BMC Microbiology

RESEARCH

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Prebiotic potential of oligosaccharides extracted from improved Ugandan varieties of millet, sesame, soybean, and sorghum: enhancing probiotic growth and enteric pathogen inhibition

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Abstract

Functional gastrointestinal disorders like diarrhea continue to affect children under five years in low-income countries. Incorporating health-enhancing bioactive compounds such as prebiotics in diet offers a promising solution. This study investigated prebiotic potential of oligosaccharides extracted from improved varieties of millet (Seremi 2, Naromil 2), sesame (Sesim 2, Sesim 3), soybean (Maksoy 3N, Maksoy 6N), and sorghum (Narosorg 2, Narosorg 4), commonly consumed in Uganda. These were compared to their respective indigenous variety. This study employed standardized methods for optical density measurement, culture preparation, and oligosaccharide extraction to evaluate prebiotic properties. We investigated whether plant-based oligosaccharides could enhance the effectiveness of probiotics, specifically *Lactiplantibacillus plantarum (*ATCC 14917*)* and *Lacticaseibacillus rhamnosus (*ATCC 7469*)*, in antagonizing common enteric pathogens (*Salmonella enterica* subsp. enterica (ATCC 13076) and *Shigella flexneri* (ATCC 12022)). Approximately 4–8 log CFU/ml of each probiotic was incubated in 2% w/v oligosaccharide extracts at 37 °C to evaluate the influence of the extracts on their growth, short-chain fatty acid (SCFA) production and antagonistic activity. Maximum cell density, which exceeded the minimum recommended probiotic cell density (6 log CFU/ml), was achieved during 24-h incubation period. The probiotics exhibited optimal growth in extracts of Sesim 2, Maksoy 3N, Narosorg 2 and indigenous millet variety resulting in a 68–84% increase in cell densities. The concentration of SCFA concentration was significantly higher ($p < 0.05$) in soybean-based oligosaccharides. Both probiotics antagonized growth of *Salmonella* and *Shigella* by more than 40% when cultured on Sesim 2, Maksoy 3N, Narosorg 2 and indigenous millet variety, while maintaining the probiotic cell densities above the minimum recommended level. These varieties show great potential as functional ingredients for developing synbiotic-rich foods to promote gut and public health. However, to evaluate the oligosaccharides prebiotic efficacy, *in vitro* fermentation using fecal microbiota and *in vivo* studies are necessary to determine gut microbiota changes and interactions.

Keywords Fructo-oligosaccharide, Galacto-oligosaccharide, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, Crop variety, Enteric pathogens, Prebiotics, Probiotics

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Background

Functional gastrointestinal diseases including diarrhea continue to be a substantial health issue for children under the age of five years in low-income countries such as those in sub-Saharan Africa (SSA), contributing significantly to child mortality. Although diarrhea only constitutes approximately 4% of child mortality worldwide, it is responsible for approximately 20% of deaths in low-income countries, highlighting a pressing health concern [1]. Particularly, in 2022, Uganda registered a 69% prevalence of diarrhea among children under five years [2]. The primary pathogens associated with diarrhea within this demographic group include Entero-toxigenic *E. coli*, *Salmonella* and *Shigella* species [3, 4]. The prevalent route of exposure for these diarrhea-inducing microorganisms is through ingestion of contaminated foods and beverages [5].

Historically, there has been a strong association between diarrheal disease occurrence in SSA and the lack of sufficient water, sanitation, and hygiene (WASH) practices [6, 7]. Despite intensive efforts by governmental and non-governmental organizations to improve WASH and vaccination coverage, diarrhea-related deaths still occur [8]. This situation is underscored by reports of over 80% adoption of improved WASH practices in the SSA region [9]. This suggests that while critical, sanitation and vaccination strategies may not sufficiently address the problem of diarrheal diseases when applied in isolation. This situation emphasizes the necessity for additional strategies to complement these essential interventions.

Adopting dietary interventions that extend beyond basic nutrition to incorporate health-promoting bioactive substances offers a feasible approach to alleviate functional gastrointestinal problems, such as diarrhea, in children under 5 years. Prebiotics, among other bioactive ingredients, are essential for enhancing gastrointestinal health. They modulate the gut microbiota by homeostatic regulation of the diversity/abundance of health-promoting gut bacteria (probiotics) [10], and reducing enteric pathogen [11]. Increased diversity and abundance of probiotics such as *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum* species in the gut plays crucial roles by maintaining a healthy balance of gut bacteria [12]; improving digestion and nutrient absorption, and boosting the immune system [13]. Maternal diets supplemented with both prebiotic oligosaccharide from milk and *Bifidobacterium* significantly influenced gut microbiota and immune system of neonatal mice [14]. Therefore, consumption of foods that have both prebiotic and probiotic potential provides synergistic effects on the overall well-being of an individual.

Prebiotics, primarily derived from minimally processed plant-based diets are selectively metabolized by probiotics in the lower gastrointestinal tract [15]. One of the primary beneficial metabolic products of this process are short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate [16]. SCFAs lower the pH of the gastrointestinal tract creating an acidic environment that is unfavorable for the growth of pathogenic microorganisms [17]. Additionally, SCFAs, especially butyrate, serve as an energy source for intestinal epithelial cells, thereby enhancing the integrity of the intestinal barrier [18]. Butyrate is also important for the maintenance of specialized gut cells, such as the interstitial cells of Cajal, which play a role in mitigating inflammation and oxidative stress in the gastrointestinal tract [19].

Furthermore, acetate administration has been shown to significantly reduce inflammation in mice with gout, as reported in a study by Vieira et al. [20]. Similarly, a dose–response study by Tayyeb et al. [21], indicated increased inhibition in the production of pro-inflammatory mediators such as nuclear factor kappa B (NF-κB) with increasing levels of propionate and butyrate, thereby strengthening the intestinal barrier function. Furthermore, SCFAs contribute to weight management and the prevention of non-communicable diseases by regulating appetite-regulating gut hormones such as peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) [22]. These studies highlight the comprehensive local and systemic benefits of SCFAs to the host produced by metabolization of dietary prebiotics.

Majorly recognized prebiotics are carbohydrate-based and are of plant-based origin, for instance, Fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS). These oligosaccharides have diverse applications in the food industry such as low-calorie sweeteners, texturizers, stabilizers [23], fat replacers [24], nutrition supplements and as prebiotic sources [25]. They can either be consumed as extracts/supplements or as part of whole foods. FOS and GOS can be obtained from plants such as millet, sorghum, soybean, and beans [26]. However, the amount of these prebiotic oligosaccharides in plants differs greatly. For instance, cereals contain higher amounts of FOS whereas legumes largely contain GOS [27]. Furthermore, FOS and GOS differ in the structural composition and degree of polymerization [28] both within and between different food types. Consequently, these variations influence the level at which the probiotic bacteria metabolize them. This in turn affects the type and quantity of metabolites such as SCFAs produced either *in vivo* or *in vitro* [29].

An *in vitro* evaluation of the growth potential of 41 prospective *Lacticaseibacillus* probiotic strains on various carbohydrate substrates revealed a spectrum of carbohydrate utilization, ranging from partial to complete [30]. Furthermore, Harris et al. [31] observed an elevated

production of butyrate and acetate by gut microbiota upon consumption of galacto-oligosaccharides, as compared to other carbohydrate sources such as polydextrose and rhamnose. This implies that different probiotic strains have varying abilities to utilize different carbohydrate types [32], which can influence their growth and survival both in the gut and the food matrix. The type of carbohydrate consumed can also impact the metabolic activity of the probiotic, including the production of beneficial metabolites like SCFAs [33].

To develop food products that integrate both prebiotics and probiotics nutritionally termed as synbiotics, achieving the right combination of prebiotic compounds and probiotic strains is essential [34]. Synbiotic food products create a synergistic effect between prebiotics and probiotics that enhances host health. The prebiotics in the synbiotic food products not only increases gut microbiota but also increases the survival rate of probiotics as they pass through the harsh gastrointestinal tract [35]. However, research in this area has largely focused on commercialized prebiotic oligosaccharides such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and inulin, paired with well-characterized probiotic strains from *Lactiplantibacillus plantarum, Lacticaseibacillus rhamnosus*, and *Bifidobacterium* [36–38]. Studies investigating the prebiotic potential of oligosaccharide extracts from various food sources have largely focused on wheat [39, 40], sea weed [41], lotus seeds [42] and human milk [43]. However, there is limited information on prebiotic potential of oligosaccharides derived from foods commonly consumed in Ugandan households including millet, sorghum, soybean, and sorghum varieties.

Additionally, variations in oligosaccharide content of cereals, legumes and oilseeds resulting from differences in variety and processing methods have been documented [44–47]. Nonetheless, the authors did not evaluate the potential of these oligosaccharides as prebiotics that can be combined with established probiotics to develop food products possessing synbiotic properties. Therefore, this study evaluated prebiotic potential of oligosaccharide extracts from millet, sorghum, sesame, and soybean varieties to determine the most appropriate oligosaccharide source that can be combined with probiotics to develop synbiotic food products using commonly consumed foods. The effect of these oligosaccharides on growth of probiotics, production of major SCFA (acetate, butyrate and propionate), and inhibition of common enteric pathogen was investigated since these aspects form the mechanism of action of prebiotics [15]. Evaluating prebiotic potential of these commonly consumed foods could create new opportunities for formulating innovative and diverse synbiotic food products, potentially enhancing accessibility, sustainability, and health benefits especially among individuals at risk of functional gastrointestinal disorders such as children under five years.

Materials and methods

Prebiotic plant sources

Four crops were used: two cereals; finger millet (*Eleusine coracana*) and sorghum (*Sorghum bicolor*); one legume; soybeans (*Glycine max*); and one oilseed; sesame (*Sesamum indicum*) were used as oligosaccharide sources. These were selected because they are commonly consumed in households in Uganda and other parts of Africa. Each crop consisted of two improved varieties and 1 indigenous variety (Table 1). The indigenous varieties were purchased from 10 different indigenous markets commonly used by most of the population in Gulu City, northern Uganda. These were then pooled together

Table 1 Oligosaccharide plant sources used in this study segregated by crop type and variety

Name of Food/crop	Variety	Unique attributes
Sorghum	Indigenous variety	N/A
	Narosorg-2	Drought tolerant, minimal bird damage, used for food and yeast/brew
	Narosorg-4	Tolerant to striga weed, used for food
Finger Millet	Seremi 2	Good aroma & taste, early maturity, high marketability used for food & brewing
	Naromil 2	High yielding, early maturity
	Indigenous Variety	N/A
Soybean	Maksoy 3 N	High yielding, large seed, high oil & protein content,
	Maksoy 6 N	Early maturity, high yielding, high oil & protein content, resistant to rust disease
	Indigenous Variety	N/A
Sesame	Sesim 2	Drought tolerant, high yielding, high oil content, resistant to pests
	Sesim 3	High yielding
	Indigenous Variety	N/A

Source: [51–54]. *N/A* No relevant information on crop attributes available on the variety

to make 20 kg for each crop type. The improved varieties of millet, sorghum, and sesame were sourced from the National Semi-Arid Resources Research Institute (NASARRI) located in Serere district, Uganda, whereas the improved soybean varieties were acquired from the Zonal Agricultural Research and Development Institute in Ngetta, Lira, Uganda. These institutes lead plant breeding and crop improvement in the country and hence have pure lines of improved varieties. Plant breeding and crop improvement alters nutritional composition including prebiotic attributes [48] in addition to enhancing agronomic traits. Therefore, pure lines of highly adopted improved varieties were selected to determine the degree of alteration of prebiotic attributes compared to indigenous varieties. Millet and sorghum were used for the study because they are the ingredients mostly used to produce fermented non-alcoholic beverages that can be consumed by children [49]. Sesame and soybean were used because they are the major plant protein sources used to enrich the nutritional quality of plant-based complementary foods [50]. Individual samples were sorted, cleaned, and sun-dried on a clean tarpaulin until a moisture content of 10–12% was achieved. Details on each crop used are shown in Table 1.

Microbial strains and growth conditions

Probiotic and pathogenic microbial strains were procured in lyophilized form from the American Type Culture Collection (ATCC) (Microbiologics, Minnesota, USA). To ensure their long-term viability, these strains were stored at −20 °C. The probiotic strains used included *Lacticaseibacillus rhamnosus* (ATCC 7469) and *Lactiplantibacillus plantarum* (ATCC 14917), while the pathogenic organisms were *Salmonella enterica* subsp. enterica (ATCC 13076) and *Shigella flexneri* (ATCC 12022). *Lb. rhamnosus* and *Lpb. plantarum* were cultured anaerobically at 5% CO_2 in de Man, Rogosa, and Sharpe (MRS) agar (HiMedia, India) for 48 h at 37 °C. The enteric pathogenic microorganisms were cultured aerobically on a selective and differential Xylose Lysine Deoxycholate (XLD) agar (HiMedia, India) at 35 °C for 24 to 48 h. This medium selectively promotes the growth of *Shigella* and *Salmonella* while inhibiting other enteric pathogens [55].

Oligosaccharide extraction from selected crops

Oligosaccharide from millet, sesame, sorghum, and soybean varieties were extracted using ethanol absolute (≥ 99.5% purity) (Millipore, Darmstadt, Germany) extraction method described by Muir et al. [56] and Pereira et al. [57]. Briefly, individual oven dried crop varieties were ground using a laboratory grinder (Neo-Tech SA, Milmort, Belgium) and passed through a 40-mesh screen sieve with diameter size of 300 µm (HT/standard sieves). Subsequently, 1 g of the ground sample was accurately weighed into a Pyrex beaker to which 80 ml of 80% ethanol absolute solution was added and placed in a water bath shaker (MaxQ 7000, Thermo Fisher Scientific, UK) at 75 °C. The mixture was shaken at 200 rpm for 60 min to ensure maximum extraction of oligosaccharides without hydrolysis [57]. Following extraction, the mixture was centrifuged (VWR Micro star 17, 521–1646, Berlin, Germany) at 3000 × g for 10 min to obtain the supernatant.

The total oligosaccharide content in the extracts was quantified using Megazyme kits and determined by UV–VIS spectrophotometer (Jenway 6705, Milmort Belgium). K-RAFGL kit (last updated August 2023) (Megazyme, Bray Ireland) was used to determine galacto-oligosaccharide content whereas total fructo-oligosaccharide was determined by K-FRUC kit (last updated November 2022) (Megazyme, Bray Ireland) according to manufacturer's instructions. Subsequently, the oligosaccharide content (Table 2) of the extracts were used to standardize the oligosaccharide concentration to 2% (w/v) across all samples by dilution using distilled water or concentration by evaporation. Concentration was done using a rotary evaporator (Hei-vap precision, Heidolph, Germany) at 43 °C, 75 mbars, and 125 rpm between 20 and 60 min depending on the degree of concentration required to achieve the standardized concentration. This was to ensure that the prebiotic effects observed were due to the oligosaccharides and not variations in extract concentration [58]. The prepared solution was subsequently filter-sterilized using a 0.22 µm nylon syringe filter (AVF-100 C-NY, Dubai, UAE) to eliminate any solid particles and bacteria, and then stored at −14°C until further analysis.

Table 2 Total oligosaccharide content of sorghum, millet, soybeans, and sesame varieties

Name of Food/crop	Variety	Initial total oligosaccharide content (% w/v)
Sorghum	Narosorg-2	0.7 ± 0.05^{g}
	Narosorg-4	0.38 ± 0.12^{i}
	Indigenous variety	1.45 ± 0.19^{f}
Finger Millet	Naromil 2	0.84 ± 0.23^{g}
	Seremi 2	0.40 ± 0.20^{hi}
	Indigenous Variety	0.58 ± 0.35^{h}
Soybeans	Maksoy 3 N	8.08 ± 0.12^{b}
	Maksoy 6 N	7.28 ± 0.24^{c}
	Indigenous Variety	9.03 ± 0.51^{a}
Sesame	Sesim 2	3.16 ± 0.29^{d}
	Sesim 3	2.59 ± 0.12^{e}
	Indigenous Variety	2.50 ± 0.34^{e}

Values indicate mean ($n = 3$) total oligosaccharide content for each crop variety used for reconstitution and standardization to 2% w/v concentration. Means in the same column followed by different letter superscripts are significantly different from each other at 5% level of significance

Probiotic growth characteristics on oligosaccharides extracted from sorghum, millet, soybeans, and sesame

The initial step involved preparation of overnight cultures of the probiotic strains *Lactiplantibacillus plantarum* (ATCC 14917) and *Lacticaseibacillus rhamnosus* (ATCC 7469). Cell pellets were harvested from the overnight cultures by centrifugation (VWR Micro Star 17, 521–1646, Berlin, Germany) at 8000 ×g for 10 min. The pellets were washed three times with phosphate-buffered saline (PBS) (Sigma-Aldrich, Chemie Taufkirchen, Germany), with centrifugation performed after each wash. Following the final wash, the pellets were re-suspended in 5 ml of PBS. Subsequently, 3 ml aliquots of the suspension were transferred into cuvettes, and the optical density (OD) at 600 nm was measured using a calibrated UV–VIS spectrophotometer (6705 Jenway, Milmort, Belgium). The OD readings were adjusted by diluting the suspension with PBS and standardized to a range of 0.8 to 1.0 at 600 nm, corresponding to approximately 1.0 $\times 10^8$ CFU/ml [59] prior to evaluation. The microbial concentration was determined using culture media as described in "Microbial strains and growth conditions" section.

To evaluate their growth in the presence of different oligosaccharide sources, MRS broth (Millipore, Germany) composition was modified by adding 2% (w/v) of individual oligosaccharide extract ("Oligosaccharide extraction from selected crops" section) and the MRS broth without any modification was used as a control. Subsequently, a 5 ml aliquot of standardized probiotic culture was serially diluted with PBS and appropriate dilutions with about 5 log CFU/ml, determined by plate count method, was mixed with 50 ml of modified MRS broth and aliquoted for further analysis. A 10 ml aliquot of the mixture was incubated anaerobically in an anaerobic incubator (Forma Series 3 Water Jacketed Co_2 incubator, model 4111, Thermo Fisher Scientific, Marietta, USA) with 5% CO_2 at 37 °C for 48 h. A colony counter (Scan-100, Interscience, Paris, France) was used to enumerate microbial growth at 0, 24 and 48 h of incubation on MRS agar (HiMedia, India). Each treatment was performed in triplicates.

Determination of short chain fatty acid content of extracted oligosaccharide and probiotic mixtures

Measurements were taken at 0, 24, and 48 h to monitor SCFAs production over time. The SCFAs quantified were butyrate, Propionate and Acetate which were summed up to obtain total SCFA. SCFA concentration was quantified using High-Performance Liquid Chromatography (HPLC) with UV detector (LC-2050, Shimadzu, Kyoto, Japan) following the method described by Harris et al. [39] with slight modifications. The HPLC analysis involved a derivatization step using 2,3,4,5,6-Pentafluorobenzyl bromide (PFB-Br) (Sigma-Aldrich Co. LLC, USA) in acetone, following a procedure compatible with a SCFA analysis kit (Sigma-Aldrich Co. LLC, USA) (catalog number SBR00030).

The kit also provided the basis for constructing a calibration curve using a SCFA mix, with dilutions ranging from 0.0156 M to 0.5 M. This curve was used to determine the limit of detection (LOD) and limit of quantification (LOQ) of the standard using Eqs. 1 and 2, respectively. The LOD was 0.07 mmol and LOQ of SCFA mix was 0.22 mmol with an R^2 value of 0.994, and equation was y= 237.15x +18.483. The derivatization procedure of the standards and the sample mixtures were made according to the manufacturer's instructions. Briefly, the derivatization reaction mixtures were prepared by combining 100 µL of SCFA standard or sample, 900 µL of acetone and 3 µL of PFB-Br. The mixtures were thoroughly homogenized by vortexing at maximum speed for 20 s. The mixtures were then placed in a water bath at 65 °C for 24 h to complete the derivatization reaction. After derivatization, the samples were filtered through 0.2 µm nylon syringe filters into 1.5 ml HPLC vials.

A slight modification for the HPLC conditions was made as follows; HPLC was conducted using a C18 column (Luna^R 5µm C18 (2) 100 Å, LC Column 250 ×4.6 mm) and UV detector at λ_{max} 245nm. Two mobile phases (80% Acetonitrile (gradient grade ≥99.9% purity, Sigma Aldrich, Chemie Taufkirchen, Germany) as solvent A and HPLC water +0.1% Trifluoro acetic acid (≥ 99.0% purity, Sigma Aldrich, Chemie Taufkirchen, Germany) as solvent B) were used in gradient mode. The gradient conditions were set as; 0–15 min 20% solvent A, and 80% of solvent B, 15–18 min, 80% of solvent A and 20% of solvent B and 18–20 min, 20% of solvent A. The flow rate was set at 1 ml/min, with 5 µL injection volume and a column temperature set at 30 °C. The sample retention times were compared to the standard retention times and their concentration was determined by calculating the area under the corresponding chromatogram using Shimadzu Lab Solutions CS software. The analysis for each mixture was conducted in triplicates.

$$LOD = 3.3\ x\ (s\tan dard\ deviation\ of\ intercept \div slope) \quad (1)$$

$$LOQ = 10\ x\ (s\tan dard\ deviation\ of\ intercept \div slope) \quad (2)$$

Potential of oligosaccharides extracted from different plant sources to enhance antagonistic activity of *Lpb. plantarum* and *Lb. rhamnosus* against *Shigella* and *Salmonella*

The antagonistic effects of probiotic strains (*Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus*) cultured with 2% w/v extracted oligosaccharide against pathogenic bacteria (*Salmonella enterica subsp. enterica* and *Shigella flexneri*), was evaluated following the method of Śliżewska & Chlebicz-Wójcik [58] with slight modifications. This current study used the total oligosaccharide content (Fructo-oligosaccharides and galacto-oligosaccharides) because these foods are consumed in their wholesome form rather than as isolated compounds. A 10 ml aliquot of the mixture prepared in "Probiotic growth characteristics on oligosaccharides extracted from sorghum, millet, soybeans, and sesame" section was incubated anaerobically for 24 h.

Overnight cultures of *Salmonella enterica subsp. enterica* and *Shigella flexneri* were prepared in Rappaport–Vassiliadis broth (Condalab, Spain) and nutrient broth (Millipore, Germany), respectively at 37 °C in a shaking incubator (AAH23433 K, Lab companion, Oxfordshire, UK). The cell density for each pathogen was standardized as described earlier in "Probiotic growth characteristics on oligosaccharides extracted from sorghum, millet, soybeans, and sesame" section. Exactly 1 ml of each standardized pathogen culture was individually added to 1 ml of the 24-h oligosaccharide extract-probiotic mixture. Individual probiotic and pathogen co-cultures without added oligosaccharide extract were used as a negative control. These samples were then incubated aerobically at 35 °C for 24 h. The impact on the growth of pathogens was periodically assessed at 6-h intervals for 24 h. The pathogenic bacterial counts were enumerated using a colony counter (Scan-100, Interscience, Paris, France) and expressed as log CFU/ml. Each experiment was performed in triplicates.

Statistical analysis

To analyze the effects of extracted oligosaccharides from different crop varieties on the growth of probiotics and their antagonistic activity, a one-way Analysis of Variance (ANOVA) was employed, using SPSS software version 25.0 (SPSS Inc., Chicago, IL). The significance level was set at 5%. Before running the ANOVA, Levene's homogeneity test of variance was performed to test for equality of variances and normal distribution was assessed using the Shapiro–Wilk test. A Post hoc Tukey's test was performed to evaluate the significant differences between the means. Additionally, to visually represent the growth of the microbial cultures and SCFA production over time, Sigma Plot version 11.0 software was used to plot a graph of microbial counts and short chain fatty acid concentration against incubation time.

Results

Preliminary results on the total oligosaccharide content in sorghum, millet, soybeans and sesame varieties.

Table 2 shows the content of oligosaccharides extracted from different varieties of sorghum, millet, soybeans, and sesame. The content of oligosaccharide was dependent on the type and variety of crop. The results show that soybeans and sesame significantly ($p < 0.05$) contained higher total oligosaccharide content compared to sorghum and millet. The indigenous variety of soybean registered the highest total oligosaccharide content of 9.03% (w/v) followed by Maksoy 3 N soybean variety with 8.08% (w/v). These results were used to standardize oligosaccharide content to 2% (w/v) used for subsequent analysis of prebiotic properties as described under "Oligosaccharide extraction from selected crops" section.

Growth characteristics of *Lpb. plantarum* ATCC 14917 and *Lb. rhamnosus* ATCC 7469 on oligosaccharides extracted from sorghum, millet, soybean, and sesame

Figures 1 and 2 show that oligosaccharide extracts from sorghum, millet, soybean, and sesame increased the cell density of both *Lpb. plantarum* ATCC 14917 and *Lacticaseibacillus rhamnosus* ATCC 7469 to levels between 8–9.3 log CFU/ml after 24 h of incubation. The increase in cell density was observed to be within the range of 68% to 84%. In every case, the recorded cell counts (log CFU/ml) exceeded the minimum recommended threshold for probiotic classification (> 6 log CFU/ml) (Figs. 1 and 2). Specifically, the organism had a comparative growth advantage in oligosaccharide extracts from all sorghum and sesame varieties when contrasted with growth in millet (Fig. 1-A) and soybean extracts (Fig. 1-B), respectively. Among the sorghum extracts, growth of *Lpb. plantarum* was highest in Narosorg 2 sorghum variety with a count of 9.24 log CFU/ml, followed by Sesim 2 sesame extracts (9.04 log CFU/ml). After 48 h, the cell density of *Lpb. plantarum* decreased in all crop extracts but only significantly ($p < 0.05$) in millet and sesame varieties with Naromil 2 and Sesim 3 exhibiting the highest decreases of 21% and 22%, respectively.

Figure 2 similarly presents a significant increase ($p < 0.05$) in growth of *Lacticaseibacillus rhamnosus* ATCC 7469 within the first 24-h incubation period. However, a notably higher growth was observed in all sorghum and millet oligosaccharide extracts than growth observed in soybeans and sesame extracts (Fig. 2-B). Sorghum and millet oligosaccharide extracts increased *Lb. rhamnosus* cell density between 70 and 80% during the first 24-h incubation period. Specifically, millet varieties

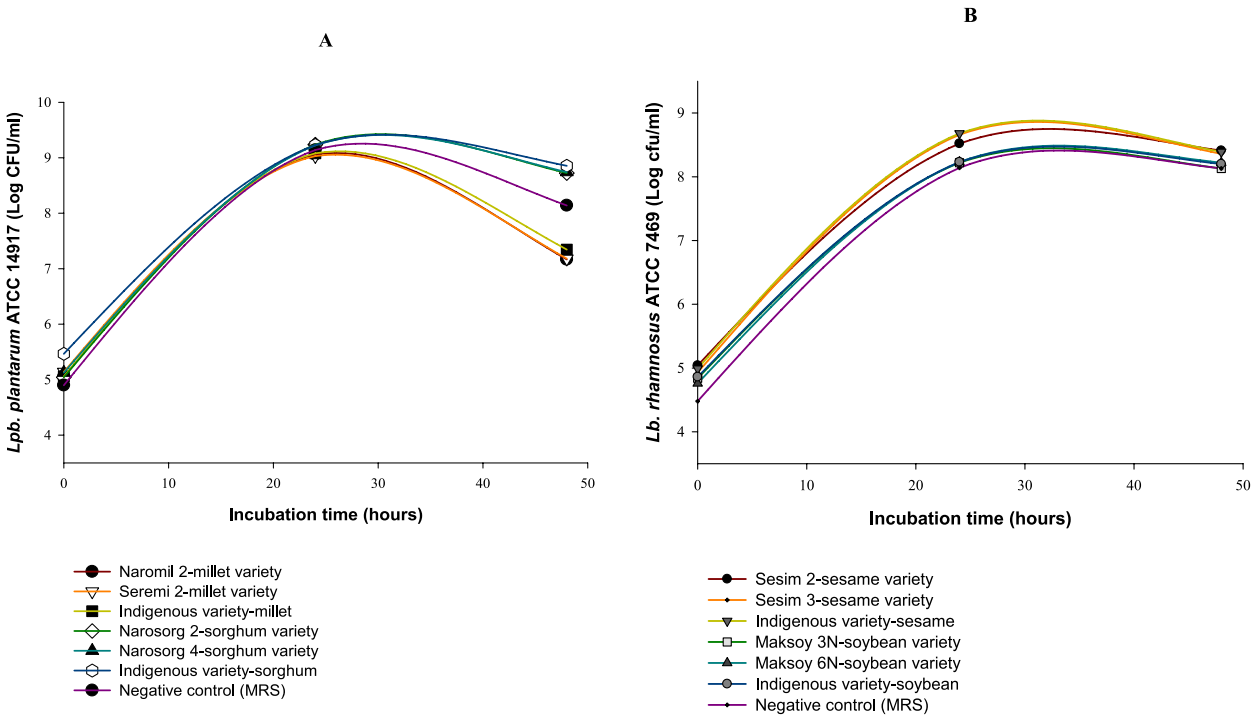

Fig. 1 *Lactiplantibacillus plantarum* ATCC 14917 colony forming unit counts during 48-h incubation on different oligosaccharide plant source. **A**-*Lpb. plantarum* growth curve on millet and sorghum crop varieties, **B**- *Lpb. plantarum* growth curve on sesame and soybean crop varieties. Error bars are excluded for clarity

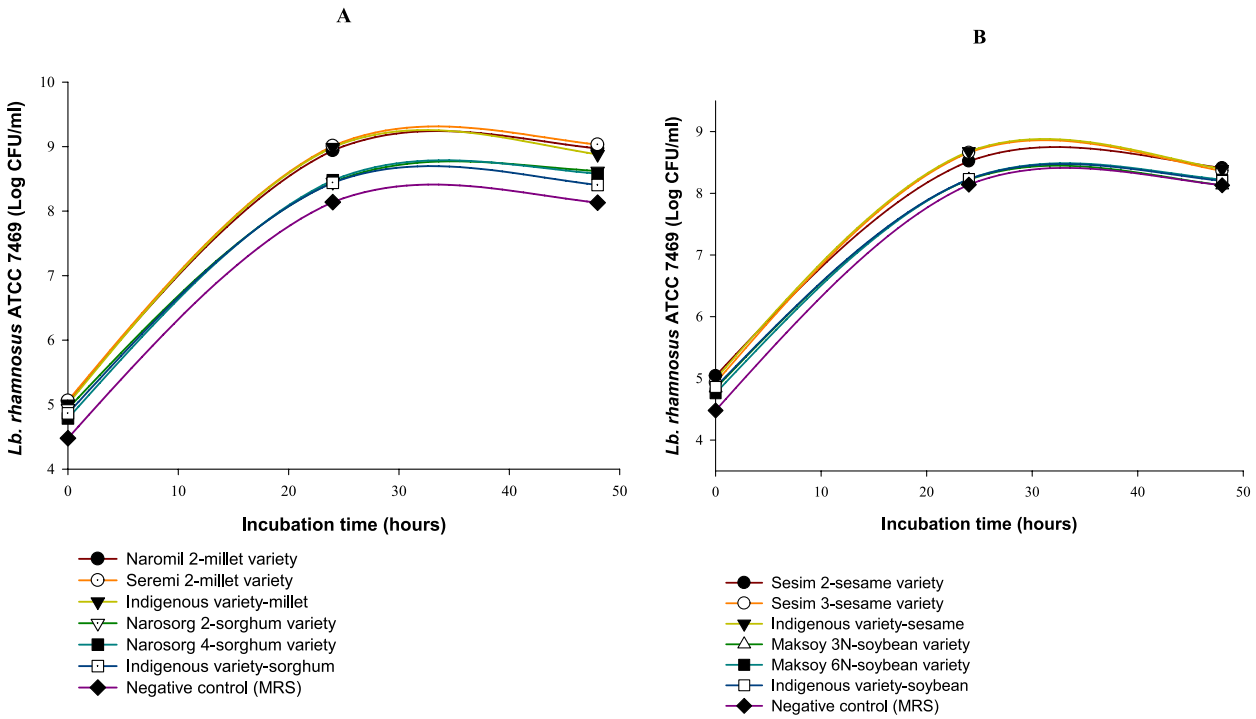

Fig. 2 *Lacticaseibacillus rhamnosus* ATCC 7469 colony forming unit counts during 48-h incubation on different oligosaccharide plant source. **A**-*Lb. rhamnosus* growth curve on millet and sorghum crop varieties, **B**- *Lb. rhamnosus* growth curve on sesame and soybean crop varieties. Error bars are excluded for clarity

demonstrated the maximum growth of *Lb. rhamnosus*, with the indigenous variety indicating the most substantial increase by 79.4%. Among sesame varieties, Sesim 3 displayed a maximum increase of 75.7% in the cell density. All crops recorded their maximum growth (> 8 log CFU/ml) of *Lb. rhamnosus* at 24 h, followed by a stationary growth phase, as indicated by a plateau curve (Fig. 2).

The data from Figs. 1 and 2 indicates that both *Lactiplantibacillus plantarum* ATCC 14917 and *Lacticaseibacillus rhamnosus* ATCC 7469 reached maximum growth within the first 24-h incubation period. This implies that 24-h incubation time is the most favorable for achieving maximum growth of both *Lpb. plantarum* and *Lb. rhamnosus*. Consequently, the 24-h incubation data were used to evaluate varietal differences among the different crop varieties concerning the growth characteristics of *Lpb. plantarum* ATCC 14917 and *Lb. rhamnosus* ATCC 7469, as indicated in Table 3.

Lpb. plantarum ATCC 14917 showed a comparative growth advantage over *Lb. rhamnosus* ATCC 7469 by more than one logarithmic cycle (Table 3) irrespective of oligosaccharide plant source. However, both bacteria had similar trend in the growth rate between 0.32 and 0.41 log CFU/ml/hour with Narosorg 2 recording the highest growth rate of 0.41 log CFU/ml/hour. Notably, the Narosorg 2 variety of sorghum significantly ($p < 0.05$) augmented the growth of *Lpb. plantarum*, with an average increase of more than 0.5 log units compared to other oligosaccharide plant extracts. Maksoy 3 N soybean variety and Sesim 2 sesame variety extracts similarly facilitated growth of *Lpb. plantarum* comparable with sorghum varieties. When comparing improved and indigenous varieties of the extracts, it was observed that improved crop varieties significantly ($p < 0.05$) enhanced the growth of *Lpb. plantarum*, except for millet varieties. However, there was no significant difference ($p < 0.05$) observed between the improved and indigenous varieties when *Lb. rhamnosus* was used as the probiotic bacteria. Therefore, oligosaccharide extracts from improved varieties (Maksoy 3 N, Narosorg 2, and Sesim 2) and indigenous millet varieties were further evaluated for their potential in short chain fatty acid production and enteric pathogen inhibition.

Production of short chain fatty acid from extracted oligosaccharide and probiotic mixtures

Figures 3 and 4 illustrate the production of SCFAs by different oligosaccharide and probiotic mixtures. The production of SCFAs (acetate, propionate, and butyrate) varied depending on the oligosaccharide source and probiotic combination during the incubation period. After incubation for 24 h, a significant ($p < 0.05$) increase in acetate concentration (ranging between 4-and > 50%) was observed in sesame, millet, and soybean oligosaccharide-*Lpb. plantarum* (Fig. 3). Acetate concentration after 24 h of incubation in these oligosaccharide extract-*Lpb. plantarum* combinations ranged between 1.39 and 2.38 mmol, with the highest increase recorded in soybean oligosaccharide. However, a significant ($p < 0.05$) increase in propionate (0.65 mmol) and butyrate (0.5 mmol)

Table 3 Growth characteristics of *Lpb. plantarum* ATCC 14917 and *Lb. rhamnosus* ATCC 7469 on different oligosaccharide sources segregated by type of crop

		Growth of *Lpb. plantarum* & *Lb. rhamnosus* (log CFU/ml) 0 h incubation		Growth of *Lpb. plantarum* & *Lb. rhamnosus* (log CFU/ml) 24 h incubation		Growth rate (μ log CFU/ml/hour)	
Crop type	**Crop variety**	***Lpb. plantarum***	***Lb. rhamnosus***	***Lpb. plantarum***	***Lb. rhamnosus***	***Lpb. plantarum***	***Lb. rhamnosus***
Soybean	Maksoy 3 N	5.09 ± 0.03^{a}	4.84 ± 0.10^{ab}	9.06 ± 0.05^{bcd}	8.22 ± 0.02^{f}	0.38 ± 0.01^{a}	0.32 ± 0.01^{a}
	Maksoy 6 N	5.06 ± 0.06^{a}	4.76 ± 0.11^{b}	9.03 ± 0.03^{bcde}	8.23 ± 0.02^{f}	0.38 ± 0.01^{a}	0.33 ± 0.01^{a}
	Indigenous variety	5.11 ± 0.12^{a}	4.86 ± 0.07^{a}	8.95 ± 0.10^{cde}	8.23 ± 0.03^{f}	0.37 ± 0.00^{a}	0.32 ± 0.01^{a}
Millet	Naromil 2	5.05 ± 0.08^{a}	5.05 ± 0.31^{a}	9.06 ± 0.05^{bcd}	8.94 ± 0.08^{a}	0.39 ± 0.01^{a}	0.37 ± 0.03^{a}
	Seremi 2	5.14 ± 0.18^{a}	5.06 ± 0.29^{a}	9.03 ± 0.02^{bcde}	9.01 ± 0.03^{a}	0.37 ± 0.02^{a}	0.38 ± 0.03^{a}
	Indigenous variety	5.06 ± 0.4^{a}	5.01 ± 0.23^{a}	9.08 ± 0.03^{abc}	8.99 ± 0.05^{a}	0.38 ± 0.04^{a}	0.38 ± 0.02^{a}
Sorghum	Narosorg 2	5.04 ± 0.14^{a}	4.94 ± 0.17^{a}	9.24 ± 0.01^{a}	8.45 ± 0.08^{d}	0.41 ± 0.01^{a}	0.34 ± 0.01^{a}
	Narosorg 4	5.13 ± 0.17^{a}	4.79 ± 0.13^{a}	9.22 ± 0.05^{a}	8.48 ± 0.04^{cd}	0.40 ± 0.02^{a}	0.35 ± 0.02^{a}
	Indigenous variety	5.46 ± 0.01^{a}	4.86 ± 0.18^{a}	9.23 ± 0.04^{a}	8.44 ± 0.06^{de}	0.36 ± 0.01^{a}	0.35 ± 0.02^{a}
Sesame	Sesim 2	5.09 ± 0.39^{a}	5.04 ± 0.30^{a}	9.04 ± 0.12^{bcde}	8.52 ± 0.07^{bcd}	0.38 ± 0.05^{a}	0.33 ± 0.03^{a}
	Sesim 3	5.08 ± 0.38^{a}	4.93 ± 0.16^{a}	8.89 ± 0.03^{e}	8.66 ± 0.13^{bc}	0.37 ± 0.04^{a}	0.36 ± 0.01^{a}
	Indigenous variety	4.96 ± 0.32^{a}	4.99 ± 0.20^{a}	8.91 ± 0.06^{de}	8.68 ± 0.04^{b}	0.38 ± 0.04^{a}	0.35 ± 0.02^{a}
Control	negative control (MRS broth)	5.18 ± 0.02^{a}	5.19 ± 0.08^{a}	9.14 ± 0.04^{ab}	8.14 ± 0.01^{f}	0.38 ± 0.01^{a}	0.28 ± 0.01^{b}

Values indicate mean ± SD ($n = 3$) for crop type under each probiotic. Means in the same column followed by different letter superscripts are significantly different from each other at 5% level of significance

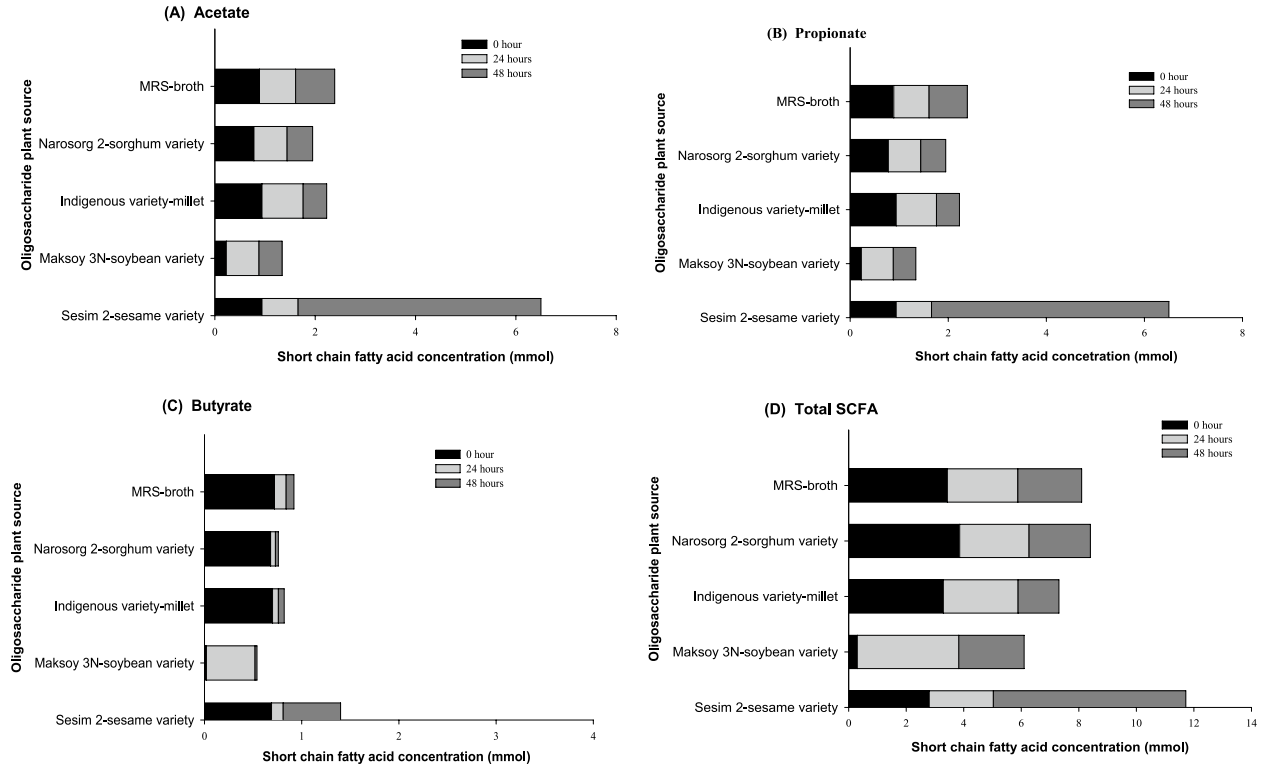

Fig. 3 Production of short chain fatty acids (**A**: acetate, **B**: propionate, **C**: butyrate and **D**: total short chain fatty acids) in different oligosaccharide plant source fermented by *Lpb. plantarum* during 48-h incubation

concentration was recorded only in soybeans. After 48 h of incubation, all the SCFAs decreased albeit significantly ($p > 0.05$) except in sesame where butyrate and propionate increased significantly ($p < 0.05$).

In contrast, oligosaccharide- *Lb. rhamnosus* combinations (Fig. 4) exhibited a significant decrease ($p < 0.05$) in butyrate concentration for the first 24 h across all oligosaccharide combinations. Propionate concentrations remained largely unchanged except in millet and sesame oligosaccharides which showed a significant ($p < 0.05$) decrease in the first 24 h followed by a significant ($p < 0.05$) increase after 48 h of incubation. Acetate concentration significantly ($p < 0.05$) increased in all oligosaccharide combinations during the first 24 h but decreased after 48 h except in soybean oligosaccharides and the negative control.

These variations in individual SCFAs consequently influenced both the total and molar proportions of the SCFAs. For instance, soybean and sesame oligosaccharides recorded the highest net increase in total SCFAs after 24 h and 48 h of incubation, respectively. Furthermore, soybean oligosaccharide extract exhibited (67:18:14-Acetate: propionate: butyrate) molar proportions of SCFAs comparable with recommended ranges (60:20:20- Acetate: propionate: butyrate) when *Lpb. plantarum* was used. However, the total SCFA concentration observed in oligosaccharide-*Lb. rhamnosus* combinations were significantly lower than MRS broth except for soybean during the first 24 h and sesame after 48 h.

Potential of oligosaccharide extracts from different plant sources to enhance antagonistic activity of *Lpb. plantarum* and *Lb. rhamnosus* against common enteric pathogens

The results of the present study demonstrate that oligosaccharide extracts from all different plant sources studied enhanced the antagonistic potential of both single and mixed cultures of *Lpb. plantarum* and *Lb. rhamnosus* against *Salmonella enterica* ATCC 13076 and *Shigella flexneri* ATCC 12022. The antagonistic activity of both probiotics was enhanced by 30–80% when combined with various oligosaccharide sources, compared to the control. This enhancement was similar in pattern but varied in intensity following a 24-h incubation period (as depicted in Figs. 5 and 6). A consistent and statistically significant ($P < 0.05$) antagonistic activity against *Salmonella* was noted for both *Lpb. plantarum* and *Lb. rhamnosus* in both single and mixed cultures in the presence of all oligosaccharide plant sources (Fig. 5).

In contrast, the antagonistic activity of the oligosaccharide-probiotic mixtures on *Shigella* growth varied

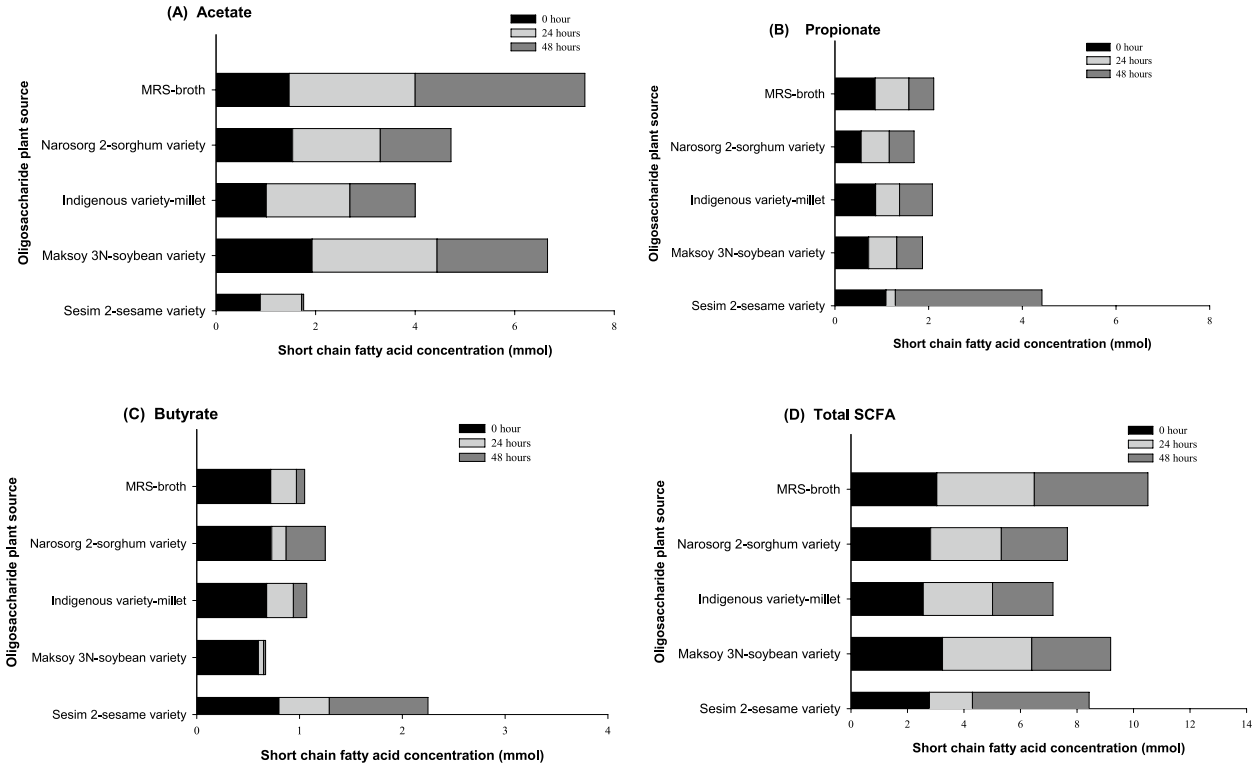

Fig. 4 Production of short chain fatty acids (**A**: acetate, **B**: propionate, **C**: butyrate and **D**: total short chain fatty acids) in different oligosaccharide plant source fermented by *Lb. rhamnosus* during 48-h incubation

(Fig. 6). For example, sesame augmented *Shigella* growth when co-cultured with *Lpb. plantarum* and *Lb. rhamnosus* after a 24-h incubation period, resulting in the bacterial count of 8.32 log CFU/ml. Conversely, millet oligosaccharide extracts did not enhance the antagonistic activity of *Lb. rhamnosus* against *Shigella* throughout the incubation period. The growth of *Shigella* ranged from 7.66 log CFU/ml to 8.13 log CFU/ml at 0- and 24-h incubation, respectively. The inhibitory effect of *Lpb. plantarum* against *Shigella* was significantly enhanced by oligosaccharides from all plant sources, resulting in colony counts that were too few to enumerate (< 30 CFU) even at the lowest dilution factor, indicating a decrease in *Shigella* growth after 24 h of incubation.

After 24 h of antagonistic activity, the cell densities of both *Lactiplantibacillus plantarum* (*Lpb. plantarum*) and *Lacticaseibacillus rhamnosus* (*Lb. rhamnosus*) remained above the recommended threshold for probiotic products (6 log CFU/ml), regardless of the oligosaccharide source or the pathogen involved. Although a decline in microbial counts was observed over the incubation period, co-cultures of oligosaccharides with *Lpb. plantarum* consistently demonstrated significantly higher cell densities compared to those with *Lb. rhamnosus*, as presented in Table 4. The decrease in the microbial counts after 24 h ranged between 0.1 and 1.5 log CFU/ml with rhamnosus co-cultures showing the greatest decrease. Among the oligosaccharides evaluated, co-cultures with indigenous millet variety recorded the lowest microbial counts for both probiotics followed by sesame. These findings underscore the superior resilience of *Lpb. plantarum* in maintaining growth under antagonistic conditions and provide valuable insights for optimizing oligosaccharide-probiotic-pathogen interactions for development of food products with synbiotic properties.

Discussion

Growth characteristics of *Lpb. plantarum* and *Lb. rhamnosus* on oligosaccharides extracted from sorghum, millet, soybean, and sesame

To qualify as a prebiotic, an oligosaccharide must demonstrate the ability to enhance the growth of probiotics to a cell density ranging from 6 to 9 log CFU/ml or CFU/g [60]. Maximum proliferation (8–9 log CFU/ml) of six *Lactobacillus* strains on 3 different prebiotic carbohydrates is reported at 24 h incubation [61]. Similar results are reported in *Lb. plantarum* MNC 21 using different varieties of sorghum malt extracts as a growth media [49]. Moreover, Di Pede et al. [62] reported significant growth of *Limosilactobacillus reuteri*, *Lactiplantibacillus*

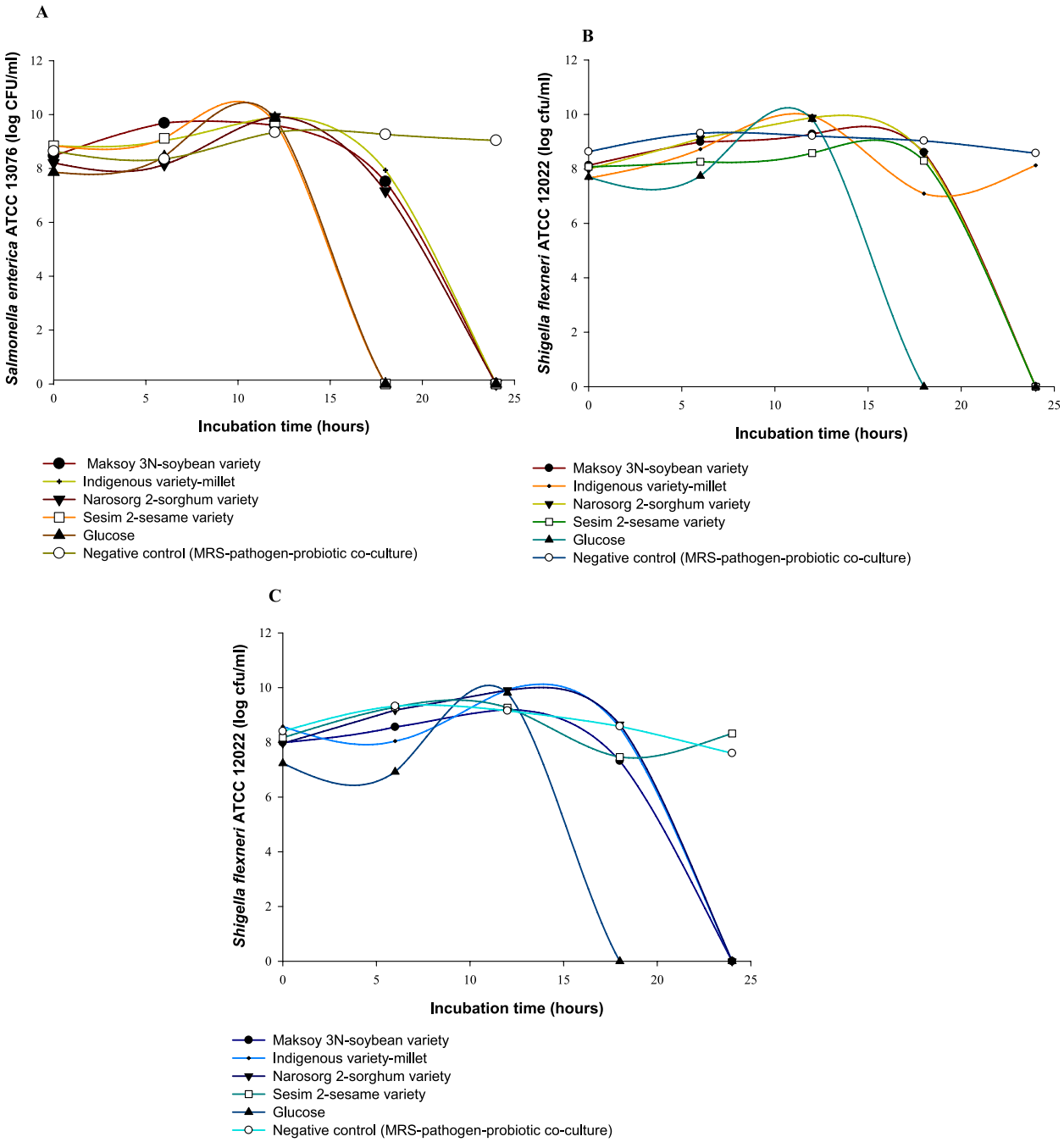

Fig. 5 Effect of oligosaccharide plant source on antagonistic activity of monoculture and co-culture strains against *Salmonella enterica* ATCC 13024 over 24-h incubation time: **A**-Antagonistic activity of *Lpb. plantarum* ATCC 14917 monoculture: **B**-antagonistic activity of *Lb. rhamnosus* ATCC 7469 monoculture: **C**-antagonistic activity of ATCC 14917 and ATCC 7469 co-culture. Error bars are excluded for clarity

plantarum and *Lacticaseibacillus rhamnosus GG* when cultured on maltooligosaacharide extracts similar to commercial FOS. These results are comparable to the maximum proliferation of both *Lpb. plantarum* ATCC 14917 and *Lb. rhamnosus* ATCC 7469 recorded in the current study for all oligosaccharide extracts after 24 h of incubation. These results could be attributed to the additional energy sources provided by the oligosaccharides to the probiotics. Sorghum, millet, soybean and sesame extracts majorly provide galacto-oligosaccharides and fructo-oligosaccharides which are good sources of energy for probiotic bacteria [47, 63]. This implies that

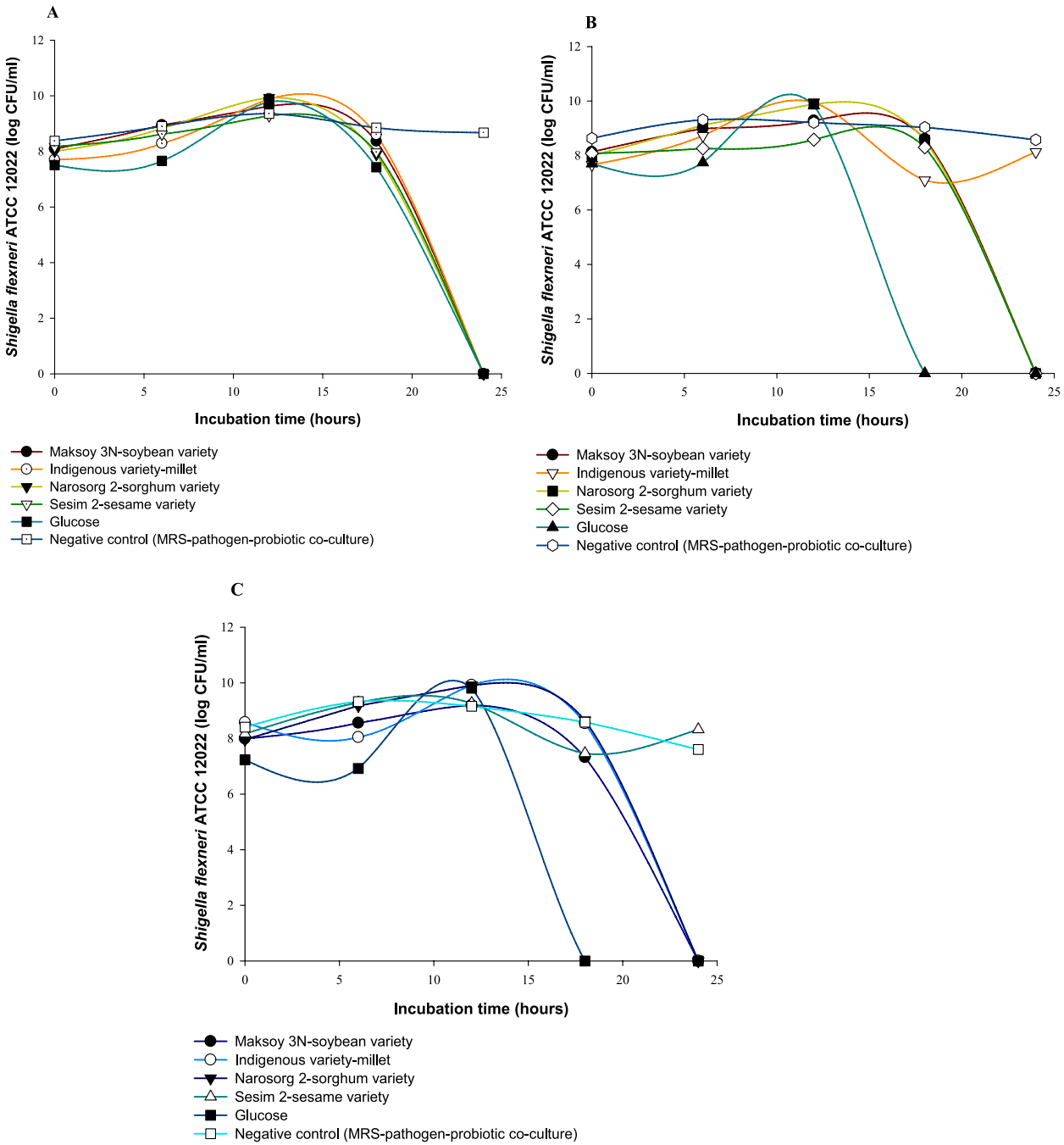

Fig. 6 Effect of oligosaccharide plant source on antagonistic activity of monoculture and co-culture strains against *Shigella flexneri* ATCC 12022 over 24-h incubation time: **A**-Antagonistic activity of *Lpb. plantarum* ATCC 14917 monoculture: **B**-antagonistic activity of *Lb. rhamnosus* ATCC 7469 monoculture: **C**-antagonistic activity of ATCC 14917 and ATCC 7469 co-culture. Error bars are excluded for clarity

these plant-based oligosaccharides have a prebiotic property of enhancing growth of beneficial bacteria such as *Lpb. plantarum* and *Lb. rhamnosus*. When consumed, they could increase the gut microflora, which in turn boosts gut health, strengthens the immune system, and improves overall well-being.

The extent to which plant-based oligosaccharides are metabolized as energy sources by probiotic microorganisms depends on the degree of polymerization and structural complexity of the plant source. The current study indicates that oligosaccharide from improved varieties of cereals, legumes and oil seed significantly enhance the

Table 4 Probiotic microbial counts following pathogen inhibition across oligosaccharide-probiotic-pathogen co-cultures

Oligosaccharide source	probiotic count across oligosaccharide-probiotic-pathogen co-cultures after 24 h incubation (log CFU/ml)							
	Lpb. plantarum-salmonella		*Lpb. plantarum-shigella*		*Lb. rhamnosus-salmonella*		*Lb. rhamnosus-shigella*	
	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
Maksoy 3 N soybean	8.03 ± 0.04^{a}	7.85 ± 1.44^{a}	8.05 ± 0.21^{a}	7.1 ± 1.13^{b}	8.47 ± 0.48^{a}	7.66 ± 1.35^{b}	8.27 ± 0.2^{a}	7.23 ± 0.5^{b}
Indigenous variety-millet	8.05 ± 0.21^{a}	7.48 ± 2.22^{b}	7.96 ± 0.08^{a}	6.79 ± 0.16^{b}	8.34 ± 0.96^{a}	6.95 ± 0.13^{b}	7.84 ± 0.25^{a}	6.81 ± 0.21^{b}
Narosorg 2-sorghum	7.95 ± 0.21^{a}	7.105 ± 1.14^{a}	8.1 ± 0.14^{a}	7.65 ± 1.06^{b}	8.4 ± 0.57^{a}	7.73 ± 1.17^{b}	7.995 ± 0.01^{a}	7.13 ± 0.95^{b}
Sesim 2-sesame	8.1 ± 0.28^{a}	7.75 ± 0.64^{a}	8.25 ± 0.21^{a}	7.65 ± 1.78^{b}	7.94 ± 0.2^{a}	6.55 ± 1.06^{b}	8.09 ± 0.01^{a}	6.9 ± 0.57^{b}
Negative control	8.15 ± 0.49^{a}	6.25 ± 0.92^{b}	8.13 ± 0.18^{a}	6.65 ± 0.21^{b}	7.69 ± 0.14^{a}	5.45 ± 0.64^{b}	8.44 ± 0.26^{a}	5.05 ± 1.48^{b}

Values indicate mean ± SD ($n = 3$) for oligosaccharide-probiotic-pathogen co-culture. Means in the same row followed by different letter superscripts are significantly different from each other at 5% level of significance

growth of *Lpb. plantarum* and *Lb. rhamnosus* compared to indigenous varieties. These results concur with those of Marotti et al. [40] who reported enhanced growth of *Lb. plantarum* L12 by improved wheat varieties compared to old varieties. Crop improvement through breeding techniques may have caused a decrease in the degree of polymerization of the oligosaccharides [64].

A low degree of polymerization increases the exposure of oligosaccharide structure to hydrolytic enzymes and probiotic micro-organisms that are responsible for their fermentation [65]. This in turn influences the quantity of metabolites produced. Therefore, oligosaccharide extracts from improved varieties (Maksoy 3 N, Narosorg 2, and Sesim 2) and indigenous millet varieties were further evaluated for their potential in short chain fatty acid production and antagonistic activity of the probiotics. This provides an opportunity for the development of fermented food products with both prebiotic and probiotic attributes (synbiotic food products).

Production of short chain fatty acid from extracted oligosaccharide and probiotic mixtures

The second key attribute of a substrate to be classified as a prebiotic is its capacity to produce bioactive metabolites that confer health benefits to the host [15]. In this study, sorghum, millet, soybean, and sesame oligosaccharides successfully produced significant amount of the three primary SCFAs (butyrate, acetate, and propionate) in the first 24 h, which are well-documented for their health-promoting properties. The concentration of SCFAs observed in oligosaccharides in this study is consistent with the findings of Harris et al. [39], who observed an elevated production of SCFAs by galacto-oligosaccharides, compared to polydextrose and rhamnose. Additionally, Roupar et al. [29] reported similar results in *Lactobacillus*-FOS mixtures. This could be due to the quantity and structural composition of the available total oligosaccharides which have rapid and moderate fermentation rates by the probiotic bacteria.

The fermentation rate and metabolite production by bacteria are dependent on the structure of the carbon source, specifically the number of glycosidic linkages and branching levels [66]. When these linkages and branches are moderately complex (with sugar residues between 3 and 10), they allow varying rates of enzymatic accessibility and utilization by different bacterial species. This differential accessibility regulates the degree of fermentation, and the diversity of metabolites generated during the process [62]. Nemska et al. [30] demonstrated that 41 candidate *Lacticaseibacillus* probiotic strains had varying degree of fermenting different carbohydrate sources in an *in vitro* assessment study. Similarly, *Lpb. plantarum* ATCC BAA-793 metabolized Fructo-oligosaccharides and human milk oligosaccharides better than cranberry xyloglucan [67].

The results of the current study demonstrate that plant oligosaccharide extracts when combined with *Lpb. plantarum* exhibits higher metabolite production compared to their combinations with *Lb. rhamnosus*. The results highlight that oligosaccharide extract-*Lb. rhamnosus* combinations are not ideal choices as prebiotic-probiotic combinations for developing a synbiotic food product. *Lpb. plantarum* is known for its versatile metabolic pathways and ability to utilize a wide range of carbohydrates including oligosaccharides from different food types [68, 69]. This adaptability allows it to efficiently ferment various plant-based oligosaccharides, leading to enhanced metabolite production. Therefore, it is recommended to optimize oligosaccharide extract-*Lpb. plantarum* combinations for the formulation and advancement of synbiotic food products.

Variations in the individual concentrations of SCFAs affect the total SCFAs and their molar ratios produced. Only soybean oligosaccharide extract exhibited (67:18:15-Acetate: propionate: butyrate) molar

proportions of SCFAs comparable with recommended ranges (60:20:20- Acetate: propionate: butyrate) [70] when *Lpb. plantarum* was used. An optimal molar ratio of SCFAs is essential for maximizing their local and systemic health benefits. For instance the local effects include reduced gut pH, inhibition of gastrointestinal pathogens through decreased adherence of enteric pathogens to the epithelial cells of the gastrointestinal tract and improved gastrointestinal barrier [71, 72]. Systemically, SCFAs enhance the absorption of minerals like calcium and magnesium, and exhibit immune-regulatory, cardio-protective, neuro-protective, anti-inflammatory, and anti-oxidant activities [73, 74]. The results of the current study indicate that the evaluated oligosaccharides particularly soybean oligosaccharides and *Lpb. plantarum* combinations have the potential to create prebiotic and probiotic product combinations that have the potential to synergistically improve host health when consumed. However, *in vivo* studies are necessary to determine the concentration of SCFAs after consumption of such food products to evaluate their associated beneficial attributes, particularly among vulnerable populations.

It also provides an opportunity to explore the potential of improving the molar proportions of total SCFA from other oligosaccharides extracts by making composite formulae. This processing technology has been used to increase the nutritional quality of plant-based foods in resource constrained settings [50, 75, 76]. However, further studies should evaluate the effect of nutrient-nutrient interaction on the fermentability and quantity of SCFA produced by the composite products.

Potential of oligosaccharide extracts from different plant sources to enhance antagonistic activity of *Lpb. plantarum* and *Lb. rhamnosus* against common enteric pathogens

The enhanced antagonistic activity of the study probiotics against enteric pathogens concur with those of Śliżewska & Chlebicz-Wójcik [58] where *Lactobacillus* species cultured in inulin as a prebiotic source significantly inhibited *Salmonella* growth to non-detectable levels compared to β-glucan and maltodextrin. Furthermore, a combination of *Lb. rhamnosus* NCDC 298 and FOS reduced risk of entero-toxigenic *E. coli* infection [77]. The enhancement of antagonistic activity is influenced by the specific probiotic strain, the type of enteric pathogen, and the available prebiotic oligosaccharide source. Piatek et al. [78] reported variation in the degree of pathogen inhibition of *Shigella* and *Salmonella* spp. by *Lb. rhamnosus GG.* This suggests that selecting the appropriate combination of probiotic and oligosaccharide source is crucial for achieving effective inhibition of enteric pathogens.

This study demonstrates that oligosaccharide extracts from sorghum, soybean and sesame selectively promoted the growth of probiotic bacteria (*Lpb. plantarum* and *Lb. rhamnosus)* while inhibiting the growth of enteric pathogenic bacteria. This selective promotion is another crucial attribute for a substrate to be classified as a prebiotic [15]. *Shigella* and *Salmonella* infections are significant contributors to childhood functional gastrointestinal disorders such as diarrhea in developing countries [3]. Traditionally, these infections have been treated with antibiotics such as azithromycin, and colistin [79]. However, recent studies indicate a rise in antibiotic resistance in these regions [80], prompting the exploration of alternative therapeutic approaches. One such approach involves the use of prebiotic oligosaccharides and probiotics, either individually or in combination, to treat FGIDs [81].

The ability of oligosaccharide extract-probiotic combinations to reduce the bacterial pathogens depends on the extent of fermentation by the probiotic and the type of fermentation metabolites. SCFAs are the major health promoting metabolites produced which exhibit antimicrobial [82], and anti-inflammatory properties [83]. Additionally, SCFAs effectively inhibit enteric pathogens through a pH, concentration and complexity-dependent pathway [84]. When these SCFAs are ingested they enhance mucus production which increases intestinal barrier integrity [85]. However, other factors such as the initial concentration of host gut bacteria, and host responses influence the prebiotic mechanistic actions [86] and hence the host's health outcomes. Therefore, the efficacy and effectiveness of the oligosaccharides with prebiotic attributes under physiological conditions need to be explored. Furthermore, to evaluate its potential as a functional food for children under the age of five, it is essential to perform *in vitro* fermentation using fecal microbiota from this age group. This will allow for the observation of microbiota changes and interactions.

Conclusion

This study highlights the superior prebiotic properties of oligosaccharides extracted from improved cereal and legume varieties when fermented with selected probiotic strains. The oligosaccharide extracts promoted proliferation of the probiotic cell density exceeding the World Health Organization's minimum recommended levels (6 log CFU/ml or log CFU/g) for probiotic products. Furthermore, the observed increase in the production of total short-chain fatty acids (SCFAs) when *Lpb. plantarum* was cultured in oligosaccharide substrates derived from Maksoy 3N soybean and Sesim 2 sesame varieties may have contributed to enhanced antagonistic activity against pathogenic species. This positions *Lpb. plantarum* as a promising candidate for targeted applications in probiotic and synbiotic food production. Future

research should focus on optimizing combinations of plant oligosaccharide extracts with *Lpb. plantarum* to maximize their production of beneficial metabolites and probiotic functionality. This could include evaluating different oligosaccharide types, composite proportions, and fermentation conditions to enhance product yields.

Incorporating oligosaccharide plant sources with prebiotic potential, such as improved cereal and legume varieties, in the diet of populations at risk of functional gastrointestinal diseases may help reduce the incidence and severity of these conditions. These findings can also be used to develop targeted nutrition interventions such as the development of food products with both prebiotic and probiotic attributes for vulnerable populations, such as children under five years old. However, it is crucial to investigate the efficacy and effectiveness of oligosaccharides with prebiotic properties under physiological conditions. Additionally, to assess their potential as functional foods for children under the age of five, it is imperative to conduct *in vitro* fermentation using fecal microbiota from this demographic. This approach will facilitate the observation of microbiota changes and interactions, providing valuable insights into their prebiotic benefits and overall improved public health.

Acknowledgements
We would like to thank the TAGDEV project team for the funds provided to conduct this research and the Gulu University Research & Multifunctional Laboratories for providing a conducive Laboratory environment to implement the study experiments.

Authors' contributions
D.A. Performed experiments, collected and analyzed data and wrote original draft, S.O. Analyzed data and wrote original draft., I.M.M., Validated experimental design and data collection and D.O. performed data curation and interpretation, D.O. Validated experimental design and provided resources to perform the experiments. All authors conceptualised, wrote, reviewed and approved the final manuscript.

Funding
This research was funded by the Regional University Forum for Capacity Building in Agriculture (RUFORUM) in partnership with MasterCard Foundation under the Transforming African Agricultural Universities to Meaningfully Contribute to Africa's Growth and Development (TAGDEV) project (grant number RU2016MCF002). The funding source did not have any involvement in; study design; collection, analysis, and interpretation of data; writing the report; and in the decision to submit the article for publication.

Data availability
All data supporting the findings of this study are available within the paper.

Declarations

Ethics approval and consent to participate
All procedures were performed in compliance with the relevant laws and institutional guidelines. Ethical approval to conduct the study was granted by Uganda National Council for Science and Technology, reference number A226ES and Gulu University Research Ethics Committee, reference number GUREC-2022–242.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 21 January 2025 Accepted: 7 May 2025
Published online: 19 May 2025

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