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Structural, antioxidant and antibacterial activities of polysaccharides extracted from olive leaves

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ABSTRACT

In the present study, hot-water extraction procedure was used to recover polysaccharides from olive leaves. Primary structural characteristics were determined by nuclear magnetic resonance spectroscopy (^{1}H NMR and ^{13}C NMR), Fourier Transform-Infrared spectroscopy (FT-IR) and X-ray diffractometry analysis. Emulsifying capacity, Zeta (ζ) potential and differential scanning calorimetric (DSC) of olive leaf polysaccharides (OLP) were investigated. Antioxidant and antibacterial activities were then examined. Infrared spectroscopy data revealed that OLP were constituted of functional groups O—H, C—O and C—H which were specific to polysaccharides. Results of ζ-potential showed that OLP possessed an anionic structure and exhibited donated electron capacities. OLP displayed strong DPPH-radical scavenging activity (IC 50 = 34.80 μg/mL). They showed also important reducing power and β-carotene bleaching inhibition activities. Besides, OLP have attractive antibacterial activity against *S.enterica* and *M.luteus* with inhibition zones of 23.5 and 21.5 mm, respectively.

1. Introduction

Olive trees (*Olea europaea* L.) provided huge economic and dietetic benefits to people of the Mediterranean basin. During the past two decades, olive tree cultivation has increased all over the world due to the health benefits attributed to the olive oil consumption. Olive leaves, one of the main olive tree by-products, are obtained in large quantities in olive tree cultivation, olive oil and olive table industries. In olive oil industries, olive leaves represent approximately 10% of the weight of olive [1–3]. In olive tree cultivation, 25 kg of by-products (twigs and leaves) is produced per tree annually [2]. Olive leaf constitutes an abundant vegetable material which presents a potential source of high added value compounds. In folk medicine, it has been associated with health and used to treat some diseases like diabetes, hypertension, fever and hypercholesterolemia [4].

Nowadays, bioactive compounds from olive leaves can be of great interest in pharmaceutical, cosmetic and food industries. Among these bioactive compounds, we found phenolic compounds, mainly oleuropein and hydroxytyrosol, flavonoids, and sugars (mannitol, oligosaccharides). It has been reported that olive leaves form a biomass of polysaccharides including cellulose, hemicelluloses and lignin [5].

Polysaccharides are heterogeneous group of carbohydrate polymers, formed of monosaccharide units joined together by glycosidic linkage (α or β configuration) [6]. This biopolymer showed various nutritional and functional activities related to its chemical structure and physicochemical properties [5]. Recent studies revealed that polysaccharides isolated from natural source have attracted so much attention in the fields of pharmacology and biochemistry due to their beneficial activities. As an example, these biomacromolecules were shown to exhibit various biological activities including anti-tumor, immunostimulation, anti-inflammatory, anti-coagulation, anticarcinogenic activities, antimicrobial and antioxidant activities [7,8]. For this reason, it becomes necessary to explore them as novel potential antioxidants. Another aspect of the benefits of polysaccharides is the improvement of food quality. Indeed, they are long utilized to great extent in food industry as thickening or gel-setting agent and film- forming [9]. Besides they are used to improve water retention and to stabilize emulsions and dispersions. These bioactive molecules are

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widely incorporated into functional foods due to their prebiotic effect which is in relation with the presence of dietary fibers [10].

Hot water extraction is a traditional technology which is widely employed to extract polysaccharides from plant material [6,7,11]. This technology has shown to present convenient and fast features [12]. So far, olive leaves have not been subjected to hot water extraction for recovering polysaccharides.

In the present study, hot water extraction technology was used to extract olive leaf polysaccharides (OLP). Then the yield, physicochemical analysis and primary structural characteristics of polysaccharides were comprehensively investigated. Antioxidant and antibacterial activities of OLP were examined by various in vitro assays.

2. Materials and methods

2.1. Plant material

10 kg of olive leaves (*O. europaea*, cultivar Chemlali) was collected from Sfax (Tunisia) in 2015, dried in a tunnel microwave dryer (Adasen, JN- 100, China) for 10 min (1200 W, 70 °C), then milled and stored at 4 °C until extraction.

2.2. Extraction of the polysaccharides

The extraction process of polysaccharides from olive leaves was performed according to the method described by Shen et al. [6] with some modifications. In a previous work we investigated the extraction of polyphenols from olive leaves [13]. Particularly, olive leaves extract was prepared by the dispersion of olive leaf powder in water at a rate of 2.5%. The mixture was stirred for 1 h at room temperature (30 °C) and decanted for 4 h. The supernatant was clarified through linen cotton to remove leaf particles. Leaf marc was pretreated in petroleum ether and then in ethanol each for 5 h at 60 °C and 90 °C, respectively using soxhlet apparatus. Pretreated powder was dried and reserved at room temperature. Sample was then mixed with deionized water (1:30; w:v) and put into a water bath at 90 °C for 4 h. the suspension was finally filtered, freeze dried and stored at 4 °C until analysis.

2.3. Determination of yield and chemical composition of OLP

Ash content of olive leaf polysaccharides (OLP) was determined according to AOAC methods [14]. Protein content was determined according to the Bradford method [15], using bovine serum albumin as a standard. Total carbohydrates were determined by the phenol–sulfuric acid colorimetric method [16] using D-glucose for the calibration curve. Results were expressed as% of freeze dried matter.

The percentage extraction yield (%) was calculated with the formula below:

$$Y = W1/W0 \times 100$$

W_1 was the polysaccharide weight of extraction (g), and W_0 represented pretreated sample weight (g).

2.4. X-ray diffraction of OLP

The X-ray diffraction (XRD) pattern of OLP was measured by an X-ray diffractometer (Siemens D 5000, Bruker, Germany) according to the method described by Bouzidia et al. [17] with some modifications. The data were collected in the 2 θ range 5–100° with a step size of 0.02° and a counting time of 0.78 s/step.

2.5. Infra-red spectroscopic analysis

The absorption spectra of OLP were obtained by FT-IR spectroscopy (Nicole Impact 410) in KBr pellet. The FT-IR spectra were recorded between 400 and 4000 cm^{-1}.

2.6. Nuclear magnetic resonance (NMR) analysis

NMR analysis was carried out according to the method of Chen et al. [18] with some modifications. 20 mg of OLP sample was dissolved in 0.5 mL D_2O and put into a 4 mm NMR tube. All NMR experiments, including ^1H NMR and ^{13}C NMR were performed on a BRUKER 600M spectrometer (Rheinstetten, Germany) at 400 and 100 MHz, respectively.

2.7. Differential scanning calorimetric (DSC) analysis

Differential scanning calorimetry (DSC 4000, PerkinElmer, Netherlands) was employed to investigate the thermodynamic properties and the stability of macromolecules. This method was performed according to the method described by Wand and Lü [19] with some modifications. 5 mg of OLP was put into a standard aluminum crucible and immediately sealed. The crucible was heated from 10 to 80 °C and then cooled from 80 to 10 °C at a rate of 5 °C/min.

2.8. Zeta potential of polysaccharide dispersions

The Zeta (ζ) potential was determined using the zetasizer Nano series ZS 90 equipement (Malvern instruments, Worcestershire, UK). Briefly, the sample was dissolved in deionized water at 1 g/L and the ζ- potential was determined at 25 °C. Experiments were performed at pH 3, 6 and 9.

2.9. Emulsifying capacity of OLP

A volume of 21.25 mL of the different OLP solutions (5 mg of polysaccharides per mL, pH 3, 6 et 9) was homogenized with 3.75 mL of soybean oil for 1 min at room temperature (25 ± 2 °C) using a Ultra-Turrax IKR® WERKE performed at 21 500 rpm. After the emulsion preparation, an aliquot of each sample (50 μL) was pipetted from the emulsion and the drop was placed between lame and lamella to be observed under light microscopy, employing a 100× objective lens, using a CX31-12C04 microscope (Motic 2048 × 1536 pixels, Olympus Co., Tokyo, Japan). The emulsion images were then captured by a charge-coupled device camera (Olympus Co., Tokyo, Japan) connected to the microscope. For the droplet size distribution, analyses were carried out using Oxford Lasers analyzer software [20].

2.10. Antioxidant activities of polysaccharides

2.10.1. DPPH assay

The antiradical activity of OLP was determined by the DPPH method as described by Bersuder et al. [21]. The reduction of DPPH radical was measured spectrophotometrically (UVmini-1240, Shimadzu, Japan) at 517 nm. The antiradical activity was expressed as IC 50 (μg/mL), the concentration required to inhibit 50% of DPPH radial. BHA was used as positive standard.

2.10.2. β-carotene-linoleate bleaching assay

The antioxidant activity using ß-carotene bleaching method was assessed as described by Ben Taârit et al. [22]. 1 mL chloroform was added to 0.5 mg of β-carotene and mixed with 25 μL of linoleic acid and 200 μL of Tween-40. The chloroform was completely evaporated in a rotator evaporator at 40 °C. 100 mL of ultra-pure water

Table 1
Chemical composition of polysaccharides from olive leaves.

Parameters	OLP
Yield (%)	7.20 ± 0.10
Ash (%)	5.41 ± 0.21
Carbohydrates (%)	92.48 ± 2.60
Protein (%)	1.07 ± 0.07

was then added and the mixture was vigorously stirred. 0.5 of sample at different concentrations was immersed in 2.5 mL of the β-carotene-linoleic acid emulsion. The tubes were incubated at 50 °C for 60 min. The absorbance was measured at 470 nm using a UV–vis spectrophotometer (UVmini-1240, Shimadzu, Japan).

2.10.3. Reducing power assay

Reducing power assay assessing the ability of OLP to reduce iron (III) was determined according to the protocol described by Yıldırım et al. [23]. A volume of 1 mL of sample at different concentrations was mixed with phosphate buffer (1.25 mL, 0.2 M pH 6.6) and potassium ferricyanide (1.25 mL, 10 g/L). The mixtures were incubated at 50 °C for 30 min. 1.25 mL of 10% (w:v) trichloroacetic acid was added. Thereafter, the mixtures were centrifuged at 3000 × *g* for 10 min. Finally, 1.25 mL of the supernatant solution was mixed with 1.25 mL of distilled water and 0.25 mL of ferric chloride (1 g/L). After 10 min reaction, the resulting solution absorbance was measured at 700 nm using a UV–vis spectrophotometer (UVmini-1240, Shimadzu, Japan). Ascorbic acid was used as reference antioxidant.

2.11. Antibacterial activity of OLP

Bacillus cereus (ATCC 11778), *Micrococcus luteus* (ATCC 4698), *Salmonella enterica* (ATCC 43972, *Escherichia coli* (ATCC 25922), *Enterobacter* sp. and *Klebsiella pneumonieae* (ATCC 13883) were selected as test bacteria in this study. Culture suspension (100 μL) of the tested microorganism (10^6 Colony Forming Units (CFU)/mL) was spread over the Luria Bertani (LB) agar. Polysaccharides were delivered into wells (7 mm depth, 6 mm diameter). The antagonistic zones were detected after incubating for 24 h at 37 °C. The inoculated plates were kept at 4 °C for 1 h and after that, they were incubated at 37 °C for 24 h. The antibacterial activities were evaluated by measuring the diameter (in millimeters) of the inhibition zone around the wells.

2.12. Statistical analysis

Analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation.

3. Results and discussion

3.1. Chemical composition of the polysaccharides extracted from olive leaves

The chemical composition of OLP is shown in Table 1. The yield of hot-water extracted polysaccharides obtained was 7.20 ± 0.10%. This result was higher than that obtained by Mokni Ghribi et al. [24]. However it was similar to that previously obtained from *Dendrobium chrysotoxum* by enzyme assisted extraction method [25].

Low protein content (1.07 ± 0.07%) was observed for polysaccharides extracted from olive leaves. Although no treatment was performed to remove proteins, a low protein level was obtained. In fact, Rahmanian et al. [26] showed that the crude protein content in olive leaves was low and it ranged between 6.31 and 10.9 g/100 g dry matter. Higher protein level was previously reported by Mokni Ghribi et al. [24] for of water-soluble polysaccharides from chickpea flours (11.22 ± 0.63%) and by Wang et al. [27] for *Phellinus linteus* polysaccharides (1.53 ± 0.12%). Indeed, protein content in the extracted polysaccharides might depend on the extraction method and deproteination process.

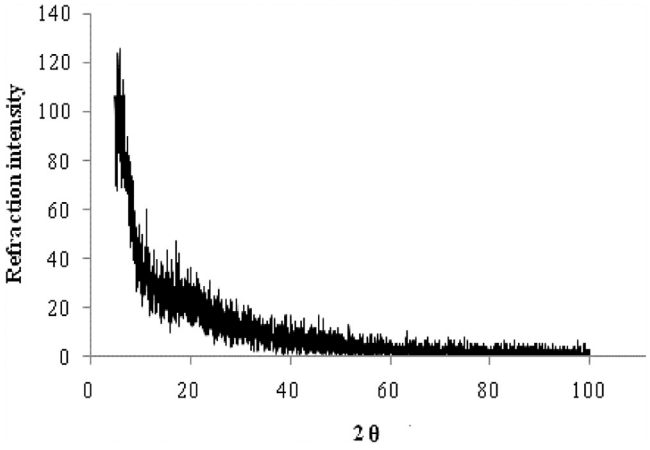

Fig. 1. X-ray diffraction pattern of polysaccharides from olive leaves.

Results presented in Table 1 showed that the ash content of OLP was 5.41 ± 0.21%. This result was higher than that obtained by ultrasonic-assisted extraction of water-soluble polysaccharides from *Lonicera macranthoides* (4.33 ± 0.14%) as reported by Zhen et al. [28].

The carbohydrate content in OLP was about 92.48 ± 2.60%, which was higher than that of polysaccharides extracted by ultrasonic treatment from mulberry leaves (83.42 ± 0.95%) [29]. This finding suggested that extraction methods played an important role in the carbohydrate content of the polysaccharides.

3.2. X-ray diffraction and infra-red spectroscopic analysis of OLP

XRD pattern of OLP was performed in order to determine its crystalline degree. In fact, the crystalline or non-crystalline characteristics of a substance play an important role in the physicochemical properties by influencing the structural arrangements, such as viscosity, solubility, swelling and flexibility. The X-ray diffractogram of OLP (Fig. 1) revealed that the polysaccharides extracted from olive leaves present diffraction peaks at 2θ = 11.38 and 17.26° which indicated the semi crystalline nature of the sample. Ben Jeddou et al. [10] found that water soluble polysaccharide extracted from potatoes peels was a semi-crystalline polymer.

FT-IR spectroscopy of OLP in a range of 4000–400 cm^{-1} is shown in Fig. 2. The strong absorption peak between 3600 and 3200 cm^{-1} corresponded to the stretching vibration of hydroxyl group of O—H [30]. The weak band recorded at 2920 cm^{-1} was related to C—H stretching vibration including CH, CH_2 and CH_3. The peak obtained at 2366 cm^{-1} was from the aliphatic C—H bonds [24]. The relatively strong absorption peak recorded at 1657 cm^{-1} indicated the presence of carbonyl group C=O [30]. The large band obtained at 1080 cm^{-1} corresponded to stretching vibration of C—O in C—O—H bonds and C— O—C glycosidic bond vibration and it is related to the presence of pyranose ring in polysaccharides [31]. Indeed, compared with others infra-red spectra of polysaccharides found in the literature [29–31], it's important to highlight that the obtained spectrum corresponds to a typical carbohydrate pattern.

3.3. NMR spectroscopy data of OLP

The structural features of OLP were analyzed using NMR spectroscopy. The ^1H and ^{13}C NMR spectra of OLP are represented in

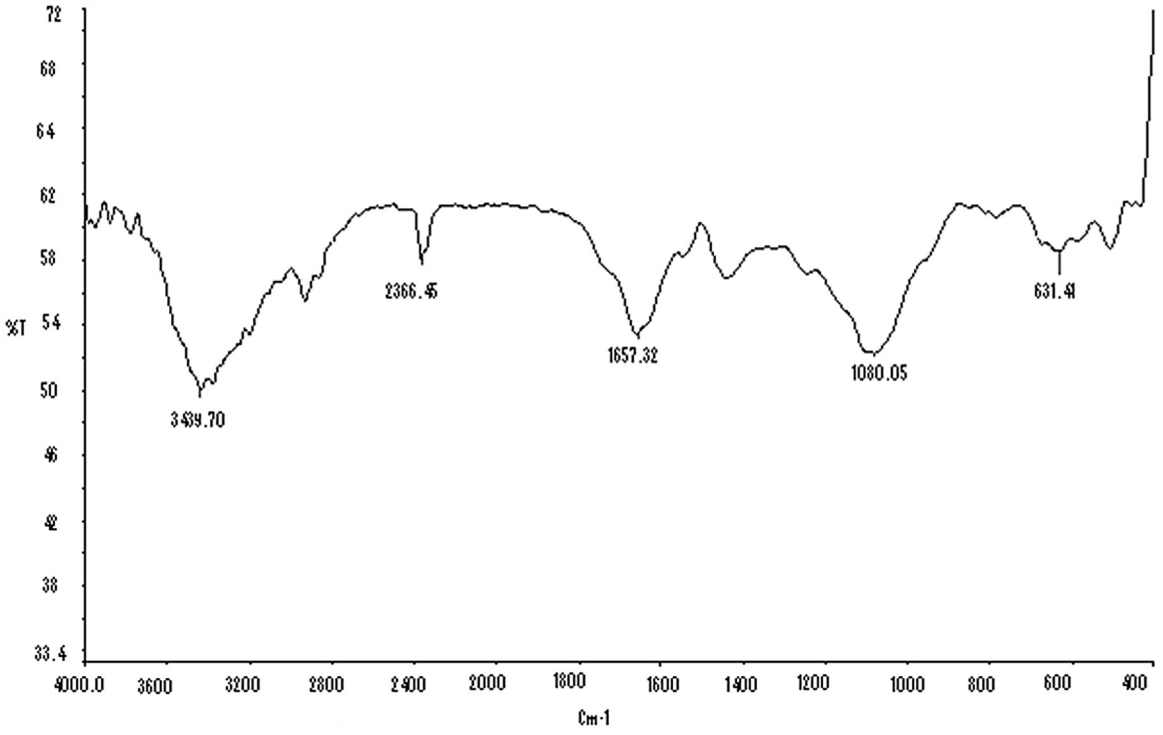

Fig. 2. FT-IR spectrum of polysaccharides from olive leaves.

Fig. 3a, b. The ^{1}H NMR spectrogram was crowded in a cramped region ranging from 3 to 5 ppm which confirms the presence of polysaccharides [32,33]. The signals from the anomeric protons were observed at 4.980 and 5.110 ppm, indicating the exists of both α and β anomers in OLP [33]. In fact, most of the α-anomeric protons appear in the 5–6 ppm range while most of the β-anomeric protons appear between 4 and 5 ppm [34]. The two peaks observed in the anomeric region belong to two sugar residues. Besides, the ^{13}C NMR spectrum revealed the existence of sugar residues in our polysaccharides. In this spectrum, two anomeric carbons were found in the regions of 95.89 ppm (α- configuration) and 107.40 ppm (β-configuration). This result confirms that of ^{1}H NMR spectrum. Signals at 60.74 and 60.58 ppm found in the ^{13}C NMR spectrum can be assigned to an O—CH_3 group [11]. The ^{1}H NMR spectrogram showed that the O—CH_3 group appeared between 3.127 and 3.397 ppm. The COO—CH_3 group of an uronic acid with methyl-esterified carboxyl group appeared in the 1.828–2.112 ppm range [34]. The absorption signal peaks of C2—C6 were found between 62 and 85 ppm. In the ^{1}H NMR, signal peaks of H2—H6 resonate at 3.5–4.5 ppm [35]. Thus, signal peaks between 3.599 and 4.179 ppm were provoked by protons on sugar rings.

3.4. Differential scanning calorimetric (DSC) analysis

DSC is a thermodynamic technique developed in the late 1960s in order to investigate not only the thermal properties but also the stability of macromolecules and their interactions [36]. It's frequently performed for the characterization of glass transition in food science due to its allowance routine measurement with the control. Heating and cooling scans of polysaccharides are presented in Fig. 4. The DSC heating trace of OLP exhibited one exothermic pick at 57 °C which corresponds to glass transition temperature (Tg). The heat of glass transition was 27.15 J/g. Generally, exothermic peak and heat of glass transition reflect the ability of the biopolymer to lose water. It was reported that Tg is obviously affected in the presence of bound-water which is strongly restrained by the hydroxyl group and the ionic group of polysaccharides [37].

3.5. ζ-potential of polysaccharide dispersions

According to McConaughy et al. [38], ζ-potential is the electrical potential existing at the hydrodynamic plane of shear surrounding a charged particle and is essentially the potential at the point in space where low molecular weight ions stop moving with the particle and remain within the surrounding solvent.

The ζ-potential of OLP determined under different pH values were presented in Fig. 5. A direct relationship exists between the charge of polysaccharide and the ζ-potential of the solution. As shown in Fig. 5, polysaccharide solutions were negatively charged across the entire pH range (−12, −15.7 and −16.9 respectively at pH 3, 6 and 9) which could suggest that OLP possessed donated electron capacities. The pH has a significant ($p < 0.05$) effect on the ζ-potential value. Indeed, the latter was increasingly negative as the pH increased until it reached a value of -16.9 ± 0.2 mV at pH 9. This decrease is related to a decrease of the intermolecular interactions when the pH increased. This result may be due to the decrease of the charge density of OLP solution which can be provoked by the reduction of the concentration of H+ ions as reported by Carneiro-Da-Cunha et al. [39]. The Increases in absolute value of charge indicated the good stability of polysaccharide solution.

3.6. Emulsifying capacity of OLP

The Emulsifying capacity of OLP was assessed by using microscopic observations of the oil-in-water emulsions realized at pH 3, 6 and 9 (Fig. 6) and by the determination of their droplet size distributions (Table 3). Generally, polysaccharides are considered as hydrophilic polymer which do not displayed significant surface activity. However, it has been reported that hydrocolloid gums can adsorb onto oil droplets and stabilize emulsions despite their character hydrophilic [40,41].

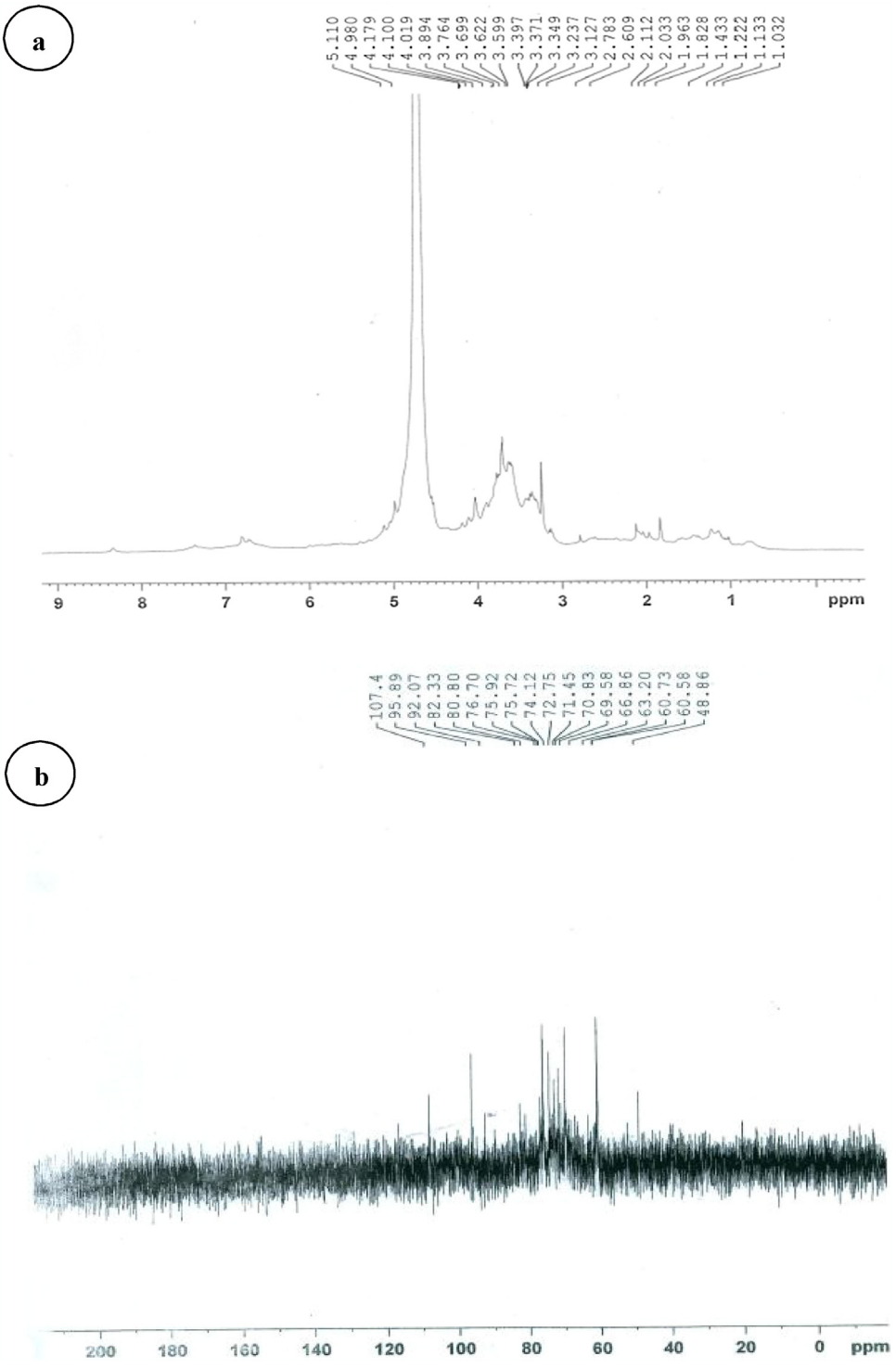

Fig. 3. ^{1}H NMR spectra (a) and ^{13}C NMR spectra (b) of polysaccharides from olive leaves.

Table 2
Antibacterial activity of polysaccharides from olive leaves.

Microbial species	Gram-negative				Gram-positive	
	S. enterica	*E. coli*	*K. pneumonieae*	*Enterobacter*	*M. luteus*	*B. cereus*
Diameter (mm)	23.5 ± 3.54	10.5 ± 0.71	–	9.5 ± 0.71	21.5 ± 0.5	10.0 ± 1.41

OLP emulsions displayed significant difference between the ranges of pH tested. Fig. 6 showed that the droplet diameter progressively decreased with the increase of pH applied during emulsion preparation. Indeed, emulsion gradually evolved from large, coarse and flocculated oil droplets at pH 3 to a small and fine appearance at pH 9. This result was proved by the determination the

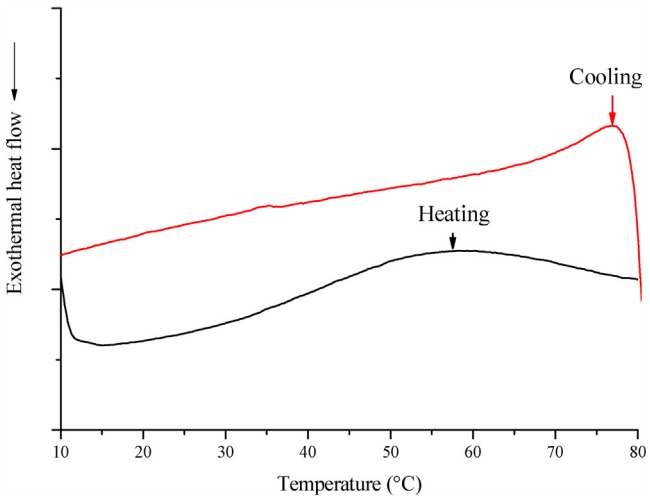

Fig. 4. Differential scanning calorimetric (DSC) analysis of polysaccharides from olive leaves.

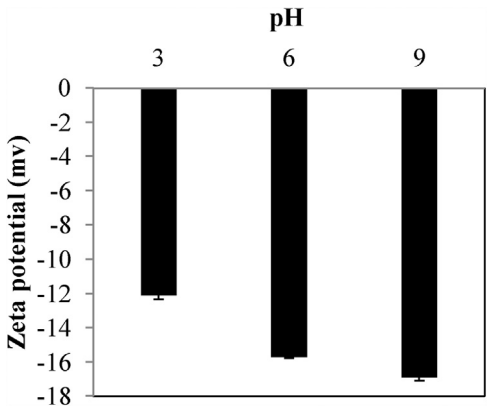

Fig. 5. Influence of pH on the ζ-potential of polysaccharides from olive leaves.

oil droplets distribution of OLP (Table 3). Indeed, oil droplets with diameter below 10 μm presented a distribution of 58.9% for the emulsion prepared at pH 9. However, they were 32.6% and 52.9% for OLP emulsions at pH 3 and 6, respectively. Moreover, the oil droplets diameter of OLP emulsion prepared at pH 9 did not exceed 30 μm. This finding was in accordance with the ζ-potential results. McClements [42] highlighted that the ζ-potential and the droplet sizes play an important role in determining the stability and the appearance of emulsions. Conventionally, the higher the absolute value of ζ-potential, the stronger stability of emulsions, which is related to the greater electrostatic repulsion among droplets.

3.7. Antioxidant activities of OLP

3.7.1. DPPH radical scavenging activity assay

DPPH radical scavenging activity of OLP was compared to that of BHA used in this test as positive standard (Fig. 7a). This assay is based on the reduction of free radical DPPH in the presence of a proton-donating substance [43].

As shown in Fig. 7, the DPPH radical scavenging activity increased along with the increased concentration of OLP between 5 and 50 μg/mL. The IC 50 values were estimated at 34.80 ± 0.37 and 12.63 ± 0.11 μg/mL for OLP and BHA, respectively. OLP showed excellent free radical scavenging activity. Indeed, the IC 50 value was lower than that some reported polysaccharides extracted from other leaves [9,31,44]. The mechanism of DPPH scavenging activity may be due to the hydrogen donation of polysaccharides from olive leaves, which combines with radicals. Thus, it forms a stable radical to terminate the radical chain. It has been found that the antioxidant activities of polysaccharides are affected by various factors such as chemical contents, molecular mass, structure and extraction methods [45].

3.7.2. β-carotene-linoleate bleaching assay

The β-carotene-linoleate bleaching assay was used to evaluate the lipid peroxidation inhibitory activity of OLP. This test is based on the disappearance of β-carotene color under thermally-induced oxidation. The presence of antioxidant in the system can neutralize free radicals, leading therefore to maintain β-carotene color for a longer period.

The antioxidant activity of OLP as measured by bleaching of β-carotene is shown in Fig. 7b. The antioxidant activity of BHA, used as positive control, was higher than that of analyzed polysaccharides. The inhibition percentage of β-carotene bleaching increased in a concentration-dependent manner of OLP up to 59.51 ± 3.46% for 500 μg/mL. Considering the antioxidant activity result, polysaccharides extracted from olive leaves could be used as a natural additive in order to prevent foods from lipid oxidation during processing and preservation.

3.7.3. Reducing power assay

The reducing power of a substance is directly correlated with its antioxidant activity. Indeed, reducing power assay is employed to assess the antioxidant activity of various compounds. In this assay, Fe^{3+}/ferricyanide complex was conversed to the ferrous form (Fe^{2+}) by the tested samples. The concentration of Fe^{2+} is monitored by determining the absorbance at 700 nm and ascorbic acid was used as positive control.

The data in Fig. 7c indicates that OLP showed reducing power activity in a concentration depended way. Higher absorbance value signifies stronger reducing power activity. Compared with ascorbic acid, the polysaccharide fraction exhibited lower antioxidant activity. The IC 50 value of OLP was found out to be 106.31 ± 1.01 μg/mL, thus being higher than that of ascorbic acid (5.63 ± 0.05 μg/mL).

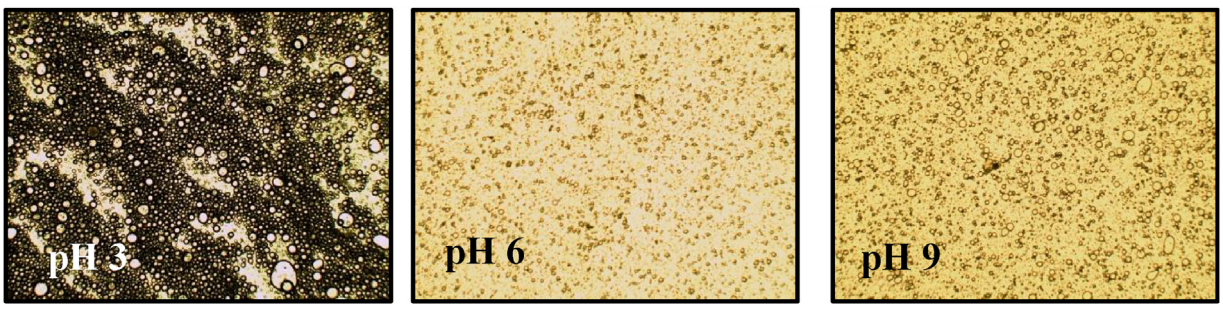

Fig. 6. Microscopic observations of the oil in water emulsions stabilized by OLP.

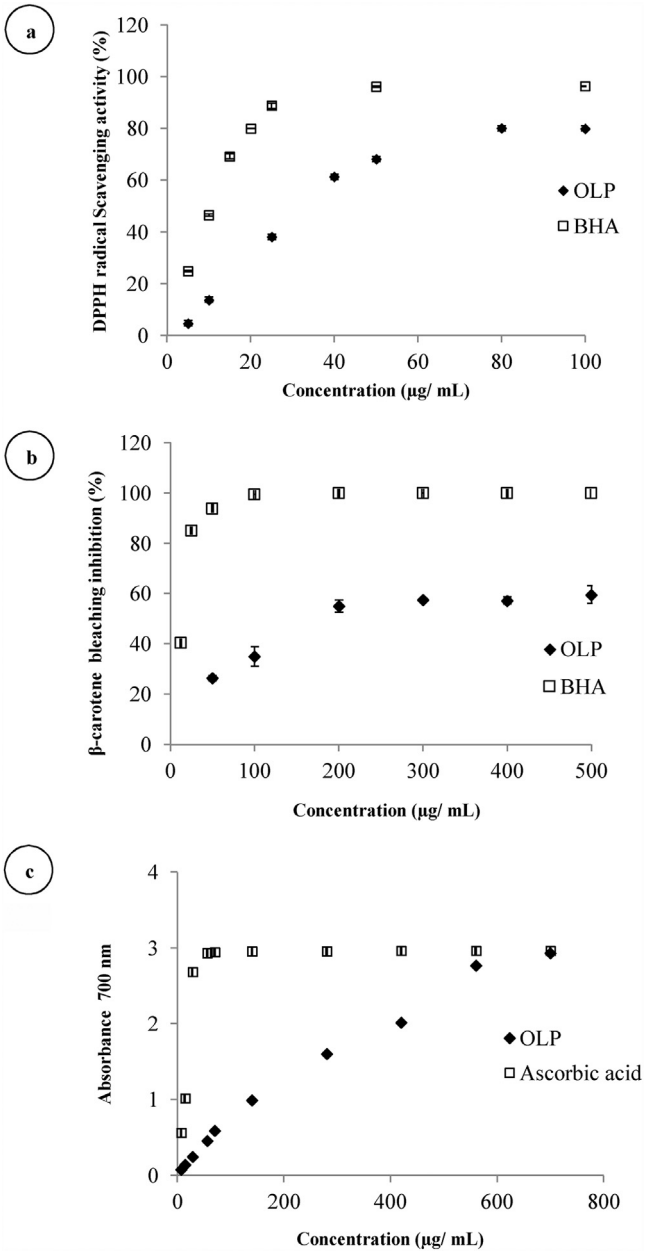

Fig. 7. Antioxidant activities of polysaccharides from olive leaves at different concentrations. a: DPPH-radical scavenging activity; b: β-carotene bleaching inhibition; c: reducing power assay.

Similar reducing power activity was reported by Bagchi and Jayaram Kumar [46]. They found an IC 50 of 110 µg/mL for polysaccharides from *Pithecellobium dulce* Benth. seeds. In particular, OLP at the concentration of 700 µg/mL showed the highest Fe^{3+} reducing ability ($OD_{700} = 2.93 \pm 0.06$) which was similar to ascorbic acid ($OD_{700} = 2.96 \pm 0.00$). The reducing power results revealed that OLP can act as electron donor compound, react with free radicals and convert them to more stable products terminating, therefore, reactions of the radical chain.

3.8. Antibacterial activity of OLP

Antibacterial activity results of OLP are presented in Table 2. Polysaccharides from olive leaves displayed inhibitory effects against various bacterial strains tested. The analyzed polysaccharides at a concentration of 50 mg/mL, exerted strong antibacterial activities towards *S. enterica* and *M. luteus* with inhibition zones of 23.5 ± 3.54 and 21.5 ± 0.5 mm, respectively. This highest antibacterial activity of polysaccharides may be caused by the disruption of cell wall and membrane of bacteria [7]. OLP also showed moderate inhibitory effect on *E.coli*, *B.cereus* and *Enterobacter*. However, the tested polysaccharide had non-inhibitory effect on *K. pneumonieae* (Table 2).

Table 3
Oil droplets size distribution of the oil in water emulsion stabilized by OLP.

Diameter	Distribution%		
	pH 3	pH 6	pH 9
>50 µm	1.6	–	–
30–50 µm	20.5	26.8	–
10–30 µm	45.3	20.3	41.1
<10 µm	32.6	52.9	58.9

4. Conclusion

In the present study, hot-water extracted polysaccharides from olive leaves were characterized. Chemical analysis showed that OLP is composed of 92% of carbohydrates. Results obtained from XRD, IR spectroscopy and RMN spectroscopy confirmed that OLP were a semi-crystalline polymer forming polysaccharides. Based on the results above, it can be obviously observed that the polymer isolated have attractive antioxidant and antibacterial activities. This study might provide useful information to guide the application of OLP in food, pharmaceutical and cosmetic fields.

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References

[1] C. Li, Y. Zheng, X. Wang, S. Feng, D. Di, Simultaneous separation and purification of flavonoids and oleuropein from Olea europaea L. (olive) leaves using macroporous resin, J. Sci. Food Agric. 91 (2011) 2826–2834.
[2] N. Talhaoui, A. Taamalli, A.M. Gómez-Caravaca, A. Fernández-Gutiérrez, A. Segura-Carretero, Phenolic compounds in olive leaves: analytical determination, biotic and abiotic influence, and health benefits, Food Res. Int. 77 (2015) 92–108, http://dx.doi.org/10.1016/j.foodres.2015.09.011.
[3] M. Bouaziz, S. Sayadi, Isolation and evaluation of antioxidants from leaves of a Tunisian cultivar olive tree, Eur. J. Lipid Sci. Technol. 107 (2005) 497–504, http://dx.doi.org/10.1002/ejlt.200501166.
[4] F. Rodrigues, F.B. Pimentel, M.B.P.P. Oliveira, Olive by-products: challenge application in cosmetic industry, Ind. Crops Prod. 70 (2015) 116–124, http://dx.doi.org/10.1016/j.indcrop.2015.03.027.
[5] J.M. Romero-garcía, A. Lama-muñoz, G. Rodríguez-gutiérrez, M. Moya, E. Ruiz, J. Fernández-bolaños, E. Castro, Obtaining sugars and natural antioxidants from olive leaves by steam-explosion, Food Chem. 210 (2016) 457–465.
[6] S. Shen, D. Chen, X. Li, T. Li, M. Yuan, Y. Zhou, C. Ding, Optimization of extraction process and antioxidant activity of polysaccharides from leaves of Paris polyphylla, Carbohydr. Polym. 104 (2014) 80–86, http://dx.doi.org/10.1016/j.carbpol.2014.01.006.
[7] S. Tahmouzi, M. Ghodsi, Optimum extraction of polysaccharides from motherwort leaf and its antioxidant and antimicrobial activities, Carbohydr. Polym. 112 (2014) 396–403, http://dx.doi.org/10.1016/j.carbpol.2014.06.024.
[8] J.H. Xie, M.Y. Shen, M.Y. Xie, S.P. Nie, Y. Chen, C. Li, D.F. Huang, Y.X. Wang, Ultrasonic-assisted extraction, antimicrobial and antioxidant activities of Cyclocarya paliurus (Batal.) Iljinskaja polysaccharides, Carbohydr. Polym. 89 (2012) 177–184, http://dx.doi.org/10.1016/j.carbpol.2012.02.068.
[9] S. Tahmouzi, Optimization of polysaccharides from Zagros oak leaf using RSM: antioxidant and antimicrobial activities, Carbohydr. Polym. 106 (2014) 238–246.
[10] K. Ben Jeddou, F. Chaari, S. Maktouf, O. Nouri-ellouz, C. Boisset, R. Ellouz, Structural, functional, and antioxidant properties of water-soluble polysaccharides from potatoes peels, Food Chem. 205 (2016) 97–105, http://dx.doi.org/10.1016/j.foodchem.2016.02.108.
[11] Z. Wu, H. Li, Y. Yang, H. Tan, Ultrasonic extraction optimization of L. macranthoides polysaccharides and its physicochemical properties, Int. J. Biol.

Macromol. 74 (2015) 224–231, http://dx.doi.org/10.1016/j.ijbiomac.2014.12.010.
[12] X. Li, L. Wang, Effect of extraction method on structure and antioxidant activity of Hohenbuehelia serotina polysaccharides, Int. J. Biol. Macromol. 83 (2016) 270–276, http://dx.doi.org/10.1016/j.ijbiomac.2015.11.060.
[13] I. Khemakhem, O.D. Gargouri, A. Dhouib, M.A. Ayadi, M. Bouaziz, Oleuropein rich extract from olive leaves by combining microfiltration, ultrafiltration and nanofiltration, Sep. Purif. Technol. 172 (2017) 310–317, http://dx.doi.org/10.1016/j.seppur.2016.08.003.
[14] W. Horwitz, G.W. Latimer, Official Methods of Analysis of AOAC International, AOAC, International, Gaithersburg, Md, 2005.
[15] M. Bradford, A rapid and sensitive method for the quantiWcation of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.
[16] M. Dubois, K.A. Gilles, J.K. Hamilton, P. a Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.
[17] N. Bouzidia, N. Salah, B. Hamdi, A. Ben Salah, Hydrothermal synthesis, structural elucidation, spectroscopic studies, thermal behavior and luminescence properties of a new 3-d compound: FeAlF 2 (C 10 H 8 N 2)(HPO 4) 2 (H 2 O), J. Mol. Struct. 1134 (2017) 797–805.
[18] T. Chen, M. Zhang, J. Li, M.M. Surhio, B. Li, M. Ye, Structural characterization and hypoglycemic activity of Trichosanthes peel polysaccharide, LWT-Food Sci. Technol. 70 (2016) 55–62.
[19] X. Wang, X. Lü, Characterization of pectic polysaccharides extracted from apple pomace by hot-compressed water, Carbohydr. Polym. 102 (2014) 174–184.
[20] M. Jridi, O. Abdelhedi, N. Souissi, M. Kammoun, M. Nasri, M.A. Ayadi, Improvement of the physicochemical, textural and sensory properties of meat sausage by edible cuttlefish gelatin addition, Food Biosci. 12 (2015) 67–72, http://dx.doi.org/10.1016/j.fbio.2015.07.007.
[21] P. Bersuder, M. Hole, G. Smith, Antioxidants from a heated histidine-glucose model system. I: investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography, J. Am. Oil Chem. Soc. 75 (1998) 181–187, http://dx.doi.org/10.1007/s11746-998-0030-y.
[22] M. Ben Taârit, K. Msaada, K. Hosni, B. Marzouk, Fatty acids, phenolic changes and antioxidant activity of clary sage (Salvia sclarea L.) rosette leaves grown under saline conditions, Ind. Crops Prod. 38 (2012) 58–63, http://dx.doi.org/10.1016/j.indcrop.2012.01.002.
[23] A. Yıldırım, A. Mavi, A.A. Kara, Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts, J. Agric. Food Chem. 49 (2001) 4083–4089, http://dx.doi.org/10.1021/jf0103572.
[24] A. Mokni Ghribi, A. Sila, I. Maklouf Gafsi, C. Blecker, S. Danthine, H. Attia, A. Bougatef, S. Besbes, Structural, functional, and ACE inhibitory properties of water-soluble polysaccharides from chickpea flours, Int. J. Biol. Macromol. 75 (2015) 276–282, http://dx.doi.org/10.1016/j.ijbiomac.2015.01.037.
[25] L.H. Pan, J. Wang, X.Q. Ye, X.Q. Zha, J.P. Luo, Enzyme-assisted extraction of polysaccharides from Dendrobium chrysotoxum and its functional properties and immunomodulatory activity, LWT-Food Sci. Technol. 60 (2015) 1149–1154, http://dx.doi.org/10.1016/j.lwt.2014.10.004.
[26] N. Rahmanian, S.M. Jafari, T.A. Wani, Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves, Trends Food Sci. Technol. 42 (2015) 150–172, http://dx.doi.org/10.1016/j.tifs.2014.12.009.
[27] Z.-B. Wang, J.-J. Pei, H.-L. Ma, P.-F. Cai, J.-K. Yan, Effect of extraction media on preliminary characterizations and antioxidant activities of Phellinus linteus polysaccharides, Carbohydr. Polym. 109 (2014) 49–55.
[28] Z. Wu, H. Li, Y. Yang, H. Tan, Ultrasonic extraction optimization of L. macranthoides polysaccharides and its physicochemical properties, Int. J. Biol. Macromol. 74 (2015) 224–231.
[29] D.-Y. Zhang, Y. Wan, J.-Y. Xu, G.H. Wu, L. Li, X.-H. Yao, Ultrasound extraction of polysaccharides from mulberry leaves and their effect on enhancing antioxidant activity, Carbohydr. Polym. 137 (2016) 473–479, http://dx.doi.org/10.1016/j.carbpol.2015.11.016.
[30] L.F. Ballesteros, M.A. Cerqueira, J.A. Teixeira, S.I. Mussatto, Characterization of polysaccharides extracted from spent coffee grounds by alkali pretreatment, Carbohydr. Polym. 127 (2015) 347–354, http://dx.doi.org/10.1016/j.carbpol.2015.03.047.
[31] L. Zhang, Z. Tu, H. Wang, Y. Kou, Q. Wen, Z. Fu, H. Chang, Response surface optimization and physicochemical properties of polysaccharides from Nelumbo nucifera leaves, Int. J. Biol. Macromol. 74 (2015) 103–110, http://dx.doi.org/10.1016/j.ijbiomac.2014.11.020.
[32] J. Gao, T. Zhang, Z.Y. Jin, X.M. Xu, J.H. Wang, X.Q. Zha, H.Q. Chen, Structural characterisation, physicochemical properties and antioxidant activity of polysaccharide from Lilium lancifolium Thunb, Food Chem. 169 (2015) 430–438, http://dx.doi.org/10.1016/j.foodchem.2014.08.016.
[33] Y. Xu, G. Liu, Z. Yu, X. Song, X. Li, Y. Yang, L. Wang, L. Liu, J. Dai, Purification, characterization and antiglycation activity of a novel polysaccharide from black currant, Food Chem. 199 (2016) 694–701, http://dx.doi.org/10.1016/j.foodchem.2015.12.078.
[34] S.W. Cui, Structural analysis of polysaccharides, in: Food Carbohydrates Chem. Phys. Prop. Appl., vol. 3, 2005.
[35] H.N. Cheng, T.G. Neiss, Solution NMR spectroscopy of food polysaccharides, Polym. Rev. 52 (2012) 81–114, http://dx.doi.org/10.1080/15583724.2012.668154.
[36] M.H. Chiu, N.S. Berezowski, E.J. Prenner, DSC Applications: Macromolecules, Woodhead Publishing Limited, Canada, 2013, http://dx.doi.org/10.1533/9781908818348.237.
[37] M. Iijima, K. Nakamura, T. Hatakeyama, H. Hatakeyama, Phase transition of pectin with sorbed water, Carbohydr. Polym. 41 (2000) 101–106, http://dx.doi.org/10.1016/S0144-8617(99)00116-2.
[38] S.D. McConaughy, P.a. Stroud, B. Boudreaux, R.D. Hester, C.L. McCormick, Structural characterization and solution properties of a galacturonate polysaccharide derived from Aloe vera capable of in situ gelation, Biomacromolecules 9 (2008) 472–480, http://dx.doi.org/10.1021/bm7009653.
[39] M.G. Carneiro-Da-Cunha, M.a. Cerqueira, B.W.S. Souza, J.a. Teixeira, A.a. Vicente, Influence of concentration, ionic strength and pH on zeta potential and mean hydrodynamic diameter of edible polysaccharide solutions envisaged for multinanolayered films production, Carbohydr. Polym. 85 (2011) 522–528, http://dx.doi.org/10.1016/j.carbpol.2011.03.001.
[40] N. Garti, Z. Madar, A. Aserin, B. Sternheim, Fenugreek galactomannans as food emulsifiers, LWT-Food Sci. Technol. 30 (1997) 305–311.
[41] N. Garti, Hydrocolloids as emulsifying agents for oil-in-water emulsions, J. Dispers. Sci. Technol. 20 (1999) 327–355.
[42] D.J. McClements, Food Emulsions: Principles, Practices, and Techniques, CRC Press, 2015.
[43] Z. Zhang, F. Kong, H. Ni, J.-B. Wan, D. Hua, C. Yan, Structural characterization, α-glucosidase inhibitory and DPPH· scavenging activities of polysaccharides from guava, Carbohydr. Polym. 144 (2016) 106–114, http://dx.doi.org/10.1016/j.carbpol.2016.02.030.
[44] Y. Wang, F. Mao, X. Wei, Characterization and antioxidant activities of polysaccharides from leaves, flowers and seeds of green tea, Carbohydr. Polym. 88 (2012) 146–153, http://dx.doi.org/10.1016/j.carbpol.2011.11.083.
[45] S. Feng, H. Cheng, L. Fu, C. Ding, L. Zhang, R. Yang, Y. Zhou, Ultrasonic-assisted extraction and antioxidant activities of polysaccharides from Camellia oleifera leaves, Int. J. Biol. Macromol. 68 (2014) 7–12, http://dx.doi.org/10.1016/j.ijbiomac.2014.04.026.
[46] S. Bagchi, K. Jayaram Kumar, Studies on water soluble polysaccharides from Pithecellobium dulce (Roxb.) Benth. seeds, Carbohydr. Polym. 138 (2016) 88–93, http://dx.doi.org/10.1016/j.carbpol.2015.11.018.