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# Isolation of bioactive polysaccharide from acorn and evaluation of its functional properties

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ABSTRACT

The aim of this study was to evaluate the prebiotic potential and some functional properties of polysaccharides isolated from acorn fruit. The FTIR spectrum of isolated acorn polysaccharide (IAP) showed the typical bands corresponding to sugars and polysaccharides. The IAP was resistant to simulated acidic and enzymatic digestion even more than Inulin (In). The prebiotic activity, which was tested using IAP as a carbon source, showed significant increase in the growth and viability of *Lactobacillus plantarum* A7 (probiotic). Viability of *Lactobacillus plantarum* A7 in IAP and In supplemented media was stable even after 72 h, whereas in glucose supplemented medium, bacterial growth showed a notable decrease after 24 h. Lipid absorption capacity (LAC) and water holding capacity (WHC) of IAP were $5.44 \pm 0.02$ (g oil/g DM) and $4.33 \pm 0.03$ (g water/g DM), respectively, which were comparable to some dietary fibers and were more than In. IAP scavenged DPPH radicals by 82.24%. IAP was found to have a high scavenging ability compared to the reference prebiotic (In), giving a scavenging ability of about 20%. Therefore, due to prebiotic capability, high WHC, LAC and good antioxidant activity, IAP can be a suitable candidate for technological applications and health improving effects in functional food.



## 1. Introduction

In recent years, natural plant compounds have received considerable attention from scholars because of health benefits from their relevant bioactive constituents [1]. Health improving claims and preventive or therapeutic effects of bioactive compounds correspond to polyphenols, proteins, lipids, vitamins, polysaccharides and other constituents [2]. Polysaccharides isolated from plants, mushrooms or seaweeds have emerged as an important class of bioactive natural products [1–4]. They exhibit various biological effects including antitumor, anticoagulant, antiviral, immune modulatory and anti-inflammatory, anti-diabetic, hepato-protective, hematopoietic, anti-oxidant or free radical scavenging activity and anti-lipidemic effects [5–9]. Some studies are now focused on the prebiotic potential of polysaccharides extracted from natural sources such as artichoke [10] seaweeds [11] bamboo shoot [12] edible burdock [13]. Prebiotics are defined as non-digestible ingredients that beneficially affect host's health by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon [14]. Prebiotics alone or with probiotic bacteria (symbiotic) can exert health improving effects by influencing the formation of blood glucose, increasing mineral absorption, reducing the cholesterol and serum lipid level, reducing the risk of colon cancer, artroscelrosis and immunomodulatory activities [15–20]. Additionally, due to their porous matrix structure, polysaccharides could absorb and hold oil or water in their matrix, and therefore could be valuable additives in food and drug industries because of their rheological properties like gelling and a thickening agent for stabilizing and modifying the texture of food or drug [21,22]. In addition, these properties are very important for helping to prevent or control obesity and abnormal blood lipid profiles [21,23,24]. Therefore, isolation of bioactive polysaccharides from new sources and evaluation of their functional property for technological applications or popular food formulation have become a hot research spot. Oak or acorn fruit is indigeneous to Italy, Spain, Iran, North America and India [25]. The species of oak belonged to *Quercus* genus and are classified into the Fagaceae family including about 200 species. Different species of oak grow in Iran, but four species of oak are found in the Zagrossian region. *Quercus branti* is the most famous and dominant

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species in the Zagros mountain chain in Iran, and this study was carried out on this species [25]. The use of acorn in local diet and empirical treatment of some human diseases such as diarrhea is not something new to Iranians. Due to its anti-nutritional substances, bitter constituents, wooden texture and astringent taste, acorn is scarcely used in normal diet [25]. However, its widespread availability and empirical use for medical purposes suggest some possibilities of using acorn in food and drug industries. Therefore, this study was carried out to isolate and characterize of acorn polysaccharide and evaluate its prebiotic and functional potential.

## 2. Material and methods

### 2.1. Materials

Acorn fruit was obtained from a local market in Baghmalek, Iran. All chemical reagents, enzyme and media used in this study were purchased from the Merck Chemical Company (Darmstadt, Germany) and Sigma-Aldrich. Inulin (In) was obtained from Sensus, USA.

### 2.2. Extraction

Acorn fruit was ground in a blender and then defatted with three volumes of 80% ethanol at 60 °C for 8 h. Ethanol extract was then separated from defatted ground acorn through filtration, and the defatted ground acorn was air dried. The pretreated sample was extracted by hot water bath (Memmert, Germany) at 90 °C for 3 h. Then, the extract containing water-soluble polysaccharide was centrifuged (Sigma k-16, Germany), to remove insoluble residues (2000 × *g* for 10 min, at 20 °C). The solution was concentrated in a rotary evaporator (BUCHI 011, Switzerland) under reduced pressure at 60 °C. The water extraction solution was separated from debris fragments (8000 rpm for 10 min, at 20 °C). The water extraction solutions were precipitated by addition of three volumes of 80% ethanol at 4 °C for 48 h. The precipitate was collected by centrifugation (2000 × *g*, 10 min, at 4 °C) and washed three times with ethanol, and then the extract was distilled by a rotary evaporator to remove the residual ethanol in it and finally lyophilized in a freeze dryer (Christ alpha-1-4, Germany) [12,22,26]. The purity of crude polysaccharide is calculated by phenol-sulphuric method [22].

### 2.3. FTIR spectroscopy

One mg of dried polysaccharide was weighed and mixed with 100 mg of potassium bromide. The mixture was made into a pellet for FTIR measurement in the frequency range of 4000–400 $cm^{-1}$ [3]. The IR specta of the polysaccharides were determined using a Fourier transform IR spectrophotometer (JASCO, Japan).

### 2.4. Resistance to acidic and enzymatic digestion

*In vitro* resistance of polysaccharide to acidic and enzymatic digestion was studied based on Jain et al. [27] and was compared with Inulin (In) as a typical prebiotic reference. Simulation of gastric intestinal transit conditions was carried out using different dissolution media. Simulated gastric fluid (SGF) pH 1.2 consisting of NaCl (2 g), HCl (7 ml) and pH level was adjusted to 1.2 ± 0.5. Simulated intestinal fluid (SIF) pH 7.4 included $KH_2PO_4$ (6.8 g), NaOH (190 ml) and α-amylase (2 unit/ml) (Sigma-Aldrich). Simulation of the mixture of simulated gastric and intestinal fluid (SMF) of pH 4.5 was performed by mixing SGF and SIF in a ratio of 39:61. The dissolution studies were carried out in 900 ml of dissolution medium, which was stirred at 100 rpm at 37 °C [27]. A sample was taken at 1, 2, 3 h to determine the percentage of hydrolysis, which was calculated based on reducing liberated sugar and total sugar content according to DNS (3,5-Dinitrosalicylic acid) and phenol-sulfuric acid method [28,29].

### 2.5. Effect of extracted polysaccharide on probiotic growth

*Lactobacillus plantarum* A7 obtained from the microbial collection of Food Science and Technology Department, Isfahan University of Technology (Isfahan, Iran). The probiotic properties of *Lactobacillus plantarum* A7 were previously reported by Mirlohi et al. [30,31]. To obtain sufficient cells for each inoculum, cultivation was performed on MRS broth (Merck, Germany) at 37 °C for overnight. MRS free carbohydrate supplemented with 2% isolated acorn polysaccharide (IAP), a reference prebiotic (In) and glucose (Glu), were used for the growth of probiotic. The culture was incubated at 37 °C for 72 h. Samples were taken at 24, 48, 72 h for bacterial enumeration and pH. To evaluate the metabolization of the extracted polysaccharide through probiotic, the effect of its different concentrations on pH changes was studied [32].

### 2.6. Functional properties

Water holding and lipid absorption capacity of the IAP and Inulin (In) (commercial prebiotic) were determined as reported by Carvalho et al. (2009) [23]. 30 ml of distilled water was added to the sample (1 g) in a centrifuge tube. The sample was agitated and left in room temperature. Then, the mixture was centrifuged (12,000 × *g*, 20 min) (Sigma k-16, Germany), The supernatant was discarded and the residue was weighed. The WRC was expressed as g of water $g^{-1}$ dry sample. For LAC, samples (3 g) were added to sunflower oil (18 ml), left overnight in room temperature (25 °C), centrifuged at 1500 × *g* for 10 min and the supernatant was disposed. LAC was expressed as grams of oil $g^{-1}$ dry sample [23].

### 2.7. Antioxidant property

The ground IAP (0.2 g) was mixed with 5 ml of methanol and was stirred vigorously using shaker incubator (200 rpm, 3 h) (IKA® KS 4000 I control, Germany), and then it was centrifuged at 3000 rpm for 20 min (Sigma k-16, Germany). The obtained supernatant was analyzed for its DPPH radical (2, 2-Diphenyl-1-picrylhydrazyl) scavenging. In the second series, IAP (0.025–0.2 g) was mixed with 5 ml of methanol and was stirred vigorously for 3 h, and then it was centrifuged at 3000 rpm for 20 min. The obtained supernatant was analyzed for its DPPH radical scavenging.

#### 2.7.1. DPPH radical scavenging activity

An aliquot of methanol extracts was measured in terms of hydrogen-donating or radical scavenging ability using the stable radical DPPH (2, 2-Diphenyl-1-picrylhydrazyl). Then, 500 μl of the extract was quickly added to 5 ml of a 0.1 mM methanol solution of DPPH. After incubating this solution in room temperature for 30 min, the absorbance was read using spectrophotometer (UV-VIS 2100, USA) against a blank at 517 nm. Ascorbic acid (As) (1000 μM) and In (40 mg/ml) were used as a positive control and reference prebiotic under the same assay conditions. The assay was carried out in triplicate [4,33]. The DPPH radical scavenging activity was calculated according to the following:

$$\text{Scavenging activity\%} = \frac{(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{blank}}} \times 100$$

where $\text{Abs}_{\text{blank}}$ is the absorbance of the control and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample.