

Chemical Characterization and Prebiotic Effect of Water-Soluble Polysaccharides from *Zizyphus lotus* Leaves

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Abstract—In order to investigate the prebiotic potential of oligosaccharides prepared by chemical hydrolysis of water-soluble polysaccharides (WSP) from *Zizyphus lotus* leaves, the effect of oligosaccharides on bacterial growth was studied. The chemical composition of WSP was evaluated by colorimetric assays revealed the average values: $7.05 \pm 0.73\%$ proteins and $86.21 \pm 0.74\%$ carbohydrates, among them $64.81 \pm 0.42\%$ is neutral sugar and the rest $16.25 \pm 1.62\%$ is uronic acids. The characterization of monosaccharides was determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was found to be composed of galactose (23.95%), glucose (21.30%), rhamnose (20.28%), arabinose (9.55%), and glucuronic acid (22.95%). The effects of oligosaccharides on the growth of lactic acid bacteria were compared with those of fructo-oligosaccharide (RP95). The oligosaccharides concentration was 1g/L of Man, Rogosa, Sharpe broth. Bacterial growth was assessed during 2, 4.5, 6.5, 9, 12, 16 and 24 h by measuring the optical density of the cultures at 600 nm (OD600) and pH values. During fermentation, pH in broth cultures decreased from 6.7 to 5.87 ± 0.15 . The enumeration of lactic acid bacteria indicated that oligosaccharides led to a significant increase in bacteria ($P \leq 0.05$) compared to the control. The fermentative metabolism appeared to be faster on RP95 than on oligosaccharides from *Zizyphus lotus* leaves. Both RP95 and oligosaccharides showed clear prebiotic effects, but had differences in fermentation kinetics because of to the different degree of polymerization. This study shows the prebiotic effectiveness of oligosaccharides, and provides proof for the selection of leaves of *Zizyphus lotus* for use as functional food ingredients.

Keywords—*Zizyphus lotus*, polysaccharides, characterization, prebiotic effects.

I. INTRODUCTION

THE leaves of *Zizyphus lotus* L. are used as the treatment of dysentery and constipation in traditional medicine [1]. *Zizyphus lotus* L. is a native plant of North Africa and belongs to the family *Rhamnaceae*, distributed widely in South Algeria [2]. The flower of *Zizyphus lotus* is a high-quality honey source and *Zizyphus lotus* leaves have been exploited as tea

and functional food ingredients [3]. Moreover, several studies have indicated that polysaccharides from many plants are responsible for wound healing [4]. Owing to the commercial and pharmaceutical usefulness of polysaccharides, such as prebiotics, non-digestible carbohydrate which selectively modulates the colonic microflora composition towards improving host health [5], for example, stimulate growth of species from *Bifidobacterium* and *Lactobacillus* genera [6]. These bacteria have the ability to metabolize prebiotic carbohydrates and produce short chain fatty acids (SCFA). It has been reported that some SCFA have many benefits, including inhibition of gut pathogens, lowering of blood lipids, prevention of colon cancer [7], stimulation of calcium absorption [8], synthesis of vitamins [9] and enhancing the immune response [10]. In the present study, we report the chemical composition and partial characterization of water-soluble polysaccharides from *Z. lotus* leaves. Hence, to evaluate the effect of oligosaccharides obtained from polysaccharides hydrolysis on potential prebiotic activity on, *in vitro* conditions.

II. MATERIALS AND METHODS

A. Plant Material

The leaves of *Z. lotus* were harvested from Oued Nechou (region of Ghardaïa) in April 2013, authenticated, air-dried at ambient temperature for three weeks and stored in cardboard boxes for later use.

B. Extraction of Water-Soluble Polysaccharides

Z. lotus leaves were ground into powder using high speed disintegrator and were pre-extracted with 80% ethanol using a soxhlet apparatus in order to remove some colored materials and some other low molecular weight compounds. When no more colored material could be observed in the ethanol extract, the procedure was ceased and the organic solvent left in the residue was allowed to dry out [11]. The pretreated dry powder was extracted twice with two volumes of deionized water under constant stirring for 3 h in a 60°C water bath [12]. The mixture was centrifuged (2000g, 20 min), then the supernatant was filtered through gauze and Whatman GF/A glass fiber filter, concentrated at 40°C in vacuum and dialyzed at cut-off 3500 Da [13]. The extract was precipitated by the addition of ethanol to a final concentration of 75% (w/w) and the precipitates were collected by centrifugation, washed with acetone, dissolved in deionized water and finally lyophilized [11]. Crude water-soluble polysaccharides were obtained.

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C. Chemical Composition Analysis

Total neutral sugar content was determined by the reaction with phenol in the presence of sulfuric acid using glucose as a standard [14]. The total uronic acid content was colorimetrically determined by the m-hydroxydiphenyl assay using galacturonic acid as a standard [15]. Proteins in the solution were estimated by the method of binding of Coomassie Brilliant Blue G-250 to protein using bovine serum albumin as a standard [16].

D. Analysis of Carbohydrate Composition by HPAEC-PAD

The monosaccharide compositions of water-soluble polysaccharides were analyzed by HPAEC after acid hydrolysis with 4 M TFA for 4 h at 80°C. The hydrolysate was washed with methanol and concentrated to dryness. A Dionex system [Dionex Corporation, Sunnyvale (CA), USA] using a Carbpac PA1 (4mm×250mm) and a guard column (3mm×25mm) was used. Detection was carried out by pulsed amperometry with a gold electrode. The hydrolysates (25 ml) were filtered by passing through a 0.45µm filter before injecting into the column with an AS3500 autosampler. Solvent A was a 16mM NaOH solution; solvent B was an 100mM NaOH solution; and solvent C was an 100mM NaOH containing 1M sodium acetate solution. Gradients of NaOH and NaAc were used simultaneously to elute the carbohydrates by mixing the three eluents. This resulted in the following gradient of NaOH: 0–15 min, 16 mM; 15–18 min, 18–100 mM; 18–35 min, 100 mM. The simultaneous gradient of NaAc was 0–18 min, 0 mM; 18–35 min, 0–300 mM. After every run, the column was re-equilibrated in 16mM NaOH for 20 min. Hydrolysates (100ul) were injected. The flow rate was 1 ml/min. The monosaccharides were eluted isocratically with 16mM NaOH at a flowrate of 1ml/min. Each carbohydrate concentration was determined after integration of respective areas [Chromleon management system (Dionex)] and their comparison with standard curves (Sigma) [17].

E. Prebiotic Activity

Water-soluble polysaccharides hydrolyzate extract was tested on potential prebiotic activity. The strain selected for tests was *Lactobacillus plantarum* 299v, human probiotic provided by Probi AB (Sweden).

F. Growth Conditions

Lactobacillus plantarum Bacteria were grown in MRS basal media (Fluka, Suisse) carbohydrate free containing (per liter). 10 g protease peptone, 10 g meat extract, 5 g yeast extract, 1 g Tween 80, 2 g ammonium citrate, 5 g Sodium acetate, 0.1 g Magnesium Sulphate, 0.05 g Manganese Sulphate, 2 g dipotassium Sulphate and 0.5 g cysteine- HCl. Glucose, Raftilose®P95 (RP95), hydrolysate were dissolved in water (10% w/v) and sterilized by filtration. This solution was added to MRS basal media to a final concentration of 1% w/v. Inoculum was prepared from 48 h MRS grown *Lactobacillus*

cells and approximately 1×10^7 CFU per mL of strain was added to the MRS basal media containing 1% w/v of glucose, galactose, RP95, Hydrolysate, and incubated under anaerobic conditions in anaerobic jars (Oxoid Ltd) with gas generating kits (Oxoid Ltd) at 37°C.

G. Measurements

Bacterial growth was assessed during 2, 4.5, 6.5, 9, 12, 16 and 24 h by measuring the optical density of the cultures at 600 nm using a micro-plate reader (Molecular device, OD600). In addition, pH of culture broth was measured using a pH-meter type PHM 26, with a combined glass electrode type GK 2401C ("Radiometer").

III. RESULTS AND DISCUSSIONS

A. Chemical Composition

The yield, neutral monosaccharide, uronic acid, and protein content of water-soluble polysaccharides from the dried leaves of *Z. lotus* are given in Table I. The yield is 3.7%, based on dried leaves. Chemical analysis revealed that water-soluble polysaccharides as a heterogeneous mixture of polysaccharides consisted $64.81 \pm 0.42\%$ of neutral monosaccharides and $16.25 \pm 1.62\%$ of uronic acids such $86.21 \pm 0.74\%$ of total carbohydrates, as well as substantial amount of proteins ($7.05 \pm 0.73\%$). The carbohydrate content was higher than that reported by [18] in leaves of *Zizyphus jujube* ($81.62 \pm 3.12\%$). Therefore, the proteins content value found were greater than that reported by [18] in *Zizyphus jujuba* leaves ($5.01 \pm 0.05\%$).

TABLE I
CHEMICAL COMPOSITION OF CRUDE WATER-SOLUBLE POLYSACCHARIDES FROM THE DRIED LEAVES OF *Z. LOTUS*

Composition	Content wt. %
Yield	3.7
Protein	7.05 ± 0.73
Carbohydrate:	
Total	86.21 ± 0.74
Neutral	64.81 ± 0.42
Uronic acid	16.25 ± 1.62

B. Monosaccharide Composition

The result of HPAEC profile of acid hydrolysis of water-soluble polysaccharides from the dried leaves of *Z. lotus* is shown in Fig. 1. Significant differences of monosaccharide composition were observed, compared to results reported in other species of *Zizyphus*. It consisted of galactose, rhamnose, glucose, arabinose and galacturonic acid with the weight percentage of 43.95%, 20.28%, 11.30%, 9.55% and 12.57%, respectively. According to [19], water soluble polysaccharides of *Zizyphus mauritiana* consist of 24.98% rhamnose, 9.29% arabinose, 8.88 % galactose, and 25.98% glucose. These last values are considerably different from the values observed in our study.

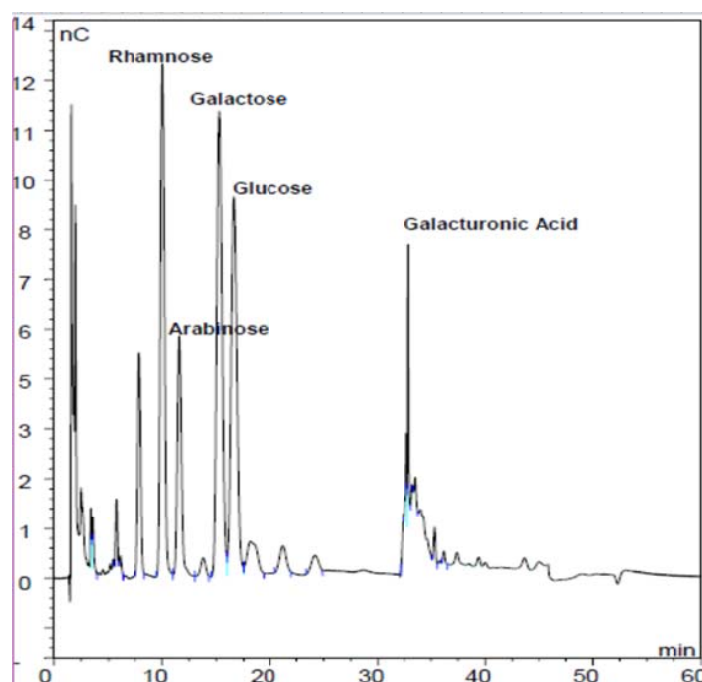


Fig. 1 HPAEC-PAD profiles of monosaccharides released from water-soluble polysaccharides of *Z. lotus* leaves by acid hydrolysis

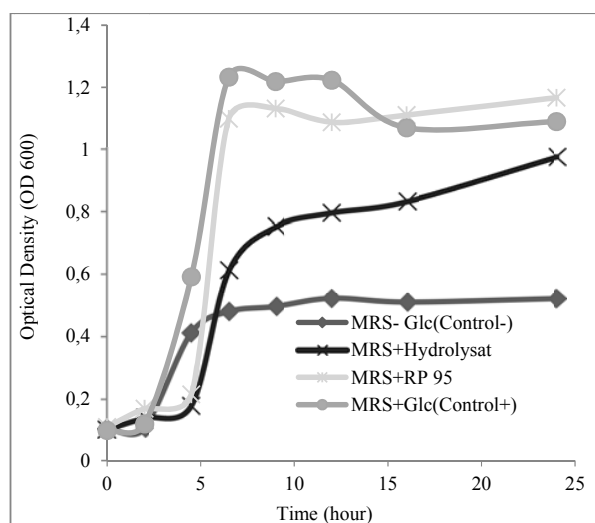


Fig. 2 Growth kinetics of probiotic *L. plantarum* strain in MRS medium enriched with carbohydrates estimated by measuring OD at 600 nm

C. Growth of *Lactobacillus plantarum* Bacteria and pH of Culture Broth

The growth densities (OD600) of *Lactobacillus plantarum* strain in basal MRS media supplemented with various carbon sources are shown in Fig. 2. The probiotic strain showed different growth characteristics in dependence on carbon sources. The growth was particularly good (1.267 OD) on RP95, glucose (1.091 OD) and hydrolysate of polysaccharides (0.975 OD). Poorer growth was observed on MRS broth without glucose. Although, glucose cannot be considered prebiotic because of its early absorption in the intestine and

therefore it is unavailable to stimulate the growth of endogenous flora in the colon. As shown in Table II, in cultures media that contained RP95 or Water-soluble polysaccharides hydrolyzate the pH progressively decreased down to about 1.7 and was significantly lower ($P \leq 0.05$) than in control cultures from 24 h. Differences between pH values on RP95 and Water-soluble polysaccharides hydrolyzate were not statistically significant.

TABLE II
PH OF CULTURE BROTH

Culture broth	<i>Lactobacillus plantarum</i>
MRS + Glc	5.19±0.15
MRS + Hydrolysate	5.87±0.15
MRS + RP95	5.29±0.20

IV. CONCLUSION

Growth of *Lactobacillus plantarum* cultures on RP95 and water-soluble polysaccharides hydrolyzate as sole carbon source was monitored by increasing of turbidity, measured as optical density at 600 nm (OD600), and lowering of pH, as result of production of lactic and acetic acids from carbohydrate fermentation. Growth was considered statistically significant ($P < 0.05$) on the basis of paired samples Student's t-test between pH and turbidity values before and after 24 h of fermentation.

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