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Evaluation of physicochemical characteristics and antioxidant property of *Prunus avium* gum exudates

Hossein Shabani [a], Gholamreza Askari [a,*], Kambiz Jahanbin [b], Faramarz Khodaeian [a]

[a] Transfer Phenomena Laboratory (TPL), Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, University Campus of Agriculture and Natural Resources, University of Tehran, Karaj 31587-77871, Iran
[b] Department of Food Science and Technology, School of Agricultural Engineering, Shahrood University of Technology, Iran

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ABSTRACT

In this study some physicochemical properties and elemental analysis of *Prunus avium* gum exudates were investigated. The gum studied had, on average, 75.14% carbohydrate, 11.3% uronic acids, 1.11% protein, 7.53% moisture content (w.b.) and 3.12% ash. Measured values for the angle of repose, Carr's index and Hausner ratio showed the good flow ability for the gum powder. The viscosity of 1% aqueous solution of the gum exhibited a Newtonian type of flow and with pH reduction the swelling index was increased. The average molecular weight of the main polysaccharide fraction was about 1.46×10^5 Da (146 kDa). GC analysis showed that the main polysaccharide was composed of four kinds of neutral monosaccharides, namely mannose (Man), arabinose (Ara), galactose (Gal) and xylose (Xyl) with a relative molar ratio of 1.0:14.7:7.1:2.4. FTIR analysis showed the presence of carboxyl and hydroxyl groups and glycosidic linkage. The antioxidant activity of the gum was evaluated by determining DPPH scavenging and total phenolic contents which showed poor antioxidant property.

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1. Introduction

Natural gums are obtained as exudates from different tree species. With wide multiplicity of uses, they have various and exclusive physicochemical properties [1]. For many years, gum exudates have been used in food applications, including, emulsification, thickening and stabilization of processed foods [2].

The exudate gums have a different component profile. They are composed mainly of polysaccharides with diverse structures. The composition of gum polysaccharides depends on species and cultivar of the plant [3]. The composition of monosaccharaides, types and patterns of linkage, chain shapes, side branches and degree of polymerization govern the structural features of polysaccharides. These features determine the physical properties of gums including solubility, flow behavior, gelling potential and interfacial properties [4].

The Biological activity of this compounds for example anticancer, immunomodulation and antioxidant properties have been reported in several investigations. [5–10]. The presence of galactose, arabinose, rhamnose, uronic acids, galacturonic acid, protein, Ca and Mg as the main structural constituents of plant gum exudates, have been reported [11].

Species of the genus *Prunus* L. are mostly found in the northern hemisphere. Most of the species occur in semiarid climates [12]. *Prunus* is economically important because they are sources of fruits, oil, timber, and ornamentals [13]. The gum exudates of cherries (*Prunus cerasus* L.), ornamental Japanese cherries (*Prunus yedoensis*), plums (*Prunus domestica* L.), apricots (*Prunus armeniaca* L.), Japanese apricots (*Prunus mume* Sieb. et Zucc.), peaches (*Prunus persica* Batsch) and almonds (*Prunus amygdalus* Batsch.) have been studied [3].

Šcerbuchin, Rosík and Kubala [14] showed that acidic polysaccharide of *Prunus avium* L. tree gum (var. *duracina* L.), composed of D-glucuronic acid, 4-0-methyl-D-glucuronic acid, D-galactose, D-mannose, L-arabinose, and D-xylose. It has been reported that the exudates gum polysaccharide of *P. avium* and *P. cerasus*, have the compact internally cross linked structure [15].

The aims of this work were to investigate the physicochemical, functional and antioxidant properties of *Prunus avium* exudate gums.

2. Materials and methods

The gum exudates of sweet cherry trees (*Prunus avium*) were collected from Marand, East Azerbaijan Province (Iran) during the

* Corresponding author.
E-mail address: iraskari@ut.ac.ir (G. Askari).

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month of July–September (2015). The gum was dried in oven at 40 °C, grounded in to powder and passed through sieve no. 50. DEAE-Cellulose A52 and Sephadex G-100 were purchased from the Pharmacia Co. (Uppsala, Sweden). Dextrans of different molecular weights, bovine serum albumin (BSA) and pure monosaccharide standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All the used chemical materials were of analytical grade.

2.1. Polysaccharide purification

The polysaccharides of gum were purified according to the method described by Pachuau, Lalhlenmawia and Mazumder [16] with slight modification. Briefly, crude gum powder was boiled with 80% ethanol with a ratio of 1:4 (w/v) to enzymes deactivation and remove low molecular weight carbohydrates and coloring substances. It was dispensed in deionized water and gently stirred overnight in a magnetic stirrer. The gum solution was then centrifuged at 12000*g* to separate any undissolved matters. The gum solution was then filtered through Whatman No.1 filter paper and precipitated with 3 volumes of 2-propanol, collected and air dried. They were passed through sieve no. 85, stored in airtight container for elemental analysis.

2.2. Physicochemical characterization

2.2.1. Determination of ash content

The ash content was determined by following the method of Yebeyen, Lemenih and Feleke [17]. About 1 g of the gum powder was first heated on a burner in air to remove its smoke. Then, it was burned in a furnace at 550 °C. The ash content was calculated as follow:

$$\text{Ashcontent}(\%) = 100 \times (\text{weightofash}/\text{weightofgumpowder})$$

2.2.2. Moisture contents (percent loss on drying)

About 1 g of ground gum powder sample was weighed and oven dried at 105 °C for 5 h. Oven dry weight was taken after allowing the sample to cool in a desiccator before reweighing. The Moisture content was expressed as a percentage of the weight loss from the original weight [17].

2.2.3. Determination of viscosity

Viscosity of 1% sample solution in distilled water was determined at 25 °C using the Brookfield viscometer (Model DV-E, Spindle ULA). Effect of shear rate (rpm) on the viscosity was studied by varying the shear rate from 300 to 6000 rpm [9].

2.2.4. Determination of angle of repose

A fixed height funnel fitted at the height of 10 cm from the base was used to measure the angle of repose (the funnel is 60°, 4.8 cm in diameter, 0.5 cm internal stem diameter with 10 cm stem length). A pile was formed at the base by flowing about 20 g of the dried powder (sample) through the funnel into the base. The angle of repose was then calculated as follows:

$$\text{Angleofrepose} = \tan^{-1}(h/r)$$

Where h and r are the height and radius of the pile, respectively [9].

2.2.5. Compressibility index

Bulk and tapped densities were used to determine the compressibility index of the sample. About 2 g of the gum powder was taken into a 10 ml graduated measuring cylinder and the initial volume (V_0) was recorded. The cylinder was then tapped 100 times using the bulk density apparatus to achieve a final volume (V_f). The experiment was performed in triplicate and is reported as means and standard deviations. The bulk density was calculated from the initial volume and tapped density from the final volume. Carr's index and Hausner ratio were then determined by the following equations [9]:

$$Carr's \quad index = \frac{tapped \quad density - bulk \quad density}{tapped \quad density} \times 100$$

$$Hausner \quad ratio = \frac{bulk \quad density}{tapped \quad density}$$

2.2.6. True density and porosity

The true densities (ρ_{true}) of gum powders were determined by the liquid displacement method using xylene as the immersion fluid, and computed according to the following equation:

$$\rho_{\text{true}} = \frac{w}{[(a+w)-b]} \times SG$$

Where w is the weight of powder, SG is the specific gravity of solvent, a is the weight of bottle + solvent and b is a weight of bottle + solvent + powder [18]. The porosity of the test powders was derived from the values of the true and tapped densities fitted into the following equation:

$$Porosity = \left[1 - \frac{tapped \ density}{true \ density}\right] \times 100$$

2.2.7. Swelling index

Swelling index of the gum powder was determined according to the WHO method [19] with a slight modification. Briefly, about 1 g of the fine sample, was introduced into the three 25 ml glass-stoppered measuring tubes. Then, 25 ml of distilled water, 0.1 N HCl and phosphate buffer (pH = 7.4) was added to each tube and the mixture was shaken every 10 min for 1 h and allowed to stand for 24 h at room temperature. The swelling index was calculated using the measured volume that occupied by the sample.

2.2.8. Foaming index

About 1 g of the sample was transferred into a 500 ml conical flask containing 100 ml of boiling water. The moderate boiling temperature was maintained for 30 min, then It was cooled and filtered. The decoction was poured into 10 glass-stoppered test tubes in successive portions of 1, 2, 3 ml, up to 10 ml the volume of liquid in each tube was adjusted to 10 ml with water. The tubes were thoroughly shaken in for 15 s at two shakes per second. It was allowed to stand for 15 min the foam height was measured [19].

2.2.9. Total carbohydrates, uronic acid and protein contents

Phenol–sulphuric acid method was used to determine of the total carbohydrate content of the gum. D-glucose was taken as standard [20]. The uronic acid content was also analyzed by carbazole method, taking D-galacturonic acid as the standard [21]. The absorbance was measured by the UV–vis spectrophotometer (SP-UV 500DB) at 490 and 535 nm for total carbohydrates and uronic acids, respectively. Crude protein content was measured using the Kjeldahl method and considering 6.25 as the conversion rate of nitrogen to crude protein [22].

2.3. DPPH radical scavenging activity

DPPH activity test was Carried out according to the Blois method [23] with minor modifications. Butylated hydroxyl anisole was taken (BHA) as the reference standard for comparison. The series of sample solutions with different pre-determined concentrations gum powder was produced by dissolving the gum powder in water. In all cases 0.5 ml of DPPH solution in methanol (0.1 mM) was mixed

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