Food Chemistry 216 (2017) 355–364

Contents lists available at ScienceDirect

Food Chemistry

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

Optimization of polysaccharides extraction from watermelon rinds: Structure, functional and biological activities

Molka Ben Romdhane [a], Anissa Haddar [a,*], Imen Ghazala [a], Khawla Ben Jeddou [a], Claire Boisset Helbert [b], Semia Ellouz-Chaabouni [a]

[a] Enzymes & Bioconversion Unit, National School of Engineers, PB 1173, 3038 Sfax, Tunisia
[b] Service de Chromatographie Purification et analyse de polysaccharides CERMAV-CNRS, 601 rue de la Chimie, 38041 Grenoble cedex 9, France

ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 29 May 2016
Accepted 19 August 2016
Available online 21 August 2016

Keywords:
Watermelon rinds
Polysaccharides
ACE inhibitory activity
Antioxidant activities
Functional proprieties

ABSTRACT

In the present work, optimization of hot water extraction, structural characteristics, functional properties, and biological activities of polysaccharides extracted from watermelon rinds (WMRP) were investigated. The physicochemical characteristics and the monosaccharide composition of these polysaccharides were then determined using chemical composition analysis, Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM) and gas chromatography-flame ionization detection (GC-FID). SEM images showed that extracted polysaccharides had a rough surface with many cavities. GC-FID results proved that galactose was the dominant sugar in the extracted polysaccharides, followed by arabinose, glucose, galacturonic acid, rhamnose, mannose, xylose and traces of glucuronic acid.

The findings revealed that WMRP displayed excellent antihypertensive and antioxidant activities. Those polysaccharides had also a protection effect against hydroxyl radical-induced DNA damage. Functional properties of extracted polysaccharides were also evaluated. WMRP showed good interfacial dose-dependent proprieties. Overall, the results suggested that WMRP presents a promising natural source of antioxidants and antihypertensive agents.

1. Introduction

Watermelon (*Citrullus lanatus*) presents a fruit of large economic value, with an international production estimated at around 93,700 million tons (Tarazona-Díaz, Viegas, Moldao-Martins, & Aguayoa, 2011). This product is enjoyed by a lot of people worldwide as a fresh fruit, due to its huge health benefits as well as low calorie intake, high nutritional value and thirst-quenching ability. This popular fruit presents also an excellent source of vitamins (A, B, C and E), mineral salts (K, Mg, Ca and Fe), specific amino acids (arginine and citrulline) and a large variety of antioxidants like phenolics and carotenoids (Perkins-Veazie, Collins, & Clevidence, 2007). Watermelon biomass is composed by three major components which are the flesh, seeds and rinds. Flesh presents about 68% of the totality of the fruit, the seeds about 2%, and the rinds approximately 30% (Kumar, 1985). Rinds present one of the main solid residues engendered by many restaurants and cottage fruit juice producers. It is dumped arbitrarily into the environment and thus representing environmental challenges. The reutilization of the rinds as valuable products still limited because of the restriction of the studies focusing on the potential conversion of this residue to other products. Consequently, it becomes necessary to develop a new environmentally friendly solution for the management of this problem.

Actually, chemical reactions, free radicals, and some redox reactions of different compounds may be a source of oxidative damage of macromolecules in living cells (Kil et al., 2009). This destruction is related to cancer, diabetes mellitus, neurodegenerative and inflammatory illnesses. To moderate the damage caused to the human body and to increase the foods storage stability, antioxidants have frequently been used in industries. The use of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), is limited since these antioxidants have been suspected to be associated with dangerous health effects, including liver damage and carcinogenesis (Lin & Tang, 2007). Natural sources of polysaccharides can be, however, explored as an alternative antioxidant. Several research studies have revealed the importance of the development of natural antioxidants for the protection of the human body from free

* Corresponding author.
E-mail address: anissa_ing@yahoo.fr (A. Haddar).

http://dx.doi.org/10.1016/j.foodchem.2016.08.056
0308-8146/

radicals and allowing then the reduction of the risk of various diseases like cancer, heart disease, arthritis, and the aging (Andrade, Gil, Breitenfeld, Domingues, & Duarte, 2009).

Hypertension presents one of the major risk factors that contribute to the pathogenesis of cardiovascular illnesses (Lavoie & Sigmund, 2003). It is predicted that more than 500 million people will be diagnosed with hypertension by 2025 (Ibrahim & Damasceno, 2012). Angiotensin-I converting enzyme (ACE), a key regulator in the renin–angiotensin system, plays a crucial role in the conversion of angiotensin I to angiotensin II, a vasoconstrictive peptide responsible for elevated blood pressure. Moreover, ACE also can inactivate bradykinin, a vasodilator in the blood pressure-reducing kallikrein–kinin system (Skeggs, Kahn, & Shumway, 1956). Accordingly, ACE inhibition has been considered as a practical approach to reduce blood pressure (Aluko, 2015; Ondetti & Cushman, 1977). Despite the effectiveness of synthetic ACE inhibitors as antihypertensive drugs, they can incite undesirable side effects for extended administration, such as cough, allergic reactions, skin rashes, taste disturbances, renal impairment and angio-neurotic edema (Acharya, Sturrock, Riordan, & Ehlers, 2003; Alderman, 1996). As a result, there is an increasing attention in identifying active compounds from natural sources that can be used as potential inhibitors of ACE with fewer adverse side effects.

In recent years, several scientific studies had revealed that polysaccharides exhibited ACE inhibitory effect, such as those extracted from chickpea (Mokni Ghribi et al., 2015), almond and pistachio (Sila et al., 2014). Some peptides had also presented an ACE inhibitory activity, like those derived from chickpea (Chang & Alli, 2012) and cereal (Cavazos & Gonzalez de Mejia, 2013).

In fact, many research proved that polysaccharides have various biological activities, like anti-viral, antioxidant, anti-tumor, anticoagulant, anti-inflammatory and immunostimulatory (Chen et al., 2011). They present also biocompatible and nontoxic polymers that can be considered as important dietary free radical scavengers for preventing oxidative damage. Moreover, many polysaccharides extracted from plants have shown good antioxidant abilities and may be explored as new promising antioxidants. Consequently, several studies have recently focused on the use of these biomolecules as a novel natural source of additives in the pharmaceutical and food industries (Sila et al., 2014).

Taking into account the promising opportunities watermelon rinds might offer for the development of novel biologically active components that can be incorporated in cosmetics, food and pharmaceutical products, the purpose of the present study was to characterize polysaccharides extracted from watermelon rinds (WMRP) and to assess their antioxidant and antihypertensive activities as well as their functional proprieties.

2. Materials and methods

2.1. Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), angiotensin I-converting enzyme from rabbit lung, ACE synthetic substrate hippuryl-1-histidyl-1-leucine (HHL), β-carotene, ascorbic acid (V_c), linoleic acid and Tween 40 were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other chemicals, namely trichloroacetic acid (TCA), potassium ferricyanide, and ferric chloride were of analytical grade. All solutions were freshly prepared in distilled water.

2.2. Plant material

Fruits used in this work were obtained from local market in Sfax, Tunisia. The waste material used was namely watermelon (*Citrullus lanatus*) rinds (WMR). WMR were cut, dried then ground until obtain a thin powder before its storage.

2.3. Polysaccharides extraction procedure

Polysaccharides from WMR were recovered according to the method of Yao et al. (2005) with some modifications. Dried WMR was defatted with 95% ethanol solution for 2 h to eliminate small lipophilic molecules and impurities. The defatted residue was extracted with distilled water at different ratios of water to raw material (5–50 ml/g) in a thermostat-controlled water-bath (from 30 °C to 70 °C) for 50–100 min. The water extract was filtered and the filtrate was concentrated under vacuum to small volumes, and then precipitated by the addition of fourfold volume of ethanol solution for 24 h at 4 °C. Subsequently, the precipitate obtained after centrifugation at 5869*g* for 15 min was dissolved in water, dialyzed against deionized water and lyophilized to obtain WMRP. The polysaccharide yield (%, w/w) was determined as follows:

$$\text{Polysaccharide extraction yield}\,(\%, w/w) = 100 \times \frac{\text{weight of dried crude polysaccharide}\,(g)}{\text{weight of dried WMR}\,(g)}$$

2.4. Chemical composition analysis

The carbohydrate content was estimated by phenol-sulfuric acid colorimetric method using glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1951). The uronic acid content was analyzed according to the vitriol-carbazole method using glucuronic acid as the standard (Bitter & Muir, 1962). The WMRP protein content was measured by the Bradford method using bovine serum albumin as the standard (Bradford, 1976). For WMR, The total nitrogen content was determined using the Kjeldahl method.

2.5. Monosaccharide composition of WMRP

The elemental monosaccharide composition (molar ratios) of the polysaccharide extracted from watermelon rinds was determined using a modified method of Kamerling, Gerwig, Vliegenthart, and Clamp (1975). 50 μg myo-inositol, used as internal standard, was added to 500 μg lyophilized polysaccharide. The mixture was hydrolyzed for 4 h at 100 °C, in screw glass tube, using 500 μL methanolic HCl (3 N). After cooling to room temperature, all solutions were neutralized with 10 mg silver carbonate. The generated methyl glycosides were then converted to their corresponding volatile trimethylsilyl derivatives. The reaction took place by adding 100 μL pyridine and 100 μL derivatization reagent; Bis (tri-methylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) (Supelco), incubated for 25 min at 80 °C. After solvent evaporation under nitrogen flow, the generated per-O-trimethylsilylmethyl glycosides were resuspended in 500 μL dichloromethane, and analyzed by gas chromatography-flame ionization detector (GC-FID). An Agilent GC 6850 A instrument equipped with HP-5MScapillary column (30 m length, 0.25 mm diameter and 0.25 μm film thickness) was used. The GC oven temperature was set to 120 °C, increased first to 180 °C at 3 °C/min, then increased to 200 °C at 2 °C/min and held for 5 min. The helium carrier gas flow was set at 1.5 mL/min and the injection volume was 0.1 μL.

2.6. FT-IR spectroscopic analysis

The FT-IR spectrum of WMR polysaccharides was obtained using a Fourier transform infrared spectrophotometer (Perkin Elmer Spectrum BX FT-IR, USA). The ground sample was