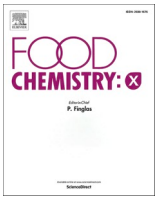
Food Chemistry: X 19 (2023) 100834

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Functional and chemical properties of *Phoenix dactylifera l.* Polysaccharides and the effect of date flesh and seed intervention on some blood biomarkers: A contrastive analysis

Hamid Noorbakhsh , Mohammad Rabbani Khorasgani *

Department of Cell and Molecular Biology & Microbiology, Faculty of Biological Sciences and Technology, University of Isfahan, Isfahan, Iran

ARTICLE INFO

Keywords:
Phoenix dactylifera L.
Polysaccharide
Xylose
Short-chain fatty acids
Prebiotics
Phenyllactic acid
Clinical trial
Inflammation

ABSTRACT

The chemical structure and bioactivity of ultrasonic-assisted alkaline extracted polysaccharides of date seed (DSP) and date flesh (DFP) were investigated. In addition, a crossover clinical trial was conducted to evaluate the effects of 28 days of date seed powder and date flesh consumption on blood biomarkers. Xylose (72.2 %) and galactose (41.6 %) were the most abundant monosaccharides in DSP and DFP, also DFP had a higher uronic acid content (12.16 ± 2.13 g/100 g) compared to DSP (5.57 ± 1.2 g/100 g). DSP had higher proliferation and antibacterial effects compared to DFP and inulin. *Bifidobacterium animalis* produced a higher short-chain fatty acid concentration during fermentation of DSP (66.98 ± 4.33 mM) and DFP (58.58 ± 5.57 mM) than inulin (19.68 ± 3.73 mM). Date seed powder could significantly reduce C-reactive proteins and triglycerides and increase red blood cell count ($p < 0.05$). DSP showed considerable prebiotic capability, antibacterial activity, and health-promoting effect; therefore, it could be considered for further investigation as nutraceuticals.

Introduction

Polysaccharides are biomacromolecule polymers characterized by glycosidic linkages with branched chains. Recently, plant-derived polysaccharides have attracted much attention in food and nutraceutical applications due to their bioactivities and health effects. Plant polysaccharides are mainly integrated into the cell wall, consisting of cellulose, hemicellulose, pectin, and lignin (Q. Wu, Wang, Fu, & Ren, 2019).

Date palm (*Phoenix dactylifera L.*) is an ancient monocotyledon plant from the *Arecaceae* family, cultivated in tropical and subtropical areas. The mesocarp of the date fruit mainly contains sugars and polysaccharides. Date seed constitutes about 10–15% of the weight of the date fruit. It has a tough texture consisting of polysaccharides, phenolics, and oil (Ghnimi, Umer, Karim, & Kamal-Eldin, 2017). Arabinoxylan and galactomannan are the most abundant polysaccharides of the date seed (Noorbakhsh & Khorasgani, 2022). Due to their diverse biological activities, date seeds have attracted much attention. Raw and roasted date seed powder, as a source of phenolics and dietary fiber, is commercially available in several countries. Although date fruit has a long history in human nutrition, a few studies have been conducted on the relation of chemical properties and biological properties of its polysaccharides.

Natural polysaccharides could positively regulate gut microbiota owing to their prebiotic properties (Blanco-Pérez, Steigerwald, Schülke, Vieths, Toda, & Scheurer, 2021). The gut microbiota can affect inflammation by producing microbial metabolites such as short-chain fatty acids (Liang, Wu, & Jin, 2018). In addition, the chemical structures of polysaccharides such as monosaccharide compositions, side-chain conformation, glycosidic bonds, and even extraction methods affect their prebiotic and antibacterial activities. Cell membrane permeability changing and preventing the adsorption of pathogenic bacteria to epithelial cells are two critical antibacterial mechanisms of polysaccharides (Zhou, Chen, Chen, & Chen, 2022). Polysaccharides also have shown a strong lipid-lowering effect and inhibited erythrocyte hemolysis (Ahmadi et al., 2019; Q. Wu et al., 2019).

Phenyllactic acid (PLA) is a phenolic acid categorized in aromatic lactic acids mainly produced by lactic acid bacteria with a broad antimicrobial spectrum. Recently, it has gained much attention because of the increasing public concern about preservatives (Armaforte, Carri, Ferri, & Caboni, 2006). PLA also can improve the human body immune system due to its gut microbiota regulating effects (Laursen et al., 2021). Although microbial fermentation is the primary process to produce natural PLA, not enough researches have studied the effects of polysaccharides on PLA production efficiency.

* Corresponding author.
E-mail addresses: h.noorbakhsh@mail.um.ac.ir (H. Noorbakhsh), m.rabbani@biol.ui.ac.ir (M. Rabbani Khorasgani).

https://doi.org/10.1016/j.fochx.2023.100834
Received 31 October 2022; Received in revised form 20 January 2023; Accepted 8 August 2023
Available online 9 August 2023

Date is an inexpensive and widely available source of numerous bioactive compounds such as polysaccharides. Date fruit and seed have polysaccharides with unique chemical structures (Ghnimi et al., 2017; Noorbakhsh & Khorasgani, 2022). Chemical analysis of the polysaccharides led to developing a hypothesis that the chemical properties of date polysaccharides affects their biological activities, which we tested using independent measurements. The relation of chemical properties and bioactivities of ultrasonic-assisted alkaline extracted polysaccharides of date seed (DSP), and date flesh (DFP) were comprehensively compared, for the first time. Moreover, the effects of the date seed powder and date flesh intervention on some blood biomarkers were evaluated using a pilot crossover clinical trial.

Materials and methods

Date and date seed

Date (*Phoenix dactylifera L.*, Zahidi variety) was obtained from the Iranian Date Palm and Tropical Fruits Research Center. Verification of plant scientific name was done in the herbarium and plant systematic laboratory of the University of Isfahan using identification keys.

Chemical properties

Moisture, ash, protein, oil, total sugar, and total polysaccharide content of the date flesh, and date seed were measured according to the corresponding AOAC methods (Horwitz & Latimer, 2000). The colorimetric method was employed to assess the uronic acid content of DSP and DFP using m-hydroxy diphenyl at 520 nm with d-galacturonic acid as the standard, as described by (Blumenkrantz & Asboe-Hansen, 1973). The total phenolic content of extracted polysaccharides, date flesh and date seed were analyzed according to the Folin-Ciocateau method with gallic acid as the standard (Woisky & Salatino, 1998). The antioxidant activity of extracted polysaccharides, date flesh, and date seed was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method with ascorbic acid as the positive control. The absorbance was measured (Shimadzu UV/VIS-Spectrometer Model UV 120–02) at 517 nm after 30 min incubation in the dark (Feriani et al., 2020). Moreover, the water holding capacity (WHC) of DFP and DSP was measured as described by (Ahmadi et al., 2019).

Polysaccharide extraction

An ultrasonic-assisted alkaline hot water extraction method was used to extract polysaccharides. Oil was extracted using the cold solvent extraction method (*n*-Hexane 1:15, 24 h, at room temperature). The residue was added to ethanol (80% w/w) 1:4 and put in the shaking water bath (BioLab BBSW-101, Canada) at 80 °C for 8 h to remove monomers and colorants, then washed three times with fresh ethanol. Distilled water (1:4) and 0.5% NaOH (w/w) were added to the remaining material and kept in the shaking water bath at 80 °C for 8 h. In this step, ultrasonic treatment (120 W, 30 m) was applied to the mixture to increase the extraction efficiency. The aqueous phase was then separated, and its pH was adjusted at 7 using 0.5 M HCl (Merck, Germany). After that, cold ethanol (4 °C, 80%, 1:4) was added and kept for 48 h to separate the polysaccharides. The mixture was filtered and washed with absolute cold ethanol several times. Finally, the residue was freeze-dried and kept at 4 °C for further analysis (Sun et al., 2019). The extraction yield of the polysaccharides was calculated as follows:

$$\text{Extraction yield}\,(\%) = \frac{w_P}{w_r} \times 100$$

where W_p refers to the extracted polysaccharides' dry weight (g) and Wr represents the source's dry weight (g).

Preliminary structural properties

Molecular morphology observation

Morphological properties of extracted polysaccharides were analyzed using the scanning electron microscopy (SEM) method (FEI Quanta 250 FEG, Hillsboro, OR, USA) (Cui, Wang, Wang, Cao, Wang, & Lü, 2022).

Fourier-transform infrared spectroscopy (FTIR) analysis

FTIR (Jasco FT/IR-6800, Japan) was used to detect the molecular structure of extracted polysaccharides (Cui et al., 2022).

X-ray diffraction (XRD)

The crystalline structure of DSP and DFP was analyzed using an XRD analyzer (Brucker, Germany). The data were collected over a 2θ range of 5–80° with a step size of 0.05° and a counting time of 5 s/step (Cui et al., 2022).

Monosaccharides composition

The extracted polysaccharides were hydrolyzed with trifluoroacetic acid (TFA) 2 N for 120 min at 120 ℃ to identify structural carbohydrate units. The monosaccharides composition of hydrolyzed polysaccharides was then analyzed by HPLC (Waters 600, Waters, equipped with Hi-Plex H column, Agilent, USA). The mobile phase was acetonitrile water (75:25, v/v) with a flow rate of 1 $ml.min^{-1}$. The monosaccharide standards (mannose, rhamnose, arabinose, xylose, glucose, and galactose) (Sigma-Aldrich) were used at 10 mg/mL (Yang et al., 2008).

Determination of prebiotic potential

Resistance to gastrointestinal juice

The resistance of extracted polysaccharides to the simulated digestion process was evaluated based on a method described by (Ahmadi et al., 2019) with some modifications. Simulated gastric juice (pH = 1.2) and simulated intestinal juice (pH = 7.4) were prepared by mixing NaCl (2 g) and HCl (7 ml) and KH_2PO_4 (6.8 g), NaOH (190 ml), and α-amylase (2 U/ml) (Sigma-Aldrich), respectively. Moreover, a combination of simulated gastric and intestinal juice (pH = 4.5) was prepared by mixing simulated gastric and intestinal juice at a ratio of 40:60. DSP, DFP or inulin were mixed with each simulated juice and kept for 3 h at 37℃ in a shaking water bath at 80 rpm (BioLab BBSW-101, Canada). The polysaccharides hydrolysis percentage was measured after 3 h, using the free reducing sugar and total sugar contents ratio based on DNS (3,5- Dinitrosalicylic acid) method and phenol–sulfuric acid method, respectively.

Growth stimulation of probiotics

The proliferation effects of extracted polysaccharides on selected strains were measured using a viable cell count assay described by (Huang et al., 2019) with some modifications. Viability of *Lactobacillus plantarum* ATCC1470 and *Bifidobacterium animalis* subsp. *lactis* DSM 10140 during 72 h in carbohydrate-free (De Man, Rogosa and Sharpe, Merck, Germany) C.F. MRS broth containing 1, 2, 3, 5 (% w/w) of DSP or DFP was measured and compared to inulin as a common prebiotic compound. MRS broth contained glucose as a carbon source, and carbohydrate-free MRS (C.F. MRS) broth was used as a positive and negative standard. To make an anaerobic growth condition for *B. animalis,* L -cysteine (0.05% w/w) was added to MRS broth using 0.45 μm Millipore syringe filters and put in an anaerobic jar with a gas pack (Merck, Germany). The proliferation rate during 72 h incubation was calculated as follows

Proliferation rate. = $logS - logE$where logS is viable cell count at the start (0 h, $CFU.g^{-1}$), and logE is viable cell count at the end (72 h, $CFU.g^{-1}$).

Short-chain fatty acids (SCFAs) production

The short-chain fatty acids (SCFAs), including lactate, acetate, propionate, and butyrate were measured at the start and end of the

incubation period (72 h, MRS broth with 5% (w/w) DSP, DFP and inulin) using HPLC (Waters 600, Waters, equipped with Agilent Hi-Plex H column, USA). A 1 mM H_2SO_4 solution was used as a mobile phase at a flow rate of 0.6 ml.min^{-1}. The difference in SCFAs concentrations before and after fermentation was reported in mM (Ahmadi et al., 2019).

Phenyllactic acid (PLA) assay

The concentration of PLA was measured after 72 h fermentation of MRS broth containing 5% (w/w) DSP, DFP or inulin with *L. plantarum* and *B. animalis*, and MRS broth was also used as blank. The HPLC method described by (Armaforte et al., 2006) with slight modifications was applied. Briefly, at the end of the incubation period, each sample was centrifuged at 4000g for 15 min at 4 °C, subsequently, cell-free supernatant (CFS) was filtered through a 0.22 μm Millipore filter and 20 μL of each CFS was directly injected in the HPLC (Waters 600, Waters, equipped with UV detector set at 210 nm, USA), as described in section 2.5.3.

Antibacterial activity of polysaccharides

Disk diffusion assay was conducted to evaluate the antibacterial activities of DSP, DFP, and inulin (as control) against common gram-negative and gram-positive pathogens, including *Staphylococcus aureus* ATCC 29213*, Escherichia coli* ATCC 25922*, Klebsiella pneumoniae* ATCC 31488 *and Bacillus cereus* ATCC 14579. The antibacterial activities were evaluated by measuring the diameter (mm) of the inhibition zone around the disks. A 2.5% (w/w) solution of each polysaccharide was prepared and filtrated using 0.45 μm Millipore filters. 10 ml of plate count agar (Merck, Germany) was added to each plate and overnight grown cultures of test organisms (10^6 CFU.g^{-1}, 100 μL) were added to the sterilized medium. A paper disk (0.6 mm diameter, 0.1 mm thickness) containing 50 μL of test solution was placed in the center of the plate and then incubated at 37 °C for 24 h (Behbahani, Yazdi, Shahidi, Mortazavi, & Mohebbi, 2017).

Effects of date seed powder and date fruit intervention on blood biomarkers

Participants

The current study was approved by the research ethics committees of the University of Isfahan, Iran (IR.UI.REC.1400.023, 2021.07.17). All participants received written information about the study protocol, and their written consent to participate was obtained. Fasting blood samples of all participants were collected at baseline and after 28 days of date fruit and seed powder consumption. At the start of the clinical trial, participants had 32.60 ± 3.38 years old, and BMI was 24.04 ± 2.75 kg.m^{-2}, the male to female ratio was 1:1. Participants with metabolic and infectious diseases, inflammation, and pregnancy were excluded. In addition, they had not taken nutritional supplements or antibiotics for three months before the intervention. During the intervention, participants were asked to keep their usual diet, exercise time, and other living habits.

Intervention and blood biomarker analysis

A randomized, pilot crossover clinical trial using 10 participants with a washout period of 28 days was conducted to compare the effects of date polysaccharides on blood biomarkers. Each participant received 30 g of date seed powder and/or 50 g of date fruit daily for 4 weeks (Evans, 2010). Fasting blood sugar (FBS), high-sensitivity c-reactive protein (hs-CRP), ferritin, erythrocyte sedimentation rate (ESR), serum triglyceride (TG), and complete blood count (CBC), including red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume of erythrocytes (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin distribution width (HDW), red cell distribution width (RDW) platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), procalcitonin (PCT), absolute neutrophil count (ANC), lymphocytes count (LYMPH), mononucleosis (MONO), eosinophil count (EOS), basophil count (BASO), and large unstained cells (LUC), were measured (X. Chen et al., 2019).

Multivariate data analysis

To compare the effects of the intervention on participants' blood biomarkers, principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using SIMCA-P + 14.1 software (Umetrics, Sweden) (Noorbakhsh, Yavarmanesh, Mortazavi, Adibi, & Moazzami, 2019).

Univariate data analysis

Univariate statistical analysis was performed by Minitab statistical software, version 17, using a general linear model (ANOVA). Their means were compared using Tukey's multiple comparison test, and the signification interval was considered at ($p < 0.05$) (Noorbakhsh et al., 2019).

Results and discussion

Chemical properties

The chemical properties of date flesh, date seed, DSP and DFP are presented in Table 1. The results showed that date seed had a higher amount of polysaccharide (17.54 ± 0.07 g/100 g) than the date flesh (7.84 ± 0.21 g/100 g). A significant difference between the chemical composition of date seed and date flesh was observed. Several studies have reported that date flesh is a rich source of sugar (mono and disaccharide) and date seed mainly contains polysaccharides. The

Table 1
Chemical properties of date flesh, date seed and extracted polysaccharides.

Chemical composition	Date Flesh	Date Seed	DFP*	DSP**
Moisture (%)	9.1 ± 0.12 ^{a}	5.2 ± 0.23^{b}	–	–
Protein (%)	1.9 ± 0.1^{b}	6.32 ± 0.2 ^{a}	–	–
Oil (%)	1.02 ±.08^{b}	9.97 ± 0.09 ^{a}	–	–
Total sugars (%)	73.01 ± 0.19 ^{a}	8.81 ± 0.21^{b}	–	–
Total polysaccharides (%)	7.84 ± 0.21^{b}	17.54 ± 0.07 ^{a}	–	–
Ash (%)	2.61 ± 0.13^{b}	3.19 ± 0.18 ^{a}	1.02 ± 0.09 ^{d}	1.87 ± 0.19^{c}
Antioxidant activity (g/l)***	0.154 ± 0.08 ^{d}	0.725 ± 0.15^{c}	4.79 ± 0.12^{b}	6.25 ± 0.13 ^{a}
Total phenolic content (mg GAE/100 g)	386 ± 9.19^{b}	1121 ± 99.24 ^{a}	2.56 ± 0.11^{c}	2.61 ± 0.13^{c}
Water holding capacity (g/g)	–	–	16.36 ± 0.03 ^{a}	3.83 ± 0.04^{b}
Uronic acid (%)	–	–	12.16 ± 2.13 ^{a}	5.57 ± 1.2^{b}
Yield (%)	–	–	5.47 ± 0.29^{b}	8.68 ± 0.48 ^{a}
Monosaccharide composition (molar ratio %)				
Mannose	–	–	1.1^{b}	6.3 ^{a}
Rhamnose	–	–	5.6 ^{a}	3.4^{b}
Arabinose	–	–	19.6 ^{a}	8.2^{b}
Xylose	–	–	15.3^{b}	72.2 ^{a}
Glucose	–	–	16.8 ^{a}	4.8^{b}
Galactose	–	–	41.6 ^{a}	5.1^{b}

Different letter in each row means significant difference ($p < 0.05$), data are mean ± SD, and values are the dry basis.
* Date Flesh Polysaccharide (DFP).
** Date Seed Polysaccharide (DSP).
*** Aqueous extract.

results showed that the WHC of DFP (16.36 ± 0.03 g/g) was significantly higher than that of DSP (3.83 ± 0.04 g/g) and inulin (0.83 ± 0.05 g/g). The higher WHC of DFP could be related to the higher uronic acid content of DFP (12.16 ± 2.13 g/100 g) compared to DSP (5.57 ± 1.2 g/100 g) (Elleuch et al., 2008). Polysaccharides with higher WHC make the stools bulkier and easier to evacuate. A bulky stool has been shown to decrease the chance of constipation, leading to reduced inflammation and enhanced excretion of free radicals (Zhao et al., 2020).

Antioxidant activity

The results of the DPPH free radical scavenging capacity of DFP and DSP showed that DSP (6.25 ± 0.13) had more potent antioxidant activity than DFP (4.79 ± 0.12 g/l) (Table 1). The antioxidant activity of polysaccharides is related to their structural properties, including the type of constituent monosaccharides, glycosidic bonds, molecular weight, and carbonyl, sulfonyl, amino, carboxyl groups. The higher antioxidant activity of DSP could be due to more carbonyl and carboxyl groups observed in FTIR Spectra (Pathania, Sharma, & Siddiqi, 2016). Moreover, the high antioxidant capacity of arabinoxylans, a non-starch polysaccharide, has been reported (H. Chen et al., 2019). Date seed (0.725 ± 0.15 g/l) showed a greater antioxidant capacity than date flesh (0.154 ± 0.08 g/l). One possible reason for the higher antioxidant capacity of date seed is the higher phenolic compound in date seed (1121 ± 99.24 mg GAE/100 g) in comparison to the date flesh (386 ± 9.19 mg GAE/100 g) (Fu et al., 2011).

Preliminary structural properties

XRD analysis

The degree of crystallinity and structure of the DSP and DFP was determined using XRD. While the spectra of the DSP showed a combination of a small crystalline region with intensities of 10° to 20° and an amorphous region of 25° to 80°, DFP spectra showed only an amorphous structure (Fig. 1). Higher WHC of DFP could be a result of its amorphous structure (Prusov, Prusova, Radugin, & Zakharov, 2014).

FTIR analysis

The FTIR spectra (8000 cm^{-1} to 400 cm^{-1}) of DFP and DSP are shown in Fig. 2. Strong absorption broad peaks appeared at about 3390 cm^{-1}, which are typical absorption peaks for O—H in the polysaccharide sugar chain. The weak absorption peaks at about 2930 cm^{-1} are regarded as the characteristic of C—H stretching, a feature of polysaccharides. Also, the peaks appearing at 2877 cm^{-1} are related to the symmetric and asymmetric frequency pattern of methyl groups, peaks at 1657 cm^{-1} are related to the stretching vibrations of the O—H bond of water molecules, peaks at 1526 cm^{-1} are related to the stretching of the C═C band, peaks at 1448 cm^{-1} are related to the asymmetric stretching of CH_3 groups, and peaks at 1115 cm^{-1} belong to CH_2. In addition, the peaks at 893 cm^{-1} could be associated with the stretching of the C—O groups of the ester groups and 783 cm^{-1} due to aromatic compounds (Pathania et al., 2016).

Monosaccharides composition

The HPLC analysis of monosaccharides composition of the DFP and

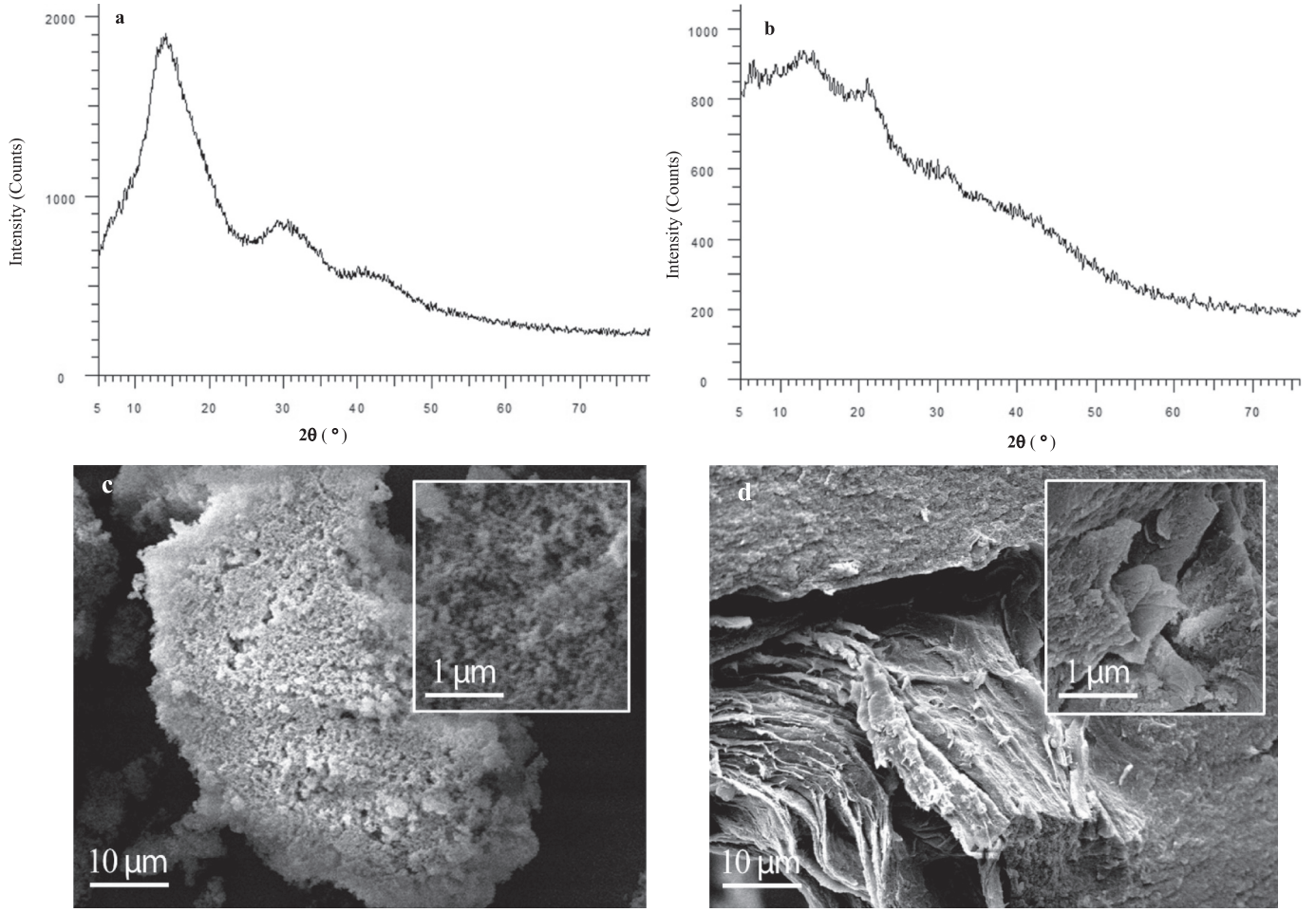


Fig. 1. XRD Curve of DSP (a) and DFP (b), SEM images of DSP (c) and DFP (d).

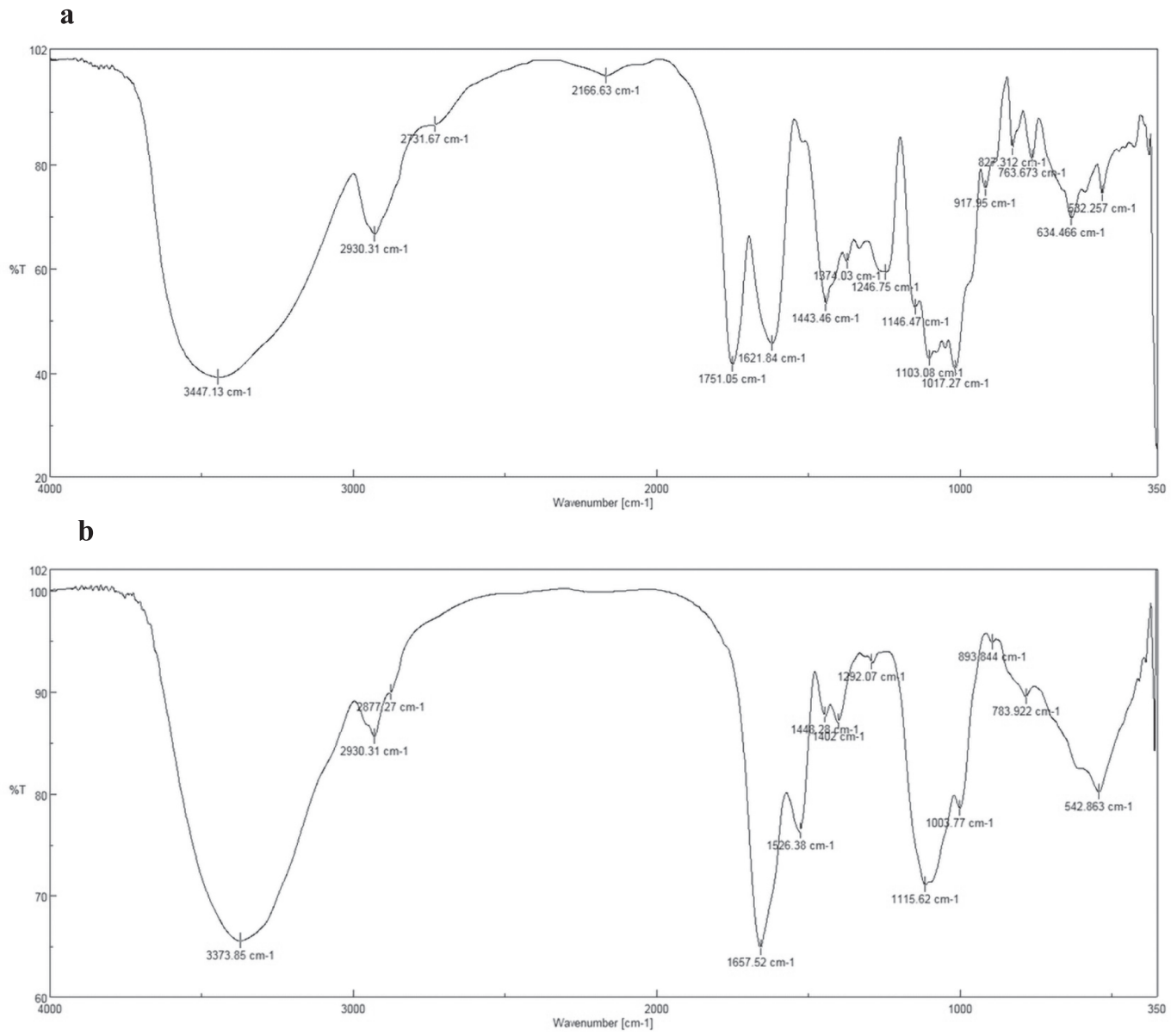


Fig. 2. FTIR spectra for DSP (a), and DFP (b).

DSP is presented in Table 1. It was found that DFP and DSP had different monosaccharide compositions, which demonstrated the diversity of date polysaccharide components. While galactose (41.6 %) was the DFP most abundant monosaccharide, xylose (72.2 %) was the highest monosaccharide in the DSP structure. Therefore, DSP mostly contained pentose, and hexose was higher in DFP. All identified monosaccharides agreed with the previously reported investigations on plant-based polysaccharides (Arab, Ghanbarzadeh, Ayaseh, & Jahanbin, 2021; Ataei, hamidi-Esfahani, & Ahmadi-Gavlighi, 2020; Noorbakhsh et al., 2022).

Determination of prebiotic potential

1 Resistance to gastrointestinal juice

Indigestibility of the DFP, DSP and inulin was investigated in simulated gastrointestinal juice by analyzing the degree of hydrolyzation (Fig. 3 A). The highest hydrolysis percentage was observed in gastric juice (pH = 1.2) for all polysaccharides. DSP with (9.45 ± 0.8 %) was significantly more resistant to acidic conditions than DFP (12.1 ± 1.24 %) and inulin (17.21 ± 1.41 %) ($P < 0.05$). Inulin is more stable in low-acid environments. Therefore in simulated intestinal juice (pH = 7.4) and mixed juice (pH = 4.5), the hydrolysis percentage declined (Glibowski & Bukowska, 2011). Resistance to gastrointestinal juices is one of the crucial factors in prebiotic activities. Prebiotic agents must be able to pass through the upper part digestive system, reach the colon, and be metabolized by the gut microbes. DSP was the most stable polysaccharide in all treatments, with more than 90% stability. The structural difference of polysaccharides, due to different sources and extraction methods, affects their resistance to digestion (Wichienchot et al., 2011). Therefore, DSP and DFP were strong enough to be used as prebiotic agents.

Growth stimulation of probiotics

The proliferation effects of DFP and DSP on *L. plantarum* and *B. animalis* are depicted in Fig. 3 B. The extracted polysaccharides generally had higher growth promoting effect than inulin. The maximum proliferation effect for *L. plantarum* (3.57 ± 0.06 log CFU.g^{-1}) was observed at 3 % DSP and declined at higher concentrations after 72 h fermentation. In contrast, the *B. animalis* population (4.32 ± 0.04 log

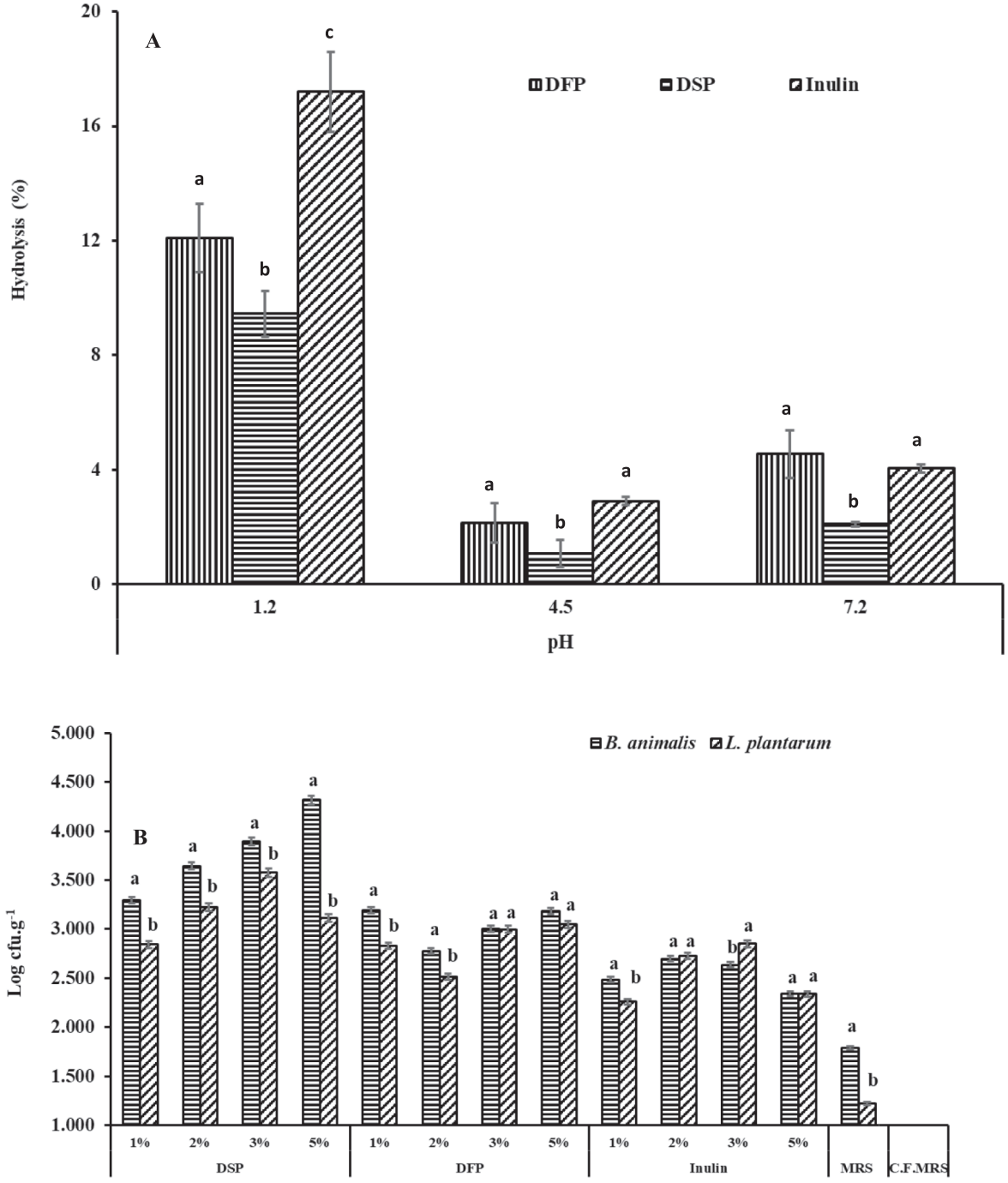


Fig. 3. A) Resistance of DSP, DFP and inulin to gastric juice, pH = 1.2, mixed juice, pH = 4.5, and intestinal juice, pH = 7.2. Data are mean hydrolysis percentage (%) ± SD. A different letter in each column means a significant difference ($p < 0.5$). B) The proliferation effects of extracted polysaccharides on selected strains during 72 h fermentation with different concentrations (1, 2, 3, and 5%) of date seed polysaccharide (DSP), date flesh polysaccharide (DFP), inulin, regular MRS, and carbohydrate-free MRS (C.F. MRS) medium. Data are mean ± SD log CFU.g^{-1}; different letters at each media mean a significant difference ($p < 0.5$). C) The Antibacterial effects on extracted polysaccharides on selected strains. Data are mean inhibition zone diameter (mm) ± SD.

CFU.g^{-1}) increased with increasing concentrations of DSP. DSP had a higher proliferation effect on both selected probiotic strains than the DFP, where the effect was more significant for the *B. animalis*. The discrepancy in the prebiotic activity of extracted polysaccharides could be interpreted by differences in their chemical characteristics. The higher arabinose and xylose content could be a reason for the better proliferation effects of DSP (Liu et al., 2021). Antioxidant activity is another critical factor that influences the prebiotic activities of polysaccharides. Free radicals lead to oxidative stress and inhabit microorganisms' growth (Liu et al., 2021). The higher antioxidant capacity of DSP compared to DFP and inulin could be attributed to proliferation effects.

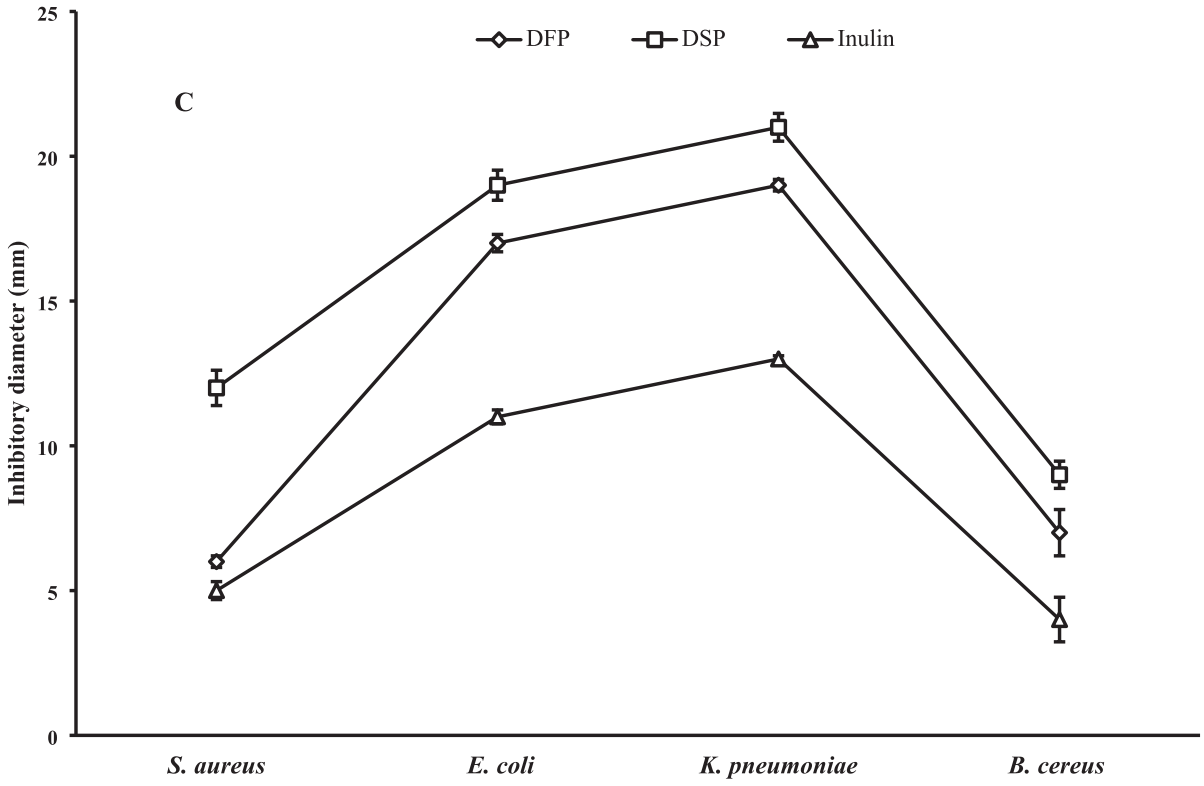


Fig. 3. (*continued*).

Short-chain fatty acids (SCFAs) production

After passing through the upper part of the digestive system and not being digested by the digestive juice, the polysaccharides are metabolized by the gut microbiota in the colon and produce a wide range of short-chain fatty acids including lactate, propionate, acetate, and butyrate. The concentration of fatty acids produced by *L. plantarum* and *B. animalis* is shown in Table 2. A significant increase in the concentration of SCFAs was observed in the MRS medium supplemented with DSP and DFP compared to inulin ($P < 0.05$). While *B. animalis* produced the highest total SCFAs in MRS + DSP (66.98 ± 4.33 mM), no significant difference was observed between MRS + DSP (52.49 ± 9.04 mM) and MRS + DFP (49.76 ± 8.21 mM) fermented by *L. plantarum*. The highest lactic acid and total SCFAs concentration were produced in the MRS + glucose medium for both strains which shows the fast metabolization of glucose by the probiotics.

Bifidobacteria naturally colonize in poor mono- and disaccharides

Table 2
Differential analysis of short-chain fatty acid profile, phenyllactic acid concentration in probiotic bacteria across various media, and blood biomarkers pre- and post-intervention with date seed powder.

SCFAs (mM)*							PLA ($mg.l^{-1}$)
Bacteria	Medium	Lactic Acid	Acetic Acid	Propionic Acid	Butyric Acid	Total SCFAs	
L. plantarum	C. F. MRS	4.87 ± 0.85^{c}	0.56 ± 0.04^{d}	1.02 ± 0.78^{c}	1.91 ± 0.71^{d}	8.36 ± 1.73^{d}	–
	MRS + Glucose	79.56 ± 4.44^{a}	1.06 ± 0.13^{c}	3.95 ± 1.01^{b}	2.61 ± 1.16^{c}	87.18 ± 8.27^{a}	98.57 ± 2.85^{c}
	MRS + Inulin	8.45 ± 1.33^{a}	3.51 ± 1.84^{b}	3.21 ± 0.89^{b}	3.06 ± 0.14^{c}	18.23 ± 0.54^{c}	102.94 ± 5.21^{b}
	MRS + DFP	9.06 ± 1.17^{b}	14.27 ± 1.47^{a}	16.47 ± 2.65^{a}	9.96 ± 1.23^{a}	49.76 ± 8.21^{b}	103.27 ± 3.48^{b}
	MRS + DSP	13.16 ± 1.88^{b}	13.51 ± 1.96^{a}	18.16 ± 1.24^{a}	7.66 ± 0.88^{b}	52.49 ± 9.04^{b}	132.56 ± 3.01^{a}
B. animalis	C. F. MRS	3.65 ± 0.63^{d}	0.48 ± 0.11^{c}	0.34 ± 0.01^{d}	2.87 ± 1.16^{d}	7.34 ± 1.95^{e}	–
	MRS + Glucose	80.01 ± 6.21^{a}	1.94 ± 0.23^{b}	2.98 ± 1.04^{c}	4.06 ± 0.09^{c}	88.99 ± 9.05^{a}	88.81 ± 2.49^{d}
	MRS + Inulin	9.21 ± 1.97^{c}	2.14 ± 0.18^{b}	3.06 ± 0.13^{c}	5.27 ± 0.83^{c}	19.68 ± 3.73^{d}	92.22 ± 7.91^{c}
	MRS + DFP	9.37 ± 1.43^{c}	16.06 ± 1.22^{a}	20.44 ± 0.87^{b}	12.71 ± 2.04^{a}	58.58 ± 5.57^{c}	100.24 ± 8.55^{b}
	MRS + DSP	17.64 ± 2.24^{b}	15.51 ± 0.91^{a}	23.71 ± 1.36^{a}	10.12 ± 1.15^{b}	66.98 ± 4.33^{b}	123.01 ± 5.25^{a}

Date Seed Powder Intervention (Blood Biomarkers) **

Biomarkers	Baseline	After Intervention	Unit
TG	103.42 ± 17.53^{a}	76.41 ± 9.47^{b}	$mg.dl^{-1}$
Hs-CRP	0.1429 ± 0.022^{a}	0.1097 ± 0.017^{b}	$mg.\ l^{-1}$
FBS	96.12 ± 5.31^{a}	86.49 ± 4.78^{b}	$mg.dl^{-1}$
RBC	4.62 ± 0.38^{a}	5.67 ± 0.42^{b}	$10^{6}.\mu l^{-1}$
RDW	12.27 ± 0.78^{a}	14.64 ± 0.86^{b}	%

Abbreviations: PLA: phenyllactic acid, C.F. MRS: Carbohydrate-free MRS; DSP: Date Seed Polysaccharide; DFP: Date Flesh Polysaccharides; FBS: Fasting Blood Sugar; TG: Serum Triglyceride; Hs-CRP: High-Sensitivity C-Reactive Protein; FBS: Fasting Blood Sugar; RBC: Red Blood Cell Count; RDW: Red Cell Distribution Width.

* The values are mean ± SD; different letters in a column for each strain mean a significant difference ($p < 0.05$).

** The values are mean ± SD ($n = 10$); a different letter in a row means a significant difference ($p < 0.05$).

environments such as the lower gastrointestinal tract., they have unique metabolic pathways, e.g., phosphoketolase pathway and bifid shunt for diverse carbohydrates metabolization (Egan & Van Sinderen, 2018). Therefore, the higher SCFAs production of *Bifidobacteria* in the current investigation could be related to their higher ability to metabolize polysaccharides. Hexose can be fermented into lactate and acetate by bifidobacteria with a theoretical yield of 1.5 mol acetate and 1 mol of lactate for every mol of glucose consumed or a 1:1 ratio of lactate and acetate in the case of pentose sugar fermentation. However, the actual ratio of acetate to lactate depends on the individual strain, pH, and growth rate, which differ depending on the carbohydrate substrate utilized (Egan et al., 2018). The monosaccharide analysis of DSP and DFP showed that they contain galactose, mannose, and xylose residue, which can be a reason for the different ratios of produced SCFAs.

In light of the importance of SCFAs produced by gut microbiota, several studies have been conducted on their health effect on the gastrointestinal tract; SCFAs can increase nutrient absorption and inhibit pathogen growth by reducing the intestinal pH. Propionate and acetate have a preventive impact on metabolic syndrome through gluconeogenesis. Moreover, butyrate is a major energy source for colonocytes, leading to pain reduction during defecation and inflammation in the gut (Ashaolu, Ashaolu, & Adeyeye, 2021). The higher SCFAs production in the MRS + DSP compared to inulin makes DSP a potent prebiotic substance which can be used in nutraceuticals.

Phenyllactic acid (PLA) assay

A comparison of PLA concentration produced by selected strains with different polysaccharide is presented in Table 2. The highest PLA concentration was produced by *L. plantarum* and *B. animalis* in MRS + DSP (132.56 ± 3.01 mg.l^{-1}) and (123.01 ± 5.25 mg.l^{-1}), respectively. The higher proliferation potential of DSP could be a reason for increase in PLA concentration after 72 fermentations. Probiotics can convert aromatic amino acids to their lactic acid derivatives such as PLA, which PLA can protect the intestinal barrier against pathogens and improve the host immune function (Laursen et al., 2021). However, PLA is an effective marker of the antimicrobial potential of LABs and has a notable impact on the formation of flavored compounds in fermented products (Armaforte et al., 2006).

Antibacterial activity

The antibacterial effects of DFP and DSP on selected strains are illustrated in Fig. 3 C. DSP and DFP showed higher antibacterial activity than inulin. Gram-negative pathogens (*E. coli* and *K. pneumoniae*) were more sensitive to DSP and DFP. In addition, DSP was more effective on gram-positive bacteria (*S. aurous* (12.2 ± 0.61 mm) and *B. cereus* (9.1 ± 0.47 mm)) compared to inulin and DFP. The antibacterial activities of polysaccharides are related to their molecular weight, structural properties, and extraction methods. As gram-negative and gram-positive bacteria differ in cell structure, e.g., thicker peptidoglycan layer in gram-positive, they are differently affected by plant polysaccharides. The cell membrane of bacteria is the main part that is influenced by polysaccharides through intermolecular forces, leading to increased permeability of the cell membrane, preventing the pathogens to attached epithelial cells or blocking the transmembrane transport of substances. (Zhou et al., 2022).

Intervention

Intervention and blood biomarkers analysis

The blood biomarkers were measured at baseline and after consuming 30 g date seed powder or 50 g seedless date fruit daily for 28 days. The PLS-DA model separated participants into two groups (date seed powder as X variable and timepoint as Y variable; model parameters $R^2Y = 0.77$, $Q^2Y = 0.61$, Cross-Validated-ANOVA $p = 0.0002$) (Fig. 4). The Variable Importance in Projection (VIP) discriminative variables, including TG, CRP, FBS, RBC, and RDW, were analyzed using univariate data analysis and found to be significantly different before and after date seed powder intervention ($p < 0.05$). The results showed a significant reduction in TG, CRP, and FBS and an increase in RBC and

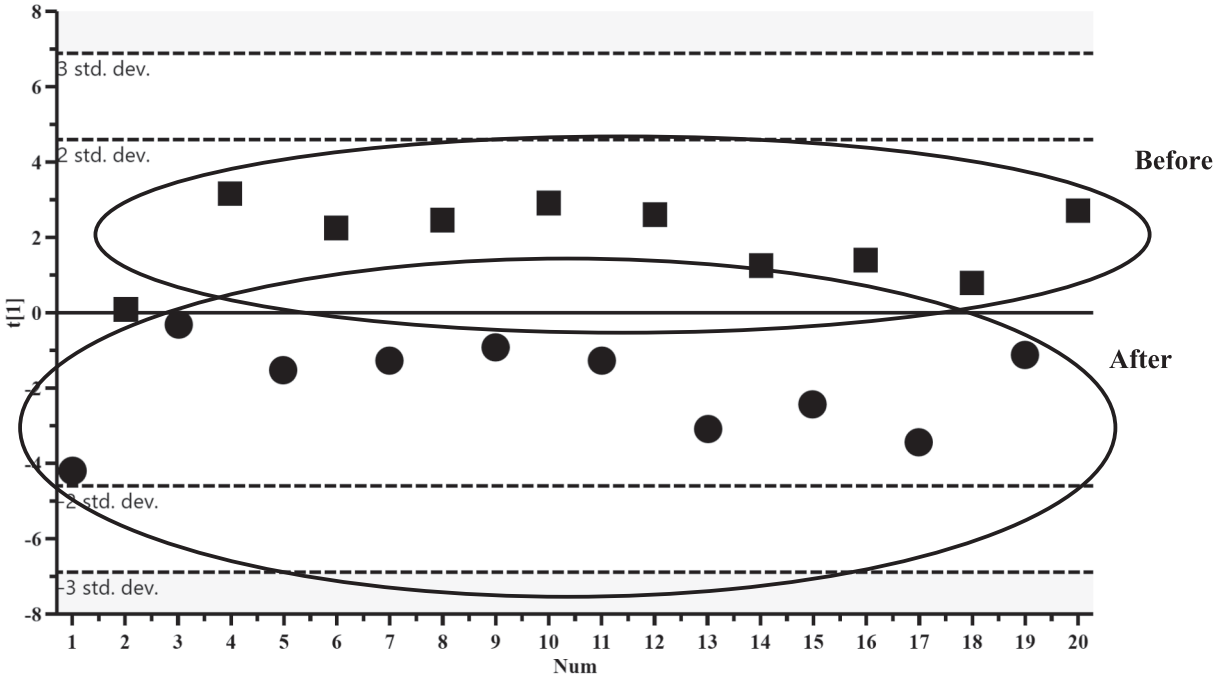


Fig. 4. The score plot for Partial Least-Square Discriminant Analysis (PLS-DA) model for blood biomarker concentrations for date seed powder intervention based on time point; two groups are separated along with the first predictive component. Model parameter were $R^2Y = 0.77$, $Q^2Y = 0.61$, and Cross-Validated ANOVA, $P = 0.020$.

RDW ($p < 0.05$) (Table 2). None of the fitted models for date fruit intervention was significant regarding R^2Y, Q^2Y, or CV-ANOVA.

CRP level is a sensitive marker of inflammation, several studies approved that a higher level of dietary fiber decreases blood CRP concentration (Jiao, Xu, Zhang, Han, & Qin, 2015). In the current study, date seed powder as a high polysaccharide content source led to a significant reduction in CRP concentration. Serum CRP concentration is recently considered as a biomarker for intestinal inflammation (Chen et al., 2020). It implies that date seed polysaccharides reduced colon pH by higher production of SCFAs and improved the growth of beneficial microorganisms in the intestine, reducing inflammation.

Recent study has reported that natural polysaccharides have a notable TG-lowering effect mainly through regulation of ATGL-(PPAR-α)/(PGC-1α), (SREBP-1c)-ACC/FAS, and ACC-CPT1 pathways (Ghribi et al., 2015). Elevated expression of CPT1 associated with high hepatic fatty acid β-oxidation and reduction of TG secretion was reported after administration of chicory polysaccharide in non-alcoholic fatty acid disease (Y. Wu, Zhou, Jiang, Wang, Hua, & Zhang, 2018). Although date seed powder significantly ($p < 0.05$) reduced blood TG, more investigation is needed to elucidate the exact TG reduction mechanism of DSP.

High FBS (≥126 mg/dl) is one of the most important biomarkers for the diagnosis of diabetes. Although, in the current study, the participants' FBS level was in the typical medical range (84 to 99 mg/dl), a significant reduction was observed after DSP intervention. The most common suggested mechanism for the hypoglycemic effect of polysaccharides is modulation of GUT microbiota, increased blood insulin levels, and increased glucose metabolism in peripheral tissues (Zhong et al., 2021).

An increase in RBC after date seed powder intervention could be described by the anti-erythrocytes' hemolysis effect of polysaccharides due to date seed powder antioxidant activity. A study on the anti-erythrocytes' hemolysis effect of *Epimedium acuminatum* polysaccharides' using an AAPH-induced hemolysis assay revealed that polysaccharides could act as antioxidants to reduce erythrocytes' oxidative membrane damage (Cheng, Feng, Jia, Li, Zhou, & Ding, 2013).

Conclusion

Date fruit and date seed polysaccharides were extracted using ultrasounic-assisted alkaline extraction. Chemical structure analysis showed that DSP and DFP are heteropolysaccharides. DSP mainly consists of xylose and mannose, while DFP consists of galactose and arabinose. Short-chain fatty acids analysis revealed that extracted polysaccharides produced a higher concentration of SCFAs than inulin during fermentation, and *B. animalis* had a higher capability to ferment DSP and DFP. Date polysaccharides showed considerable antibacterial activity, mainly on gram-negative pathogens. Moreover, the date seed intervention significantly reduced TG, CRP, FBS, and erythrocyte hemolysis. The results revealed that each part of date fruit has different polysaccharides with unique chemical properties. Therefore, the higher prebiotic and antioxidant characteristics of DSP, which led to improved health status blood biomarkers, could be resulted from different chemical properties compared to DFP. Consequently, date seed polysaccharides can be considered as inexpensive resource for further investigation of bioactive properties.

CRediT authorship contribution statement

Hamid Noorbakhsh: Investigation, Methodology, Formal analysis, Data curation. **Mohammad Rabbani Khorasgani:** Conceptualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

The authors thank Professor Ahmad Yaraghi for help in the collection of human samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100834.

References

Ahmadi, S., Nagpal, R., Wang, S., Gagliano, J., Kitzman, D. W., Soleimanian-Zad, S., … Yadav, H. (2019). Prebiotics from acorn and sago prevent high-fat-diet-induced insulin resistance via microbiome–gut–brain axis modulation. *The Journal of nutritional biochemistry, 67*, 1–13.

Arab, K., Ghanbarzadeh, B., Ayaseh, A., & Jahanbin, K. (2021). Extraction, purification, physicochemical properties and antioxidant activity of a new polysaccharide from Ocimum album L. seed. *International Journal of Biological Macromolecules, 180*, 643–653.

Armaforte, E., Carri, S., Ferri, G., & Caboni, M. F. (2006). High-performance liquid chromatography determination of phenyllactic acid in MRS broth. *Journal of Chromatography A, 1131*(1), 281–284. https://doi.org/10.1016/j.chroma.2006.07.095

Ashaolu, T., Ashaolu, J., & Adeyeye, S. (2021). Fermentation of prebiotics by human colonic microbiota in vitro and short-chain fatty acids production: A critical review. *Journal of Applied Microbiology, 130*(3), 677–687.

Ataei, D., hamidi-Esfahani, Z., & Ahmadi-Gavlighi, H. (2020). Enzymatic production of xylooligosaccharide from date (Phoenix dactylifera L.) seed. *Food Science & Nutrition, 8*(12), 6699–6707.

Behbahani, B. A., Yazdi, F. T., Shahidi, F., Mortazavi, S. A., & Mohebbi, M. (2017). Principle component analysis (PCA) for investigation of relationship between population dynamics of microbial pathogenesis, chemical and sensory characteristics in beef slices containing Tarragon essential oil. *Microbial Pathogenesis, 105*, 37–50.

Blanco-Pérez, F., Steigerwald, H., Schülke, S., Vieths, S., Toda, M., & Scheurer, S. (2021). The dietary fiber pectin: Health benefits and potential for the treatment of allergies by modulation of gut microbiota. *Current allergy and asthma reports, 21*(10), 1–19.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical biochemistry, 54*(2), 484–489.

Chen, H., Chen, Z., Fu, Y., Liu, J., Lin, S., Zhang, Q., … Han, G. (2019). Structure, antioxidant, and hypoglycemic activities of arabinoxylans extracted by multiple methods from triticale. *Antioxidants, 8*(12), 584.

Chen, P., Zhou, G., Lin, J., Li, L., Zeng, Z., Chen, M., & Zhang, S. (2020). Serum biomarkers for inflammatory bowel disease. *Frontiers in Medicine, 7*, 123.

Chen, X., Crimmins, E., Hu, P. P., Kim, J. K., Meng, Q., Strauss, J., … Zhao, Y. (2019). Venous Blood-Based Biomarkers in the China Health and Retirement Longitudinal Study: Rationale, Design, and Results From the 2015 Wave. *American Journal of Epidemiology, 188*(11), 1871–1877. https://doi.org/10.1093/aje/kwz170

Cheng, H., Feng, S., Jia, X., Li, Q., Zhou, Y., & Ding, C. (2013). Structural characterization and antioxidant activities of polysaccharides extracted from Epimedium acuminatum. *Carbohydrate Polymers, 92*(1), 63–68.

Cui, Y., Wang, S., Wang, S., Cao, S., Wang, X., & Lü, X. (2022). Extraction optimization and characterization of persimmon peel pectin extracted by subcritical water. *Food Chemistry: X, 16*, Article 100486. https://doi.org/10.1016/j.fochx.2022.100486

Egan, M., & Van Sinderen, D. (2018). Carbohydrate metabolism in bifidobacteria. In *The bifidobacteria and related organisms* (pp. 145–164). Elsevier.

Elleuch, M., Besbes, S., Roiseux, O., Blecker, C., Deroanne, C., Drira, N.-E., & Attia, H. (2008). Date flesh: Chemical composition and characteristics of the dietary fibre. *Food Chemistry, 111*(3), 676–682.

Evans, S. R. (2010). Clinical trial structures. *Journal of experimental stroke & translational medicine, 3*(1), 8.

Feriani, A., Tir, M., Hamed, M., Sila, A., Nahdi, S., Alwasel, S., … Tlili, N. (2020). Multidirectional insights on polysaccharides from Schinus terebinthifolius and Schinus molle fruits: Physicochemical and functional profiles, in vitro antioxidant, anti-genotoxicity, antidiabetic, and antihemolytic capacities, and in vivo anti-inflammatory and anti-nociceptive properties. *International Journal of Biological Macromolecules, 165*, 2576–2587.

Fu, L., Xu, B.-T., Xu, X.-R., Gan, R.-Y., Zhang, Y., Xia, E.-Q., & Li, H.-B. (2011). Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry, 129*(2), 345–350.

Ghnimi, S., Umer, S., Karim, A., & Kamal-Eldin, A. (2017). Date fruit (Phoenix dactylifera L.): An underutilized food seeking industrial valorization. *NFS journal, 6*, 1–10.

Ghribi, A. M., Sila, A., Gafsi, I. M., Blecker, C., Danthine, S., Attia, H., … Besbes, S. (2015). Structural, functional, and ACE inhibitory properties of water-soluble

polysaccharides from chickpea flours. *International Journal of Biological Macromolecules, 75*, 276–282.
Glibowski, P., & Bukowska, A. (2011). The effect of pH, temperature and heating time on inulin chemical stability. *Acta Scientiarum Polonorum Technologia Alimentaria, 10*(2), 189–196.
Horwitz, W., & Latimer, G. (2000). AOAC official methods of analysis. *Gaithersburg, MD: Association of Official Analytical Chemists International. Sections, 50*(21), 992.905.
Huang, F., Liu, H., Zhang, R., Dong, L., Liu, L., Ma, Y., … Zhang, M. (2019). Physicochemical properties and prebiotic activities of polysaccharides from longan pulp based on different extraction techniques. *Carbohydrate Polymers, 206*, 344–351.
Jiao, J., Xu, J.-Y., Zhang, W., Han, S., & Qin, L.-Q. (2015). Effect of dietary fiber on circulating C-reactive protein in overweight and obese adults: A meta-analysis of randomized controlled trials. *International Journal of Food Sciences and Nutrition, 66*(1), 114–119.
Laursen, M. F., Sakanaka, M., von Burg, N., Mörbe, U., Andersen, D., Moll, J. M., … Mølgaard, C. (2021). Bifidobacterium species associated with breastfeeding produce aromatic lactic acids in the infant gut. *Nature Microbiology, 6*(11), 1367–1382.
Liang, S., Wu, X., & Jin, F. (2018). Gut-brain psychology: Rethinking psychology from the microbiota–gut–brain axis. *Frontiers in Integrative Neuroscience, 12*, 33.
Liu, H., Wei, X., Zu, S., Lin, X., Zhang, J., Shi, A., … He, N. (2021). Separation and identification of neutral oligosaccharides with prebiotic activities from apple pectin. *Food Hydrocolloids, 121*, Article 107062.
Noorbakhsh, H., & Khorasgani, M. R. (2022). Date (Phoenix dactylifera L.) polysaccharides: A review on Chemical structure and nutritional properties. *Journal of Food Measurement and Characterization*, 1–11.
Noorbakhsh, H., Yavarmanesh, M., Mortazavi, S. A., Adibi, P., & Moazzami, A. A. (2019). Metabolomics analysis revealed metabolic changes in patients with diarrhea-predominant irritable bowel syndrome and metabolic responses to a synbiotic yogurt intervention. *European Journal of Nutrition, 58*(8), 3109–3119.
Pathania, D., Sharma, A., & Siddiqi, Z.-M. (2016). Removal of congo red dye from aqueous system using Phoenix dactylifera seeds. *Journal of Molecular Liquids, 219*, 359–367.
Prusov, A., Prusova, S., Radugin, M., & Zakharov, A. (2014). Interrelation between the crystallinity of polysaccharides and water absorption. *Russian Journal of Physical Chemistry A, 88*(5), 813–818.
Sun, H., Li, C., Ni, Y., Yao, L., Jiang, H., Ren, X., … Zhao, C. (2019). Ultrasonic/microwave-assisted extraction of polysaccharides from Camptotheca acuminata fruits and its antitumor activity. *Carbohydrate Polymers, 206*, 557–564.
Wichienchot, S., Thammarutwasik, P., Jongjareonrak, A., Chansuwan, W., Hmadhlu, P., Hongpattarakere, T., … Ooraikul, B. (2011). Extraction and analysis of prebiotics from selected plants from southern Thailand. *Songklanakarin Journal of Science & Technology, 33*(5).
Woisky, R. G., & Salatino, A. (1998). Analysis of propolis: Some parameters and procedures for chemical quality control. *Journal of Apicultural Research, 37*(2), 99–105.
Wu, Q., Wang, Q., Fu, J., & Ren, R. (2019). Polysaccharides derived from natural sources regulate triglyceride and cholesterol metabolism: A review of the mechanisms. *Food & function, 10*(5), 2330–2339.
Wu, Y., Zhou, F., Jiang, H., Wang, Z., Hua, C., & Zhang, Y. (2018). Chicory (Cichorium intybus L.) polysaccharides attenuate high-fat diet induced non-alcoholic fatty liver disease via AMPK activation. *International Journal of Biological Macromolecules, 118*, 886–895.
Yang, C., He, N., Ling, X., Ye, M., Zhang, C., Shao, W., … Li, Q. (2008). The isolation and characterization of polysaccharides from longan pulp. *Separation and Purification Technology, 63*(1), 226–230.
Zhao, R., Ji, Y., Chen, X., Su, A., Ma, G., Chen, G., … Zhao, L. (2020). Effects of a β-type glycosidic polysaccharide from Flammulina velutipes on anti-inflammation and gut microbiota modulation in colitis mice. *Food & Function, 11*(5), 4259–4274.
Zhong, Q.-W., Zhou, T.-S., Qiu, W.-H., Wang, Y.-K., Xu, Q.-L., Ke, S.-Z., … Zhang, H.-W. (2021). Characterization and hypoglycemic effects of sulfated polysaccharides derived from brown seaweed Undaria pinnatifida. *Food Chemistry, 341*, Article 128148.
Zhou, Y., Chen, X., Chen, T., & Chen, X. (2022). A review of the antibacterial activity and mechanisms of plant polysaccharides. *Trends in Food Science & Technology*.