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Structural data and biological properties of almond gum oligosaccharide: Application to beef meat preservation

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ABSTRACT

Enzymatic hydrolysis of almond gum generates low molecular weight oligosaccharides (*OAG*) with a yield of 33.5%. The generated oligosaccharides were purified and identified. *OAG* analyses show that the most prominent residues were galactose and arabinose with traces of xylose, rhamnose, glucose and mannose. The glycosyl linkage positions were analyzed using gas chromatography–mass spectrometry showing a main chain composed of galactose units [→3)-Gal-(1→] branched mainly with arabinose residues [Ara-(1→]. The antioxidant and antimicrobial activities of *OAG* were investigated. As regards the *in vitro* antioxidant activities, the *OAG* showed a high total antioxidant activity (347 μg ascorbic acid equivalent/mL), an important DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity (IC_{50} = 0.64 mg/mL) and a high reducing capacity (RP0.5AU = 3.6 mg/mL). Furthermore, *OAG* had a high antimicrobial activity against *Salmonella thyphimirium, Bacillus cereus, Actinomycetes sp, Klebsiella pneumoniae, Escherichia coli, Alternaria alternate* and *Candidat albicans*. Finally, *OAG* efficiency was tested using 0.5%; 0.75% and 1% concentrations in beef meat preservation. Microbial growth and lipid oxidation were monitored during 9 days at 4 °C. The results showed significant inhibitions ($p < 0.05$) of lipid oxidation and microbial growth in ground beef meat containing *OAG*.

1. Introduction

Food quality is defined in terms of consumer acceptability: flavor, color, texture characteristics and nutritional value. Many foodstuffs such as meat and meat products contain unsaturated fatty acids that are responsible for food deterioration and shelf-life reduction [1]. For this reason, efforts to reduce oxidation have been increased. Most often, the best strategy is the addition of antioxidants [2]. Moreover, some antioxidants may additionally exhibit antibacterial activities [3,4]. To deal with lipid oxidation issues and microbial growth in meat products; either synthetic or natural food additives are commonly used in the meat industry [5–8]. Regarding the health hazard and the toxic effects associated with numerous synthetic antioxidant molecules such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), as well as their restriction in some countries [9–11], their replacement by natural molecules has been widely investigated. Natural antioxidants are safe, can protect the human body from free radicals and delay the progress of many chronic diseases [12,13]. Antioxidants can prevent lipid peroxidation by scavenging initiating radicals, breaking chain reaction, decomposing peroxides, decreasing localized oxygen concentrations and binding chain initiating catalysts, such as metal ions [14].

Recently, the antioxidant and antimicrobial activities of some polysaccharides and oligosaccharides have been reported [15–17]. Chitosan and chitooligosaccharides were proven to have antibacterial and antioxidants activities [18], oligosaccharides isolated from roots, flowers and leaves of *Panax ginseng* showed strong antioxidant activities [19]. Although the investigation of antioxidant and antimicrobial activities of some oligosaccharide gum derivatives (e.g. peach gum oligosaccharides [20]), the biological activities of almond gum polysaccharides and their oligosaccharide derivatives remain undetermined. In fact, almond trees are widely available in Tunisia as well as throughout the Mediterranean countries [21]

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and represent a potential natural resource of hydrocolloid gums; mainly composed of polysaccharides (>90%).

In the present work, oligosaccharide almond gum (*OAG*) derivatives were first; prepared enzymatically from almond gum polysaccharides, then their monosaccharide composition were determined using gas chromatography–mass spectrometry. Afterwards, total antioxidant activity, free radical scavenging activity, reducing capacity, and antibacterial activities of *OAG* were assessed. Finally, their efficiency on preventing food spoilage has been demonstrated on beef meat during chilled storage.

2. Materials and methods

2.1. Plant material and chemicals

Almond gum was collected from almond trunks (Achaak's variety) in the suburb of Sfax City (Tunisia). Oligosaccharides were generated enzymatically from almond gum. Zinc acetate and potassium ferrocyanure were purchased from LOBA Chemie (Mumbai, India). DNS (3,5-dinitro salicylic acid), DPPH (1,1-diphenyl-2-pycril hydrazil), BHA, phosphomolybdate and Strictol were purchased from Sigma Chemical Co. (St Louis, MO). Potassium ferricyanide, trichloroacetic acid, ferric trichloride and sodium hydroxide were obtained at analytical grade.

2.2. Chemical analyses

Dry matter was determined according to the AOAC [22]. Total nitrogen content was determined by Kjeldahl's method [23] and the protein content was calculated using the general factor (6.25). Sugar content was determined according to Dubois et al. [24]. Fat content was determined according to the French Association of Standardization [25]. Total ash was determined by sample combustion in a muffle furnace at 550 °C for 4 h. Mineral levels (Na, Mg, Ca, Zn and Cd) were measured by flame atomic absorption spectrometry (Analytic Jena ZEEnit 700 spectrometer, USA), according to [26].

2.3. Fungal strain and culture conditions

Penicilluim occitanis Pol6 mutant was provided by Cayla company (Toulouse, France). The Pol6 strain is a hypercellulolytic mutant, selected after eight rounds of mutagenesis from the CL100 wild type strain [27]. This fungus was cultivated at 30 °C in a modified Mandels liquid medium: KH_2PO_4; 2 g/L, $NaNO_3$; 5 g/L, $MgSO_4$, 7 H_2O; 0.3 g/L, $CaCl_2$; 0.3 g/L, yeast extract; 1 g/L, tween 80; 0.1%, and 2% almond gum powder. The pH value was adjusted to 5.5 with NaOH (1 N) and the medium was supplemented with 1 mL oligoelement solution ($CoCl_2$; 2 g/L, $MnSO_4$ H_2O; 1.6 g/L, $ZnSO_4$ H_2O; 1.4 g/L, and $FeSO_4$ 7 H_2O; 5 g/L) [28,29].

2.4. Enzyme production in controlled fermentor

The enzyme production was carried out in a 30-L fermentor (Infors, Suisse) containing almond gum as carbon source. The fermentor was operated at 30 °C, 250 rpm and 1 vvm (volume per volume per minute) aeration. The pH of the medium was maintained at 5.5 using sodium hydroxide (2 N) and orthophosphoric acid (2 M). Antifoam (Strictol 0.1%) was added automatically when required. The fermentor was fed with almond gum (2%) after 3 days of the batch culture. After fourteen days of fermentation, the mixture was centrifuged for 20 min at 7000 rpm. The supernatant was stored at 4 °C and used as the enzyme source. The enzyme activity, performed on the crude enzymatic extract (cellulase, xylanase, mannanase, amylase, pectinase, β-glucanase, β-glucosidase, β-xylosidase), was estimated to 4 U/mL (one unit (U) was defined as the quantity of reducing sugars (μmol) liberated from almond gum per min).

2.5. OAG extraction procedure

Almond gum was ground using mortar and pestle, then sifted through 0.5 mm sieve. Afterwards, a solution of 1% almond gum prepared in sodium acetate buffer (50 mM, pH 5.5) has been treated for 15 min at 100 °C. The first step of oligosaccharide production was conducted using a 10-L reactor. For this experiment, 2.5 L of almond gum solution (1%) was mixed with 1.5 L of crude enzymatic extract (4 U/mL). The mixture was adjusted to 10 L with sodium acetate buffer (50 mM, pH 5.5). After enzymatic hydrolysis of almond gum during 30 min at 50 °C, proteins were removed from the supernatant by adding 1/10 (v/v) zinc acetate (30%) and 2/10 (v/v) potassium ferrocyanure (15%) [30]. The resulting solution was centrifuged at 7000 rpm for 20 min. In order to remove the insoluble fraction containing undigested polysaccharides, the supernatant was precipitated with 2 volumes of isopropanol (91%) for 24 h at room temperature. The oligosaccharide fraction dissolved in the supernatant was concentrated by rotary evaporator at 70 °C. The remaining solution was freeze-dried overnight and stored at −20 °C.

2.6. OAG purification

In order to further clarify the generated solution, the lyophilized fraction was loaded into a Sephacryl S-200 resin (14 × 1.6 cm) pre-equilibrated with 5 volumes of water. Oligosaccharides were eluted using deionized water at 0.5 mL/min during 2 h. All fractions were collected following the quantification of total sugars as described previously [24], and freeze-dried overnight.

2.7. Monosaccharide analysis

The elemental monosaccharide composition (molar ratios) of the purified oligosaccharides was determined using a modified method of Kamerling et al. [31]. 50 μg *myo*-inositol, used as internal standard, was added to 1 mg lyophilized oligosaccharide fractions (*OAG1* and *OAG2*). 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 fractions 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 80 μL pyridine and 80 μL derivatization reagent; Bis (trimethylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) (Supelco), incubated for 25 min at 80 °C. After solvent evaporation under nitrogen flow, the generated per-O-trimethylsilyl methyl glycosides were resuspended in 500 μL dichloromethane, and analyzed by gas chromatography-flame ionization detector (GC-FID). An Agilent GC 6850A instrument equipped with HP-5MS capillary 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.

2.8. Glycosyl linkage position analysis

Glycosyl linkage positions of the purified oligosaccharides were determined according to Hakomori [32] with slight modifications. For this experiment, 2 mg of lyophilized powder were resuspended in 500 μL anhydrous dimethyl sulfoxide and stirred until complete dissolution. After tube cooling on ice, 500 μL butyl lithium