Exploration of Polysaccharides and Oligosaccharides from Jali (*Coix Lacryma-jobi*) and Its Potential as Prebiotic

Devi Arianty[2*], Aji Sutrisno[1], Agustin Krisna Wardani[1]

[1]Department of Agricultural Product Technology, Faculty of Agricultural Technology, University of Brawijaya, Jl. Veteran, Ketawanggede, Malang, 65145, Indonesia
[2]Fisheries Resource Utilization, Faculty of Fisheries, Muhammadiyah University Kupang, Jl. K.H. Ahmad Dahlan, Oebobo, Kupang, 85228, Indonesia
[*]Corresponding author: Devi arianty, Email: devi.arianty@unmuhkupang.ac.id

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ABSTRACT

Jali (*Coix lacryma-jobi*) is a cereal plant widely used as a functional food because it contains carbohydrate compounds, such as polysaccharides and oligosaccharides with a positive impact on the digestive system. This study was divided into two stages, namely extraction Jali and prebiotic analysis. The extraction method used was hot water extraction and alkali extraction according to their solubility in solvents. The prebiotic activity of oligosaccharide and polysaccharide extracts from jali was evaluated using in-vitro analysis. Therefore, this study aimed to explore polysaccharides and oligosaccharides in jali and their potential to act as prebiotic. The results showed that the extraction process affected the types of oligosaccharides, namely Fructooligosaccharides (FOS), as well as polysaccharides, including α-glucan and arabinoxylan. In this study, FOS and α-glucan were obtained by heating at 80 °C for 60 minutes, while arabinoxylan was extracted by heating at 80 °C for 120 minutes. The results of crude extracts of FOS and arabinoxylan were tested for HPLC analysis, while α-glucan was explored using FTIR. The jali seeds exhibited a remarkable FOS content of 40.78%, while their arabinoxylan composition included 22.4% arabinose and 4.8% xylose. In addition, the FTIR analysis revealed the presence of (1→4) (1→6) -α-D-glucan bond in jali seeds. The results showed that the extraction from the polysaccharide group, namely α-Glucan and Arabinoxylan, as well as from the oligosaccharides (FOS) had potential as prebiotic for the growth of *Bifidobacterium longum* and *Lactobacillus casei.* However, the highest results were based on OD and SCFA from the FOS extract. The addition of FOS affected the growth of *Bifidobacterium longum* more significantly (OD 0,871) compared to *Lactobacillus casei* (OD 0,725). *Bifidobacterium longum* exhibited SCFA levels of 243,827 mmol/L, while *Lactobacillus casei* showed levels of 140,942 mmol/L.

Keywords: Arabinoxylan; *Coix lachryma-jobi* (jali); fructooligosaccharides (FOS); glucan; prebiotic

INTRODUCTION

Jali (*Coix lachryma-jobi*) is a cereal plant containing oligosaccharides and polysaccharides-type carbohydrates with the potential to act as prebiotic in the digestive tract. In addition, these compounds can be fermented by intestinal bacteria and hydrolyzed into short-chain fatty acids, leading to their use as a source of nutrition for growth (Djaja, 2022). "Both polysaccharides and oligosaccharides have a number of repeating monosaccharide units in the carbohydrate molecular chain (degree of polymerization); oligosaccharides consist of 2-10 monosaccharide units, while polysaccharides contain more than 10 units. According to Rusdi et al. (2021) the well-known oligosaccharides are Fructosoligosaccharides (FOS),

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Galactooligosaccharides (GOS), Lactulose which derive galactooligosaccharides (LDGOS), xylooligosaccharides (XOS), while polysaccharides include α/β-glucan and arabinoxylan. These oligosaccharides type are widely used as dietary fiber for human digestion. For example, it is a source of nutrition for probiotic bacteria growth, which are capable of producing organic compounds and have an important role in inhibiting the growth of pathogenic microbes. Similarly, certain polysaccharides exhibit prebiotic properties such as hemicellulose, pectin, cellulose, and resistant starch. These compounds are commonly found in a variety of plant materials, including cereals, mushrooms, soybeans, and potatoes. Jali has also been reported to contain 1.88% protein, 0.04% fat, 5.53% dietary fiber, and 25.73% polysaccharide (Husna et al., 2018; Laxmisha et al., 2022).

Glucan typically acts as a structural component of cell walls and energy reserves in tissue. Based on their stereochemical composition, these compounds can be divided into α-glucan and β-glucan, where the hydroxyl group is located in the axial (vertical) and equatorial (horizontal) positions, respectively (Venkatachalam et al., 2021). In this context, α-glucan is one of the components in starch composed of glucose monomers and is used as a reserve energy supply needed by plant cells. Meanwhile, β-glucan is a non-starch polysaccharides component, which acts as dietary fiber because it is difficult for human digestive enzymes to digest (Lovegrove et al., 2017). Several studies have shown the benefits of glucan as an anti-cancer by increasing immunity and lowering blood cholesterol (Murphy et al., 2020). These compounds can also be hydrolyzed using acids, alkalis, enzymes, and water. According to Maheshwari, (2017), glucan extraction using the hot water method produces a greater yield and higher purity.

Apart from glucan, another type of polysaccharides, namely Arabinoxylan, is a constituent of the cell wall structure, which is cross-linked with cellulose connective tissue. In addition, it consists of a D-xylopyranoxyl "backbone" with β-1,4 glycosidic bonds. Arabinoxylan has a replacement α-L arabinofuranosyl residue in the D-xylopyranoxyl branch chain at position C-2 (2mXyl), C-3 (3mXyl) or both C-2 and C-3 (dXyl) (Zannini et al., 2022). The branch chain proportion has been reported to show the ratio of arabinose to Xylose (Ara/Xyl). This ratio indicates the degree of branching of the arabinose chain that binds to the side chain of the α-L arabinofuranosyl group on the main xylan framework. Zhang et al. (2014) stated that the extraction of arabinoxylan in several cereal plants using alkaline solvents could produce a higher yield, namely 16–57% compared to enzymatic methods (1.08–50.7%) and water (0.33–1. 4%). During the process, alkaline solvents are commonly used due to their cost-effectiveness, safety, and ease of breaking the bonds of arabinoxylan with cell wall tissue compared to the use of water or enzyme solvents.

In line with these findings, FOS, α-glucan, and arabinoxylan have the potential to act as prebiotic. This is primarily due to the ability to increase probiotic bacteria, such as the *Lactobacillus* and *Bifidobacterium* groups as well as inhibit pathogenic bacteria, including *E. coli*. Parhi et al. (2022) reported that the addition of FOS mixed in various types of sugar solutions can increase the growth index of *Bifidobacterium* bacteria by 78.5%. According to Zhao & Cheung (2011), glucan compounds increased the growth of *Bifidobacterium infantis, Bifidobacterium longum,* and *Bifidobacterium adolescentis* during fermentation. Pandey et al. (2016) also revealed that arabinoxylan compounds were able to increase the number of *Lactobacillus casei* by 0.419 g/g from *physilium* mushroom extract compared to only 0.25 g/g glucose substrate. Building on this idea, several methods of probiotic bacteria in maintaining the health of the digestive tract include colonization, attachment to intestinal mucosal tissue, and stimulation of the immunity of the intestinal mucosa "host" to regulate the balance of the number of probiotic bacteria against pathogenic bacteria. In addition, these microbes produce antimicrobials and reduce pH in the lumen, which can inhibit pathogens (Gorreja & Walker, 2022).

Probiotic bacteria, such as *Lactobacillus casei* and *Bifidobacterium longum* can produce main metabolites in the form of organic acids (SCFA) during the fermentation process. Acetic and propionic acid typically act as energy sources in cells, while butyric acid is used for cell regeneration (Louis & Flint, 2017). The accumulation of acid from SCFA can cause a decrease in pH during fermentation due to increased H+ ions dissociating from the total acid. Therefore, this study aimed to explore polysaccharides and oligosaccharides compounds from jali *(Coix lachryma-jobi)* and their potential as prebiotic.

METHODS

Materials

Jali *(Coix Lachryma-jobi)* used came from originating from the experimental farm of Padjadjaran University (Jatinangor), *Lactobacillus casei* and *Bifidobacterium longum* bacteria were obtained from PAU Gadjah Mada University (Yogyakarta). This study used MRS broth and MRS agar (Merck), peptone (Merck), Fructooligosaccharides standard (Wako, Osaka, Japan), arabinose, and xylose standard (Sigma-Arldrich, Inc.,

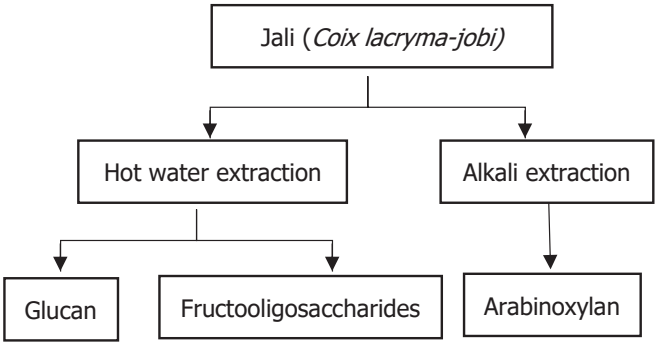

Figure 1. Flowchart of jali extraction

USA). The β-glucan standard was from the "Yeast β-glucan Assay Kit" (Megazyme., Bray, Ireland).

The tools used included a Universal oven (Memmert, Germany), centrifuge (Hettich Zentrifugen EBA 20), UV-VIS Spectro 190 to 1100 nm (Los Angeles, U.S.A), Autoclave 0.14-0.16 MPa (Tony, Taiwan), and Shaker water bath 10-95 °C (Germany).

Extraction of Jali (*Coix lacryma-jobi*)

Jali extraction was performed using two methods: hot water extraction and alkali extraction. Each method aims to produce specific compounds—glucan and fructooligosaccharides (hot water extraction) and arabinoxylan (alkali extraction). The flowchart is illustrated in Figure 1.

Extraction of FOS (Fructooligosaccharides) and α-Glucan

Fructooligosaccharides (FOS) and α-glucan extraction from jali seeds used a modified hot water extraction method (Manosroi & Khositsuntiwong, 2014, Kayal and Many, 2014). FOS was extracted from Jali seeds by removing the skin from the seeds and then grinding it with a blender. The ground flour was filtered using mesh 60 and extracted using distilled water with a ratio of 1:5 (w/v) at a temperature of 80 °C, for 1 hour. Furthermore, the sample was cooled to room temperature and centrifuged at a speed of 5000 g, 40 °C for 20 minutes. The supernatant was separated into 2, namely supernatants 1 and 2, and was dried in an oven at 50 °C for 24 hours. The drying yielded from supernatant one was analyzed as glucan. Moreover, supernatant II was precipitated with cold 90% ethanol solution (40 °C) for 24 hours using a ratio of (1:1) v/v at a temperature of (-15 °C). The precipitation results were centrifuged at 7500 rpm and 40 °C for 20 minutes. The residue from the supernatant 2 containing FOS was then dried at 60 °C for 120 minutes.

Alkaline Extraction of Arabinoxylan

Extraction of arabinoxylan from Jali seeds was carried out using a modified alkali method (Zhou et al., 2010), and the seeds were cleaned from the skin and blended to form flour. This was then filtered with 60 mesh and precipitated with distilled water at 4 °C for 60 min. The resulting precipitate was washed with distilled water at a ratio of 1:5 (w/v) to remove starch. Moreover, this was dried using an oven for 12 hours at a temperature of 50 °C. The drying yield was extracted in a water bath at a temperature of 80 °C for 120 minutes using a solvent with a concentration of (0.15 N NaOH and 0.5% H2O2). The ratio between sample and solvent yield was 1: 15 (w/v). Furthermore, the sample was centrifuged at a speed of 5000 g for 30 minutes, then neutralized with 2 M HCL until it reached pH 4.5, and then the residue was centrifuged again at a speed of 5000 g for 30 minutes. Further treatment was carried out on the supernatant with 90% ethanol for 24 hours at 4 °C, and the mixture was centrifuged again at 5000 g for 30 minutes at 4 °C. Sediment (residue) was dissolved in water 1:5 (w/v), and the supernatant containing Arabinoxylan was then dried in an oven at 50 °C for 1 night.

Sugar Composition Analysis (HPLC)

Fructooligosaccharides and arabinoxylan levels in Jali were analyzed using High-Performance Liquid Chromatography (HPLC) (Zhou et al., 2010). The HPLC used an Aminex HPX-87C column (300 x 7.8 mm) and a Refractive index detector (Bio-Rad Laboratories, USA) (Manosroi and Khositsuntiwong, 2014). The flow rate was 0.6 mL/minute using water as the mobile phase with a maximum column temperature of 80 °C. The standard used for FOS was a mixture of FOS consisting of a mixture of 1-ketose (GF2), nystose (GF3), and 1-β-D-fructofuranosylnystose (GF4). The standards used for arabinoxylan were arabinose and xylose.

FTIR (Fourier Transform Infrared)

Analysis of α-glucan was carried out using an infrared spectrophotometer (FTIR-8400S/SHIMADZU) (Synytsya and Novak, 2014). The FTIR used a wavelength of 500-4000 cm-1 with a resolution of 4 cm-1, and a temperature-controlled high sensitivity detector (DLATGS detector). The α-glucan standard was from the "Yeast β-glucan Assay Kit" (Megazyme., Bray, Ireland).

Optical Density Analysis

Analysis of total bacterial cells was carried out using the optical density method (Margino et al., 2015). For each treatment, 3 mL of MRSB media was taken

which had been given a crude extract of FOS, α-glucan, arabinoxylan, and no crude extract (control) into a visible spectrophotometer cuvette with a wavelength of 600 nm, and OD analysis every 3 hours for 24 hours.

pH

Each sample of FOS, glucan, and arabinoxylan extract grown on *Bifidobacterium longum* and *Lactobacillus casei* was measured using a pH meter every 3 hours during 24-hour fermentation (Rasbawati et al., 2019)

SCFA Analysis

Short-chain fatty acids were analyzed using gas chromatography analysis (Holmes et al., 2022). The liquid sample treatment consisted of control/no extract (B1), the addition of FOS Jali extract (B2), glucan extract (B3), and arabinoxylan extract (B4). Respectively, *Lactobacillus casei* (A1) and *Bifidobacterium longum* (B2) were added at 2%. Furthermore, 1 mL of each liquid sample was taken and then centrifuged at a speed of 300 rpm for 5 minutes. The supernatant was filtered with 0.45 µL millipure and then 0.5 µL was taken to be injected into the gas chromatography system. The gas chromatography equipment used was Shimadzu GC 8, column type (10% Sp 1200 on 1% H3P04) inner diameter of 2.5 mm and with column temperature of 130 °C. The detector used was a Flame Ionisation Detector (FID) with a temperature of 230 °C with Nitrogen (N2) as a carrier gas with a pressure of 1.5 kg/cm2. Analysis of acetic, propionic, and butyric acids was calculated based on the area of each acid with a standard.

Statistical Analysis

The study used a randomized block design with treatment consisting of 2 factors, namely factor a) type of bacteria (*Bifidobacterium longum* and *Lactobacillus casei*) and factor b) type of arabinoxylan extract, glucan, fructooligosaccharides (FOS), as well as control (without extract) with 3 repetitions each. The data were analyzed using two-way analyses of variance (two-way ANOVA) and continued with the Least Significance Difference (LSD) test using the SPSS 16 program.

RESULTS AND DISCUSSION

Yield and Characteristics of Fructooligosaccharides (FOS) Extract

The yield of FOS extract using the hot water extraction method was 14.43%. Fructooligosaccharides (FOS) contained hydroxyl groups which were easily soluble in water (hydrophilic). FOS extraction using heated water could increase the reaction rate, hence causing a higher yield of FOS (Puminat and Teangpook, 2013). According to Viet Bui et al. (2016), FOS were bound to protein, but during extraction, the protein content decreased. Heating the water during extraction could break the hydrogen bonds in the protein, thereby decreasing its ability to bind water and making the FOS structure easier to decompose and dissolve in water. The FOS yield was then processed through centrifugation to obtain a filtrate that had been separated from other impurity compounds such as fat and protein. Centrifugation speed could affect the amount of filtrate obtained due to the centrifugal force acting on the solvent to speed up separation. Fructooligosaccharides standards were analyzed using HPLC which could be seen in Figure 2.

The chromatogram in Figure 2 showed 3 peaks from standard FOS, namely GF4 (1-β-D-fructofuranosylnystose) at RT 6.617, GF3 (nystose) at RT 7.020, and GF2 (1-ketose) RT 7.803. The chromatogram of the FOS standard indicated that the GF4 peak appeared earlier than GF3 and GF2. According to Hogarth et al. (2000), the GF4 compound from FOS contained a longer OH group than GF2 and GF3. The

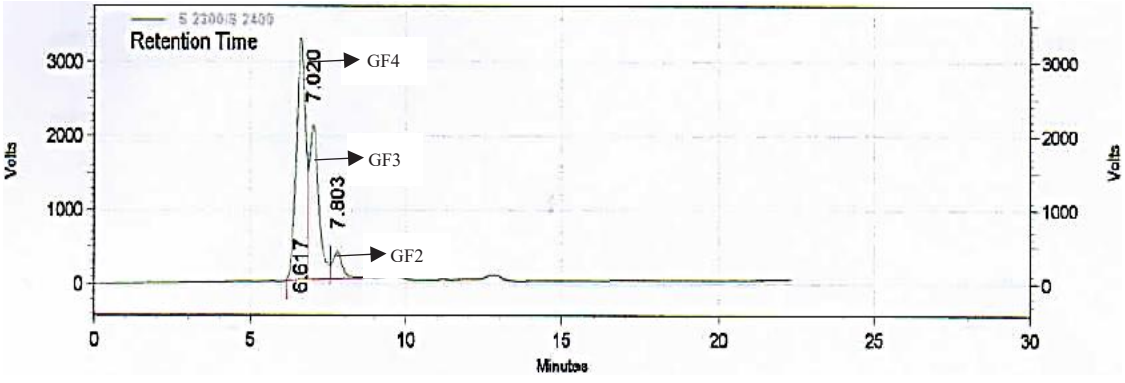

Figure 2. Standard chromatogram of fructooligosaccharides (FOS)

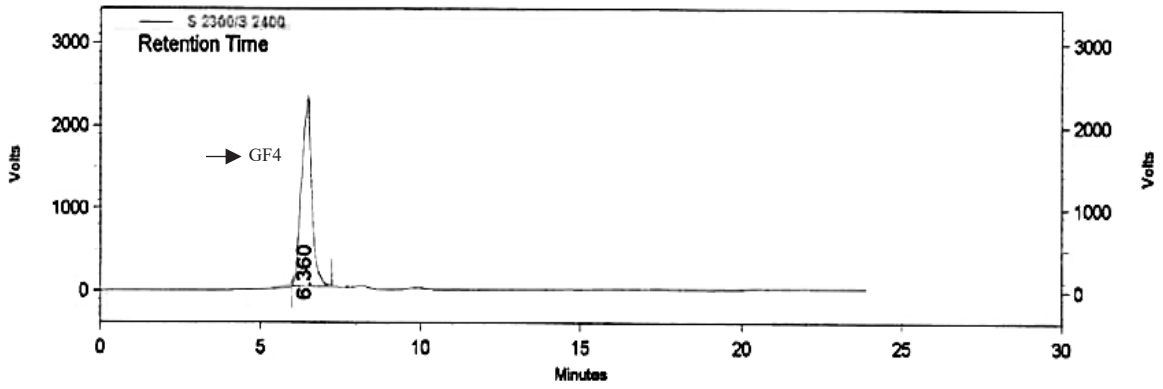

Figure 3. Chromatogram on fructooligosaccharides (FOS) of Jali (*Coix lachrymal-jobi*)

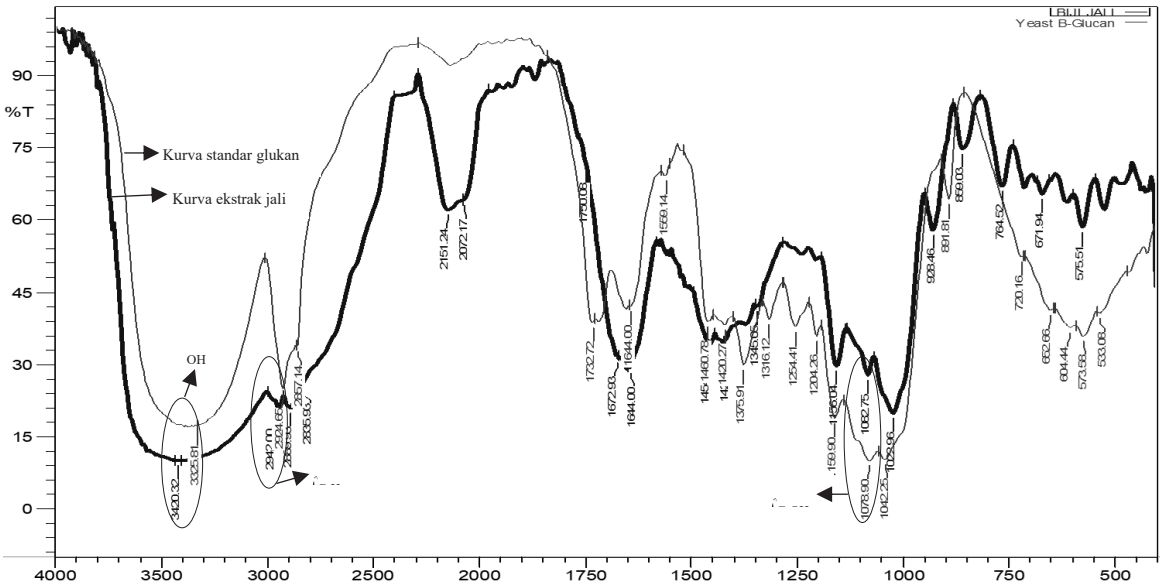

Figure 4. FTIR spectrum between standard glucan (commercial) and glucan extract from Jali

strong interaction between the mobile phase (water) and the longer OH groups in GF4 indicated a faster retention time. Jali crude extract was analyzed using HPLC as shown in Figure 3.

Based on the GF2, GF3, and GF4 standards, the FOS extract in Jali was at a retention RT of 6.36, indicated as 1-β-D-fructofuranosylnystose (GF4) of 40.78% (Figure 3). In the HPLC chromatogram, peaks GF3 (nystose) and GF2 (kestose) were not detected presumably because the levels of these 2 compounds were smaller than the GF4 compound in jali seeds.

Yield and FTIR Characteristics of Glucan Extract

The extract yield is fundamental in plant calculations extract ratio, while extract yield depends on extraction process and quantity extractable material in initial plant biomass. Yield is a comparison of the weight of the extract produced with the weight of Jali seeds as the raw material (Olawuyi et al., 2020). The α-glucan extract yield was 10.80% produced through hot water extraction after a precipitation process with ethanol. The yield in Jali showed higher yields compared to other types of cereal such as jerawut or foxtail millet with an α-glucan content of 6.96 – 9.2% (Anna et al., 2017). In obtaining α-glucan extract, the precipitation process precipitated the glucan compound due to its low solubility. Giving ethanol during precipitation could substitute water and glucan to become water and ethanol. The 90% level used as a co-solvent could affect the water solubility in ethanol, therefore the tension between water and glucan decreased and caused an increase in driving force and hence more precipitate. However, when the ethanol content was low, only partial glucan deposition occurred.

In line with Zhang et al. (2018), the type of glucan in Jali produced through extraction using water had the same structure as amylopectin. The amylopectin characteristics were a branched structure, more soluble in water, and a larger molecular weight than amylose. The branch chains of amylopectin were connected to the main chain via α-(1→6)-glycosidic bonds and formed a stable structure. Furthermore, with the large molecular weight of amylopectin, the granule size tended to be larger but had a lower gelatinization temperature than amylose.

The FTIR results in Figure 4 produced a glucan spectrum pattern with the presence of glycosidic bonds which indicated the presence of alcohol with an ether group. This consisted of hydroxyl (–OH), alkene (–CH), and ether (C-O) groups. Jali crude extract consisted of glucan with (1→4)(1→6)-α-D-glucan bonds. The 1→4-α-D-glucan bond had a structure resembling amylopectin with a branch chain at oxygen atom no-6, namely (1→6)-α-D-glucan. The (1→4)-α-D-glucan compound was located at wave numbers 928.46, 859.03, and 764.52 while (1→6)-α-D-glucan was at waves 1156.04 and 1022.9 (Synytsya and Novak, 2014). Various functional groups indicated the presence of glycosidic bonds in α-glucan, namely the presence of an OH group (alcohol or hydroxyl) in the wave number 3200-3600 cm^{-1} attached to the glucose ring, alkane CH (stretching) in the range of 2900-3000 cm^{-1} and C-O (ether) namely 1078.90 cm^{-1}. The results of the functional groups formed indicated the presence of glycosidic bonds forming glucan, where the OH and CH groups (stretching) were at 3405 and 2930 cm^{-1}, and for the C-O group in the range of 800-1300 cm^{-1} (Synytsya and Novak, 2014).

Yield and Characteristics of Arabinoxylan Extract

The arabinoxylan yield from Jali extract was (3.18%), which was lower than the arabinoxylan yielded from wheat flour at 6% (Zhang et al., 2014). The difference in temperature and interaction between the solute and alkaline solvent affected the extraction speed in increasing the material solubility. High temperatures could cause the extraction rate to run fast, while when the temperature was low the extraction speed could be slow and produce low material solubility (Andriani et al., 2019). Sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were the solvents used in this study to produce arabinoxylan. The sodium hydroxide function could break down the ester group in arabinoxylan with other components such as lignin (Kanani et al., 2018).

Figure 5 showed the chromatogram results of arabinoxylan from Jali seeds which produced hydrolyzate in the form of arabinose and xylose with concentrations of 22.4% and 4.8%. Standard HPLC chromatography of arabinose and xylose displayed a retention time of RT 13.013 and xylose at RT 10.763. Samples containing arabinoxylan in Jali on the chromatogram showed 5 peaks at RT 13.023 and RT 10.687 which indicated arabinose and xylose. Based on the substitution of arabinose branches in the xylan "backbone", the A/X ratio was 4.6%. The A/X ratio in the Jali samples was higher in other cereals. The high ratio value between the 2 was based on the degree of branching produced in the arabinoxylan extract. According to Sternemalm et al. (2008), the high value of Ara/Xyl substitution was due to the aggregation of unsubstituted areas, showing an Ara/Xyl ratio of around 4 and increased to above 10. This was because the arabinose side group was related to the ability to bind water during the hydrolysis reaction, therefore it caused a decrease in viscosity and the breaking of the arabinose group.

Effect of Jali Extract on Optical Density of *Lactobacillus casei* and *Bifidobacterium longum*

Jali was a cereal plant rich in nutritional content such as polysaccharides and oligosaccharides carbohydrates

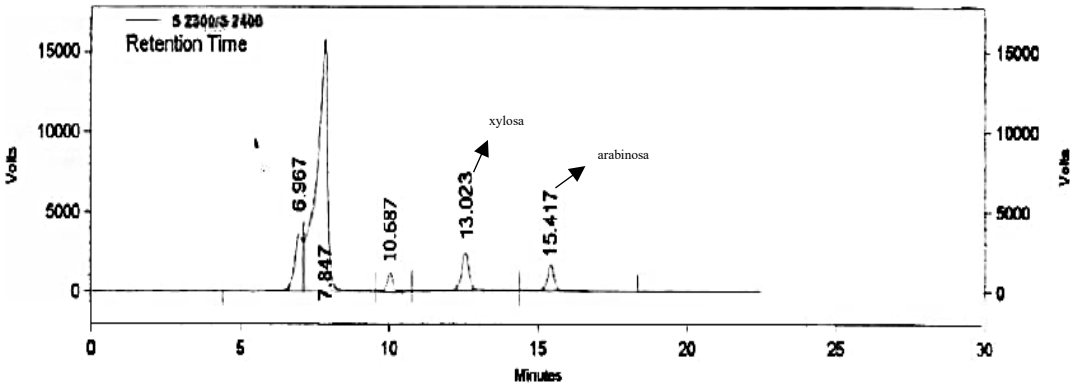

Figure 5. Chromatogram of arabinoxylan jali (*Coix lachrymal-jobi*)

for the growth of bacteria in the digestive tract. *Bifidobacterium longum* and *Lactobacillus casei* bacteria grew naturally in the digestive tract. These 2 bacteria had the potential to be prebiotic because of the presence of antimicrobial properties, could adapt to low pH, and bile salts, and grow well in simple media. The growth of these 2 bacteria could be seen based on OD or optical density, namely the bacteria density seen as turbidity in the medium. *Bifidobacterium longum* bacteria showed the highest OD in the fructooligosaccharides (FOS) addition treatment of 0.871 compared to *Lactobacillus casei* bacteria of 0.725 (Table 1). This was following Figueiredoa et al. (2020) that the fructooligosaccharides addition could increase the OD value of *Bifidobacterium longum* by 1.5-2 compared to the genus Lactobacillus by 0.5-1. The low OD value produced by *Lactobacillus casei* could be due to the sub-optimal use of the β-fructofuranosidase enzyme in utilizing FOS. This followed the statement of Watson et al. (2013) where several β-fructofuranosidase enzymes in *Lactobacillus casei* had lower hydrolysis capabilities in breaking FOS glycosidic bonds compared to *Bifidobacterium longum*.

Bifidobacterium longum bacteria utilized FOS using the enzyme fructofuranosidase, through the Bifidum transport system. FOS were hydrolyzed into glucose and fructose into lactic acid and other acids, such as acetic and formic acid by breaking the β-(1→2) glycosidic bond by the enzyme β-fructofuranoside (Chen et al., 2015). However, *Bifidobacterium longum* could degrade complex carbon sources into low molecular weight monosaccharides via the bifidum or hexose-phosphate pathway. In the control (without extract), the bacteria *Bifidobacterium longum* and *Lactobacillus casei* obtained the lowest OD. This was because the nutrient source contained in the control was different from other media which contained additional nutrients from Jali extract. The MRSB media in the control contained protein, minerals, vitamins, and yeast extract peptone as a carbon source, therefore this caused slower bacterial growth compared to other treatments given Jali extract which each contained FOS, α-glucan, and arabinoxylan.

Table 1 showed that the addition of arabinoxylan alkaline extract and α-glucan water extract produced almost the same OD values, but were higher for *Bifidobacterium longum* bacteria at 0.743 and 0.758 (p>0.05) compared to *Lactobacillus casei* at 0.549 and 0.710 (p< 0.05) and control (without extract) of 0.550 and 0.549 (p>0.05). This was in line with Mendez et al. (2021), that arabinoxylan extract from corn flour could increase the OD value in the genus *Bifidobacterium* by 0.4-0.55 compared to without the addition of the extract at 0.1. *Bifidobacterium longum* bacteria could use the pentose sugar group contained in arabinoxylan as an energy source because it had the extracellular enzyme arabinofuranosidase to produce acetic acid and lactic acid (Komeno et al., 2022). The breakdown of arabinoxylan compounds was carried out using the bifidum or fructose-6-phosphate phospoketolase pathway by *Bifidobacterium longum* bacteria to produce xylose and arabinose. In the next stage, ribulose undergoes isomerization with xylulose through the oxidative phosphorylation stage to produce ribulose-5-phosphate. A total of 2 xylulose-5-phosphate molecules were broken down into 2-glyceraldehyde-3-phosphate and 2 acetyl phosphate to produce 3 acetate and 2 lactate (O'Callaghan and van Sinderen, 2016).

Bifidobacterium group bacteria utilized carbohydrate compounds such as amylose,

Table 1. The optical density of FOS, glucan, and arabinoxylan jali extracts on the growth of *Lactobacillus casei* and *Bifidobacterium longum*

Bacteria	Extract	Optical density (OD)
Lactobacillus casei	Control	$0.550 \pm 0.02_{a}$
	FOS	$0.725 \pm 0.01_{b}$
	Glucan	$0.710 \pm 0.02_{b}$
	Arabinoxylan	$0.549 \pm 0.02_{a}$
Bifidobacterium longum	Control	$0.594 \pm 0.05_{a}$
	FOS	$0.871 \pm 0.02_{c}$
	Glucan	$0.758 \pm 0.03_{b}$
	Arabinoxylan	$0.743 \pm 0.04_{b}$

Description: Different letters on the same line indicated significant differences (p<0.05)

Table 2. pH value of Jali extract (fos, glucan, and arabinoxylan) fermented by *Lactobacillus casei* and *Bifidobacterium longum*

Bacteria	Extract	pH
Lactobacillus casei	Control	$3.56 \pm 0.12_{a}$
	FOS	$2.60 \pm 0.00_{b}$
	Glucan	$2.56 \pm 0.15_{b}$
	Arabinoxylan	$3.30 \pm 0.20_{a}$
Bifidobacterium longum	Control	$3.73 \pm 0.06_{a}$
	FOS	$2.22 \pm 0.06_{c}$
	Glucan	$2.53 \pm 0.15_{bc}$
	Arabinoxylan	$2.50 \pm 0.17_{bc}$

Description. Different letters on the same line indicated significant differences (p<0.05)

amylopectin, D-galactosamine, and D-glucosamine as a source of nutrition. This type was known as a microorganism that tended to be picky or fastidious in choosing the substrate as a place to grow (Chandan, 2007). *Bifidobacterium longum* bacteria could utilize polysaccharides compounds with high molecular weights such as α-glucan because each had several enzymes such as α-amylase and amylopululanase which easily break down these polysaccharides components for absorption into cells (Møller et al., 2014).

pH of Jali Extract (FOS, Glucan, and Arabinoxylan) Fermented by *Lactobacillus casei* and *Bifidobacterium longum*

The pH value in each treatment produced low values for both *Lactobacillus casei* and *Bifidobacterium longum* bacteria, between 2.22 to 3.73. This showed that the 2 bacteria with potential as prebiotic could create environmental conditions with a low pH as an optimal place for their growth. During fermentation, the pH value decreased due to the presence of H+ ions from the accumulation of short-chain organic acids such as acetic, propionic, and butyric acids (Goranov et al., 2013).

Table 2 indicated the lowest pH in FOS utilization by *Bifidobacterium longum* bacteria at 2.22. *Lactobacillus casei* bacteria was given with FOS extract and produced a pH of 2.60, which could be because of the number of *Bifidobacterium longum* in total bacteria produceds a higher value with the FOS addition compared to *Lactobacillus casei*, therefore the organic acid produced was higher to produce a low pH value. This was lower than in the study by Yao et al. (2022) where the FOS addition to a medium that grew *Bifidobacterium longum* bacteria and the genus *Lactobacilus* produced a pH value of 5.5 for 24 hours, and this could be caused by different FOS concentrations. The decrease in pH correlated with the OD value of *Bifidobacterium longum* during fermentation because the high growth of *Bifidobacterium longum* could produce more organic acids and cause a decrease in the pH value. The mechanism for *Bifidobacterium longum* to lower pH was through the bifidum cycle which had more energy (2.5 ATP) than *Lactobacillus casei* (2ATP). ATP functions to build cell materials from nutrients in the environment to hydrolysis of substrates becoming metabolite products in the form of organic acid accumulation with an increase in dissociated H+ ions which caused a decrease in the pH value (Puminat, W. and Teangpook, 2013).

SCFA content from Jali Extract fermented by *Lactobacillus casei* and *Bifidobacterium longum*

Lactobacillus casei and *Bifidobacterium longum* could produce the main metabolite in the form of short-chain fatty acids (SCFA) during the fermentation process. Acetic acid and propionic acid acted as energy sources in cells, while butyric acid was used for cell regeneration (Louis and Flint, 2017). Table 3 showed that the FOS extract addition to *Bifidobacterium longum* bacteria produced acetic acid with the highest value reaching 243.827 mmol/L. In line with Zhang et al. (2022), FOS combined with the *Bifidobacterium* genus produced a total acid value of up to 24.52 ± 3.66 μ mol/mL with the highest acetic acid value of >20 μmol/mL compared to butyric or propionic acid. FOS extract was used by *Bifidobacterium longum* through the bifidum pathway to be converted into glucose and fructose, which with the help of the enzyme β-fructofuranosidase produced higher acetic acid levels (Mahalak et al., 2023). The presence of FOS as a nutritional source could help *Bifidobacterium longum* in producing acetic acid in intestinal epithelial tissue which could act as a barrier against pathogenic bacteria. Acetic acid levels showed the highest values for each treatment compared to other types of acid (propionic and butyric). This could be caused by the breakdown of FOS in Jali extract into acetic acid through a short pathway during the *Bifidobacterium longum* bacteria metabolism (Liu et al., 2021).

According to the research Rozali, (2018), the production of short-chain fatty acids (SCFAs) from resistant starch substrates with potential prebiotic properties results in higher levels of acetic acid compared to propionic and butyric acids. Acetic acid is produced from the breakdown of carbohydrates into glucose, along with other gases such as hydrogen, carbon dioxide, and methane. The greatest absorption of acetic acid occurred in liver cells where 70% of acetic acid was used as a substrate for forming cholesterol and long-chain fatty acids. Furthermore, it became a cosubstrate in the formation of glutamine and glutamate, and the rest of the acetic acid was used in other tissues in the body. According to Hernández et al. (2019), acetic acid could also cause changes in fatty acid levels in the blood to reduce the lipolysis process in adipose tissue.

Propionic acid was a short-chain fatty acid from the metabolism of prebiotic components which was degraded in the liver. Propionic acid was not detected in any treatment given jali extract except in the AIBI control (MRSB + *Lactobacillus casei*) and A2B1 control (MRSB + *Bifidobacterium longum*) which were 4.536 ± 6.42 mmol/L and 3.383 ± 4.78 respectively. This could be caused by *Lactobacillus casei* and *Bifidobacterium longum* dominantly utilized Jali extract to produce acetic acid and butyric acid. Additionally, Zhang et al. (2022) stated that propionic acid produced high values of up to >4 μ mol/mL in yeast casitone fatty acids (YCFA)

Table 3. Effect of Jali extract on short fatty acid (SCFA) values of *Lactobacillus casei* and *Bifidobacterium longum*

Treatment (in SCFA methodology)	Acetate	Propionate	Butyrate
A1B1	$130.771 \pm 13.46_{bcd}$	$4.536 \pm 6.42_{a}$	$1.919 \pm 1.67_{a}$
A1B2	$140.942 \pm 12.86_{bcd}$	-	$8.991 \pm 12.72_{b}$
A1B3	$166.241 \pm 12.75_{d}$	-	$0.276 \pm 0.39_{a}$
A1B4	$152.272 \pm 7.07_{bcd}$	-	$0.920 \pm 1.30_{a}$
A2B1	$116.137 \pm 16.02_{b}$	$3.383 \pm 4.78_{a}$	$2.434 \pm 0.20_{a}$
A2B2	$243.827 \pm 11.17_{e}$	-	$0.417 \pm 0.59_{a}$
A2B3	$158.825 \pm 15.02_{cd}$	-	$0.256 \pm 0.36_{a}$
A2B4	$142.980 \pm 18.98_{bcd}$	-	$1.760 \pm 2.49_{a}$

Description. Different letters on the same line indicated significant differences (p<0.05)

A1B1: *Lactobacillus casei* – control, A1B2: *Lactobacillus casei* – fos, A1B3: *Lactobacillus casei* – glucan, A1B4: *Lactobacillus casei* – arabinoxylan, A2B1: *Bifidobacterium longum*- control, A2B2: *Bifidobacterium longum* – fos, A2B3: *Bifidobacterium longum*- glucan, and A2B4, *Bifidobacterium longum* – arabinoxylan

media compared to media added with FOS extract or the synergy between FOS and *Bifidobacterium longum*. Propionic acid in the liver played a role in suppressing fatty acid production thereby influencing the reduction of triacylglycerol. Furthermore, propionic acid could control cholesterol production and the rate of its formation, therefore it produced a decrease in cholesterol levels in the liver (Haghikia et al., 2022).

Each treatment produced butyric acid starting from the lowest at 0.256 mmol/L to the highest at 8.991 mmol/L. In line with Renye et al. (2021) *Lactobacillus* genus could utilize FOS to produce butyric acid between 0.5 – 1.3 mM. The ability of *Lactobacillus casei* to produce butyric acid was due to the butyrogenic effect from the addition of FOS. This was in line with Kang et al. (2020) that the addition of fructooligosaccharides compounds could increase SCFA, especially butyric acid, which had the potential as a carbon source in the growth of Lactobacillus bacteria. Moreover, the high production of butyric acid in *Lactobacillus casei* bacteria could be caused by the metabolism of acetic and lactic acid using the butyryl CoA or acetyl CoA transferase pathway (Tang et al., 2023). Butyric acid was used in the intestine and played a role in cell repair, suppressing the increase in pathogenic bacteria and also the spread of cancer cells in the intestine.

CONCLUSION

In conclusion, Jali contains oligosaccharide compounds, specifically fructooligosaccharides such as 1-β-D-fructofuranosyl nystose (GF4) with a concentration of 40.78%. Additionally, it includes polysaccharide compounds such as α-glucan with (1→4)(1→6)-α-D-glucan bonds at 10.8% and arabinoxylan with an A/X ratio of 4.6%. These 3 compounds had the potential to act as prebiotic because of capable increasing the growth of *Bifidobacterium longum* and *Lactobacillus casei* bacteria compared to without jali extract (control) addition. Additionally, out of the 3 compounds, the FOS compound from jali produced the highest growth on *Bifidobacterium longum* with OD at 0.871, pH at 2.22, and SCFA at 243.827 mmol/L. Further studies were needed on Jali extract with the potential to be developed into functional food processing products.

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CONFLICT OF INTEREST

The authors hereby declared that the published data had no conflict of interest for any party.

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