toward α-amylase and lipase but did not separate proanthocyanidins into different DP. Sugiyama et al. (2007) isolated apple oligomeric procyanidins with different degrees of polymerization, but only their inhibitory activity on lipase was determined and no mechanism was explored. Miao et al. (2014) studied the mechanism of inhibition of α-amylase by grape skin extract, but only the molecular docking of resveratrol-3-O-glucoside and α-amylase was studied. In addition, their studies on the inhibition of digestive enzymes have not been compared with antioxidant experiments, and there is no specific guidance on the daily use of procyanidins functional foods. To improve the safety of nutraceutical products, the inhibitory effects of procyanidins on key digestive enzymes should be evaluated to verify whether the effects are different depending on the DPs of the procyanidins.

Molecular docking is a well-known method used to predict the predominant binding pattern of a ligand with a protein of known three-dimensional structure. Therefore, the potential mechanism of anti-digestion was explored by molecular docking technology in order to further explain the inhibition mechanism of procyanidins with different degree of polymerization on digestive enzymes.

The objective of this study was to evaluate the anti-digestive effects and compare the antioxidant effects of GSPs according to their DPs. For this purpose, GSPs with different DPs were first prepared, then the anti-nutritional properties of GSPs were evaluated using a multi-spectroscopic method and docking studies to better determine the use of GSPs.

2. Experimental

2.1. Materials and reagents

Grape seeds (Fernao Pires, *vitis vinifera*) were provided by laboratory of polyphenols – Polo Dois Portos/INIAV of Portugal. (+)-Catechin (Cat) was purchased from Chengdu Must Bio-Technology Co., Ltd. (Chengdu, China). Hemoglobin from bovine blood dried erythrocytes, 4-methylumbelliferyl oleate (4-MUO), α-amylase from porcine pancreas (Type VI-B), lipase from porcine pancreas (Type II), pepsin from porcine gastric mucosa, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4, 6-Tris (2-pyridyl)-s-triazine (TPTZ) and (±)-6-Hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid (trolox) were purchased from Sigma Aldrich (St. Louis, MO, USA). Orlistat (≥98.0%, BR), acarbose (> 97%, BR), pepstatin A (≥90%), Folin-Ciocalteu's phenol reagent and Tris-HCl (pH 8) were purchased from Dalian Meilun Biotech Co., Ltd. All organic solvents used for high speed counter-current chromatography (HSCCC) and soluble starch were purchased from Chemical Branch of Shandong Yuwang Industrial Co., Ltd. (Shandong, China).

2.2. Preparation of grape seed phenolic extract

The frozen seeds were ground finely under liquid N_2 and the powder obtained immediately used for the extraction of phenolic compounds using the method described previously (Sun, Belchior, Ricardo-Da-Silva, & Spranger, 1999). Briefly, a 300-g portion of the powder was extracted using 1.5 L of methanol-water (80/20; v/v) followed by 1.5 L of acetone-water (75/25; v/v). Each solvent extraction was performed by stirring for 3 h under a nitrogen atmosphere at room temperature. The combined supernatants were evaporated at < 30 °C to remove organic solvents, followed by extraction with hexane (3 × 300 mL) to eliminate fatty materials, and then filtered through a membrane filter (0.45 μm). The aqueous phenolic solution was lyophilised (Temperature: cold trap 54.4 °C, sample −20.6 °C) for 48 h. The yellow-brown powder thus obtained, referred as grape seed phenolic extract, was stored at −20 °C under darkness until needed.

2.3. Isolation and purification of total procyanidins fractions from grape seed phenolic extract

A hundred milliliters of the aqueous phenolic solution (20 mg dry matter/mL) or an equivalent aqueous solution prepared from the grape seed phenolic extract was loaded onto a open column (200 × 25 mm i.d.) packed with Lichroprep RP-18 (25–40 μm particle size) already preconditioned with distilled water adjusted to pH 7.0. The fractionation procedures were similar to those already described by using C18 Sep-Pak cartridges (Sun, Leandro, Ricardo-da-Silva, & Spranger, 1998). Elution began with 100 mL of distilled water adjusted to pH 7.0 to eliminate phenolic acids and flavonols, followed by 100 mL methanol to recover the total procyanidins fraction. After lyophilization for 72 h, the powder of total procyanidins fraction, verified to have high purity (> 92%) using the method described (Spranger, Sun, Mateus, Freitas, & Ricardo-da-Silva, 2008), was stored at −20 °C under darkness until its use for further fractionation.

2.4. Preparative fractionation of procyanidins according to their polymerization degrees

The preparative separation of the isolated total procyanidins fraction based on the degree of polymerization was performed according to the method described previously (Zhang et al., 2015) using HSCCC (model TBE-300B; Tauto Biotechnique Company, Shanghai, China). Solvent system was composed of n-hexane-ethyl acetate-water (1:50:50, v/v/v), the equipment was controlled by water bath at 25 °C, the apparatus was run at 950 rpm, flow rate was 3 mL/min. During the process of separation, effluents were monitored at 280 nm. Seven peaks (from F1 to F7) were collected manually. After a number of repeated experiments, each collected fraction was mixed with distilled water, evaporated to remove organic solvents and then lyophilized. The powders obtained were stored at −20 °C under darkness, ready to be used for further experiments.