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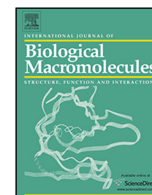

Water-soluble polysaccharides from agro-industrial by-products: Functional and biological properties

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ABSTRACT

Water-soluble polysaccharides were isolated from almond (AWSP) and pistachio (PWSP) juice processing by-products. Their chemical and physical characteristics were determined using NMR and Infrared spectroscopic analysis. The complexities of the spectra reflected the heterogeneity of these polysaccharides. The ACE inhibitory activities ($IC_{50\ AWSP}$ = 2.81 mg mL^{-1} and $IC_{50\ PWSP}$ = 2.59 mg mL^{-1}) and antioxidant properties of AWSP and PWSP were investigated based on the DPPH radical-scavenging capacity assay ($IC_{50\ AWSP}$ = 2.87 mg mL^{-1} and $IC_{50\ PWSP}$ = 1.61 mg mL^{-1}). Reducing power, β-carotene bleaching inhibition ($IC_{50\ AWSP}$ = 4.46 mg mL^{-1} and $IC_{50\ PWSP}$ = 3.39 mg mL^{-1}), and ferrous chelating assays ($IC_{50\ AWSP}$ = 0.22 mg mL^{-1} and $IC_{50\ PWSP}$ = 0.19 mg mL^{-1}) were also performed. The findings revealed that water-soluble polysaccharides exhibited antioxidant and antihypertensive activities. AWSP and PWSP showed excellent interfacial concentration-dependent properties. Overall, the results suggested that both AWSP and PWSP are promising sources of natural antioxidants and ACE inhibitory agents and could, therefore, be used as alternative additives in food, pharmaceutical and cosmetic preparations.

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1. Introduction

Large volumes of wastes are produced by the agricultural and food-processing industries annually worldwide. Those wastes, including coffee pulp from the coffee industry, bagasse and peels from the beverage and juice industries, and husks from the cereal industry, cause serious environmental and disposal problems [1]. Due to their large availability and rich composition, those residues have received increasing attention as renewable resources from which several useful biological products can be derived [2]. Recent research indicates that those agro-industrial by-products offer cheap and efficient alternative substrates and support materials for use in various industries, including the food and medical industries, and can, therefore, help reduce the costs and environmental concerns associated with their disposal.

Free radicals, chemical reactions, and several redox reactions of various compounds may cause DNA damage, protein oxidation, and lipid peroxidation in living cells [3]. Antioxidants have often been used in industrial processing systems to reduce the damage caused to the human body and prolong the storage stability of foods. Since several synthetic antioxidants, such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), have often been reported to be associated with harmful health effects, including carcinogenesis and liver damage, there has been increasing interest in finding alternative antioxidants from natural origins [4,5]. Several studies have, therefore, expressed the need for the development and use of effective natural antioxidants to protect the human body from free radicals and reduce the risk of several diseases, such as cancer, arthritis, heart disease, and the aging [6].

Polysaccharides are present in large quantities in nature and have multiple applications. Several polysaccharides have been obtained from bacterial, fungal, plant, and marine organisms. As naturally occurring biological constituents, these high molecular weight polymers are highly appreciated for their multipurpose therapeutic properties, i.e., immune-modulating, antitumor, anti-inflammatory, antioxidant and anti-pathogenic activities [7–11]. Polysaccharides have long been used in the food and medical industries. They are nontoxic and biocompatible polymers that play important roles as dietary free radical scavengers for oxidative damage prevention. In general, many plant polysaccharides have exhibited strong antioxidant properties and can be explored as novel promising antioxidants. Polysaccharides extracted from plants are interesting additives for several industries, particularly for the food industry, because consumers prefer natural

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ingredients. Accordingly, intensive efforts have recently been channeled toward the search for novel natural sources of polysaccharides for application in different industries.

Considering the promising opportunities agro-industrial by-products might offer for the development of new biologically active and functional components for application in the food and pharmaceutical industries, the present study aimed to characterize water-soluble polysaccharides (WSP) isolated from the almond (AWSP) and pistachio (PWSP) juice processing by-products. Their antihypertensive and antioxidant activities were also investigated.

2. Materials and methods

2.1. Reagents

Chemicals required for the assays including 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), β-carotene, L-ascorbic acid and Angiotensin converting enzyme (ACE) from rabbit lung, hipouril histidine leucine (HHL) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, namely potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, sodium hydroxide, $FeCl_3$, ferrozine and other solvents, were of analytical grade.

2.2. Materials

The pistachio (*Pistacia vera*) and bitter almond (*Prunus amygdalus* var. *amara*) by-products were obtained in fresh condition from a juices processing plant "TiGrain" located in Sfax, Tunisia. The samples were packed in polyethylene bags, placed in ice with a sample/ice ratio of approximately 1:1 (w/w) and transported to the research laboratory within 30 min. The samples were stored in sealed plastic bags at −20 °C.

2.3. Extraction of water-soluble polysaccharides

Water-soluble polysaccharides, from pistachio (PWSP) and bitter almond (AWSP) by-products were recovered by the method of Liu et al. [12] The dry sample was pre-extracted with six volumes of 95% ethanol at room temperature to remove small molecules. The dry residue was extracted twice with twenty volumes of deionized water at 90 °C for 4 h with stirring. The extract was combined and filtered. The filtrates were evaporated under vacuum. Subsequently, the concentrated liquids were precipitated with 95% (v/v) ethanol at 4 °C for 24 h, and then centrifuged (4500 × *g*) for 15 min using a refrigerated centrifuge (Hettich Zentrifugen, ROTINA 380R, Germany). The final precipitate was re-dissolved in double-distilled water. After removed the proteins, the water phase was dialyzed against running tap water for 2 days, and then against distilled water for another day. The dialysate was concentrated by rotary evaporation under reduced pressure (Rotary evaporator, Heidolph, Germany) and freeze-dried at −50 °C and 121 mbar (CHRIST, ALPHA 1-2 LD plus, Germany) to obtain water-soluble polysaccharides (WSP).

2.4. Determination of chemical composition

The moisture and ash content were determined according to the AOAC standard methods 930.15 and 942.05, respectively [13]. Total nitrogen content was determined by using the Kjeldahl method. Crude protein was estimated by multiplying total nitrogen content by the factor of 5.18 for bitter almond [14] and 6.25 for pistachio [15]. Crude fat was determined gravimetrically after Soxhlet extraction of dried samples with hexane. All measurements were performed in triplicate. Total carbohydrates were determined by the phenol-sulphuric acid method [16].

2.5. ^{13}C CP/MAS-NMR spectroscopic analysis

Chitin structural analysis was carried out by ^{13}C NMR with CP/MAS technique (cross-polarization, magic-angle-spinning) using a BRUKER-ASX300 instrument. NMR spectra were recorded at a ^{13}C frequency of 75.5 MHz (field of 7.04 T). CP/MAS sequence was used with the following parameters: the ^{13}C spin lattice relaxation time was 5 s, powdered samples were placed in an alumina rotor used for the double air-bearing-type MAS system and spun as fast as 8 kHz. Contact time was 8 ms.

2.6. Infra-red spectroscopic analysis

The absorption spectra of the samples were obtained using FTIR spectroscopy (Analect Instruments fx-6 160). The FTIR spectra of the prepared materials were recorded between 400 and 4000 cm^{-1} in a NICOET spectrometer. The transmission spectra of the samples were recorded by using the KBr pallet containing 0.1% of sample.

2.7. Functional properties

2.7.1. Water-holding capacity

Water-holding capacity was measured by a partially modified method of Lin et al. [17] WSP (0.5 g) was placed in a centrifuge tube and weighed (tube with WSP). Distilled water (50 mL) were added, and held at room temperature for 1 h. The WSP solutions were mixed for 5 s every 15 min. The WSP solutions were then centrifuged at 8000 rpm for 20 min using a refrigerated centrifuge (Hettich Zentrifugen, ROTINA 380R, Germany). The upper phase was removed and the centrifuge tube was drained for 30 min on a filter paper after tilting to a 45° angle. Water-holding capacity was calculated as the weight of the contents of the tube after draining divided by the weight of the dried WSP, and expressed as the weight % of dried WSP.

2.7.2. Fat-binding capacity

Fat-binding capacity was measured by a partially modified method of Lin et al. [17]. WSP (0.5 g) was placed in a centrifuge tube and weighed (tube with WSP). Ten milliliters of soybean oil were added, and held at room temperature for 1 h. The WSP solutions were mixed with vortex mixer for 5 s every 15 min. The WSP solutions were then centrifuged at 5000 × *g* for 20 min using a refrigerated centrifuge (Hettich Zentrifugen, ROTINA 380R, Germany). The upper phase was removed and the centrifuge tube was drained for 30 min on a filter paper after tilting to a 45° angle. Their capacities were calculated as the weight of the contents of the tube after draining divided by the weight of the dried WSP, and expressed as the weight% of dried WSP.

2.7.3. Emulsion properties

WSP suspensions (20 mL) at different concentrations (0.5, 1, 2 and 4%, w/w) were mixed with 2 mL of soybean oil using a Moulinex R62 homogenizer (Organotechnie, Courneuve, France) for 1 min and then centrifuged for 10 min at 8000 × *g*. The emulsion capacity (EC) was calculated as follows:

$$EC = \left(\frac{V_f}{V_i}\right) \times 100$$

where V_f is the emulsion volume, and V_i is the total volume. The emulsion stability (ES) was determined in an emulsion that had been incubated at room temperature for 30 min and centrifuged

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