Food Chemistry 143 (2014) 106–113

Contents lists available at ScienceDirect

Food Chemistry

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

Pectic polysaccharides of the fresh plum *Prunus domestica* L. isolated with a simulated gastric fluid and their anti-inflammatory and antioxidant activities

Sergey V. Popov [a,*], Raisa G. Ovodova [a], Victoria V. Golovchenko [a], Daria S. Khramova [a], Pavel A. Markov [a], Vasily V. Smirnov [a], Alexandre S. Shashkov [b], Yury S. Ovodov [a]

[a] Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, 50, Pervomaiskaya Str., Syktyvkar 167982, Russia
[b] N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47, Leninsky Prospect, Moscow 119991, Russia

ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form 23 May 2013
Accepted 10 July 2013
Available online 26 July 2013

Keywords:
Plums
Pectin structure
Simulated gastric fluid
NMR spectroscopy of polysaccharides
Leukocyte adhesion
Superoxide anion radical scavenging

ABSTRACT

A pectic polysaccharide, designated as PD, was extracted from fresh plums (*Prunus domestica* L.) with a simulated gastric fluid. Galacturonan, which was partially substituted with methyl and *O*-acetyl ester groups, and rhamnogalacturonan were the main constituents of the linear regions of the sugar chains of PD. The ramified region contained mainly 1,4-linked β-D-galactopyranose residues and, to a lesser extent, 1,5-linked α-L-arabinofuranose residues. The separation of PD, by DEAE-cellulose column chromatography, yielded two pectic fractions: PD-1 and PD-2, eluted with 0.1 and 0.2 M NaCl, respectively. Enzymatic digestion of PD with 1,4-α-D-polygalacturonase yielded the fraction PD-E. The parent pectin PD and the PD-1 fraction were found to diminish the adhesion of peritoneal leukocytes at the concentrations of 0.05–1.0 mg/ml. However, the PD-E fraction failed to have an effect on cell adhesion at the concentrations of 0.05–0.1 mg/ml. PD, PD-1 and PD-E were found to inhibit the production of superoxide anion radicals by reducing xanthine oxidase activity by 38%, 97% and 47%, respectively. Therefore, the PD-1 fraction appeared to be an active fragment of pectic macromolecule isolated from fresh plum with a simulated gastric fluid.

1. Introduction

Epidemiological studies have shown that a diet rich in fruits, significantly reduces the incidence of chronic diseases, including gastrointestinal disorders (Gonzalez et al., 2006). The beneficial health effects of fruits are believed to be attributed to the constituents with anti-inflammatory and antioxidant activity. The antioxidant compositions of fruits have been widely studied. Most of the research has been limited to secondary metabolites (phytochemicals) of low molecular weights such as carotenoids, tocopherols, ascorbic acid and flavonoids; however, secondary metabolites of low molecular weights appear to possess an insufficient antioxidant capacity against extracellular reactive oxygen species (ROS) formed in the intestinal lumen and mucosa. Indeed, ROS have been found to be generated extracellularly from pro-oxidants found within food, such as iron, copper, heme and lipid peroxides (Halliwell, Zhao, & Whiteman, 2000). In addition, local infection, ischemia/reperfusion, gastric acid production and non-steroidal anti-inflammatory drugs, may also promote the formation of ROS in the gastrointestinal milieu (Sasaki & Joh, 2007). Structural modifications of phytochemicals and/or their rapid elimination from the lumen, due to absorption, have been suggested to limit the intraluminal antioxidant capacity of phytochemicals. Moreover, phytochemicals have been shown to exert pro-oxidant effects under certain conditions (Mdel Pinto & Macias, 2005). Identification within fruits of antioxidants resistant to digestion and absorption in the gastrointestinal tract, is of great interest. One promising group of such compounds is thought to be the pectic polysaccharides, which are a component of dietary fibres that have been generally defined as the portion of edible plants that are resistant to digestion in the human stomach and small intestine (Lunn & Buttriss, 2007).

Pectin macromolecules are known to include various fragments of linear and ramified regions. The linear region consists of a backbone of (1 → 4)-linked α-D-galacturonic acid (GalA) residues. The homogalacturonic (HG) regions are interrupted by rhamnogalacturonic (RG) regions containing (1 → 2)-linked α-L-rhamnose (Rha) residues. In the ramified region the rhamnosyl units are substituted by side chains containing mainly arabinose (Ara) and galactose (Gal) (Voragen, Coenen, Verhoef, & Schols, 2009). Pectins have previously been shown to possess diverse biological activities, which may have a role in the beneficial effects of fruit and

* Corresponding author. Tel./fax: +7 8212 241001.
E-mail address: popov@physiol.komisc.ru (S.V. Popov).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.07.049

vegetable diets. Specifically, pectins have been found to possess anti-inflammatory (Chen et al., 2006; Salman, Bergman, Djaldetti, Orlin, & Bessler, 2008) and antioxidant (Mateos-Aparicio, Mateos-Peinado, Jimenez-Escrig, & Ruperez, 2010; Yang, Zhao, Prasad, Jiang, & Jiang, 2010) activities. Biological activity of pectins has been shown to depend on the structural features of the linear and the branched polysaccharide chains.

Plums represent an excellent source of nutrients and compounds that influence human health and prevent the occurrence of many diseases. Fresh plums have been shown to have a high content of phenolic compounds (111 mg/100 g) and carotenoids (1.3–2.3 mg/100 g). Characteristically for plums predominant phenolics (86% of total phenolics) are neochlorogenic (3′-caffeoylquinic) and chlorogenic (3′-caffeoylquinic) acids (Stacewicz-Sapuntzakis, Bowen, Hussain, Damayanti-Wood, & Farnsworth, 2001). The antioxidant capacity of phenolics is believed to provide anti-inflammatory, cytoprotective and neurologic effects of plum consumption (Bouayed, Rammal, Dick, Younos, & Soulimani, 2007; Shukitt-Hale et al., 2009; Zaidi et al., 2012).

Fresh plums are a rich source of dietary fibre and pectin. Plums were found to contain 0.6–1.5 g of dietary fibre including 0.5–1.0 g of pectin per 100 g of fresh pulp. So, the proportion of the main phytochemicals and pectin content of fresh plum can be estimated as 1/10 (Rani & Kawatra, 1994; Stacewicz-Sapuntzakis et al., 2001; Vicente, Manganaris, Sozzi, & Crisoto, 2009). The high content of Gal and Ara residues, as well as the low content of GalA residues, has been reported to be a structural feature of plum pectin (Nunes, Saraiva, & Coimbra, 2008; Nunes et al., 2012; Raynal, Mourgues, & Conte, 1991; Renard & Ginies, 2009). The relatively higher proportion of Ara and Gal, in relation to GalA, allowed inferring the occurrence of highly branched pectic polysaccharides in plum pectin. Antioxidant effect of pectins has been found to be associated with Ara and Gal residues in the side chains of the branched area (Yang et al., 2010). Therefore, plum is supposed to be a source of biologically active pectin.

The aim of the present study was to isolate and characterise pectin from fresh plum, using simulated gastric fluid and to assess its anti-inflammatory and antioxidant activities *in vitro*.

2. Materials and methods

2.1. Isolation of polysaccharides from plums

Plums, *Prunus domestica* L., were purchased at a local market and the stones were removed. Plum fleshes, including the skin (3 kg), were chopped into small pieces and homogenised in 1 l of distilled water, using a domestic blender, for 5 min at medium speed. The obtained mixture was added to a solution of simulated gastric fluid (19 l, pH 1.5). The simulated gastric fluid was composed of HCl (37 mM), NaCl (37 mM), KH_2PO_4 (4.6 mM), $CaCl_2$ (1.1 mM), KCl (5.2 mM), and pepsin (0.50 g/l) (Corcoran, Stanton, Fitzgerald, & Ross, 2007). The extraction was performed at 37 °C for 4 h. The extract was filtered and centrifuged with a flow centrifuge at 10,000 rpm (Avanti J-25I, Beckman Coulter) for 1 h, at 4 °C. The supernatant was collected and passed through an ultrafiltration cell with membrane pores of 300 kDa (Vladisart, Russia). The ultrafiltration was performed until all of the chemicals used during isolation were removed. The residual material, with a molecular mass above 300 kDa, was collected and lyophilised to yield pectin designated as PD (13.2 g).

2.2. General analytical methods

The monosaccharide composition was determined after the hydrolysis of the polysaccharides, where 2 M aqueous trifluoroacetic acid (TFA) (1 ml) containing myo-inositol (0.5 mg/ml), was added to a weighed portion (3–5 mg) of polysaccharide fractions. The mixture was incubated for 5 h at 100 °C. The excess acid was removed by the repeated evaporation of the hydrolysate to dryness with methanol. The mixture of monosaccharides was transformed into their alditol acetates and identified by gas–liquid chromatography (GLC) on a Varian 450-GC chromatograph (Varian, USA) equipped with a flame-ionisation detector. GLC was run on a VF-5 ms capillary column (Varian, USA; 0.25 mm, 30 m) using the temperature regime of 175 °C (1 min) to 250 °C (2 min), at a rate of 3 °C/min.

The uronic acid content was determined by a reaction with 3,5-dimethylphenol in the presence of concentrated sulphuric acid. A calibration plot was constructed for D-galacturonic acid (D-GalA), and photocolourimetry was carried out at 400 and 450 nm.

The protein concentration was calculated using the Lowry procedure and using bovine serum albumin (BSA) to generate a standard curve.

Spectra were measured on an Ultrospec 3000 spectrophotometer (Pharmacia Biotech, England).

The solutions were concentrated with a Laborota 4002 rotary evaporator (Heidolph, Germany), under a reduced pressure at 40–45 °C. The samples were centrifuged with a 6 K 15 centrifuge (Sigma, Germany) at 5000–11,000*g* for 10–20 min and then lyophilised in the frozen state using a VirTis lyophiliser (The VirTis company, USA) under a constant vacuum of <10 mTorr at −65 °C. Throughout the 6 h lyophilisation process, the flack containing the samples were periodically detached from the vacuum and weighed to assure a constant mass. The samples were dried further if their mass changed by more than 5% during the last 2 h of lyophilisation.

NMR spectra were recorded using a Bruker DPX-500 spectrometer (Germany) for 3–5% solutions of polysaccharides in D_2O, at 30 °C (internal standard – acetone, δ_H 2.225 ppm, δ_C 31.45 ppm). The number of scans was 20,000. The two-dimensional spectra were run using standard Bruker procedures.

The molecular weight of the polysaccharide samples (3 mg/ml) was determined by high-performance liquid chromatography (HPLC). The chromatographic system for the analysis included a LC-20AD pump (Shimadzu, Japan), a DGU-20A3 degasser (Shimadzu, Japan), a CTO-10AS thermostat (Shimadzu, Japan), a RID-10A refractometer (Shimadzu, Japan) as a detector, a Shodex OH-pak SB-804 HQ column (7.6 mm × 30 cm) and a Shodex GS-2G 7B pre-column (7.6 mm × 5 cm) (Shimadzu, Japan). The HPLC experiments were performed at 40 °C, with a flow rate of 0.3 ml/min. The column was equilibrated with 0.15 M sodium chloride containing 0.02% NaN_3 as a preservative, and the elution was carried out with the same solution. Deionised water supplied by the Simplicity 185 Millipore water purification system (France) was used to prepare the eluents and samples. Pullulans (Fluka, Germany) (1.3, 6, 12, 22, 50, 110, 200, 400 and 800 kDa) were used as standards. The weight-average molecular weight (Mw), the number-average molecular weight (Mn) and the polydispersity index (Mw/Mn) were calculated by the LCsolution GPC program (LCsolution, version 1.24 SP1). The samples and standards were injected in duplicate.

2.3. Separation of the polysaccharide fraction PD by anion-exchange chromatography

The polysaccharide fraction PD (79.9 mg) dissolved in 0.01 M NaCl (7 ml) was fractionated with a DEAE-cellulose (OH^- form) column (3.5 × 41 cm). The column was eluted by a stepwise elution of NaCl solutions (0.01, 0.1, 0.2, 0.3 and 1.0 M NaCl) at a flow rate of 1.0 ml/min. The obtained fractions were combined based on the total sugar content quantified using the phenol–sulphuric acid