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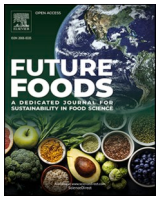

Synergistic potential of agrobiomass-derived xylooligosaccharides (XOS) and antioxidants as pioneering prebiotics for probiotic cultivation

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ABSTRACT

Prebiotic resources, such as xylooligosaccharides (XOS), which are resistant to acidity and temperature, can be derived from lignocellulosic agrobiomass. Hydrolysates containing prebiotic XOS were produced from fruit, rice, and sugarcane biomass using acid and hydrothermal pretreatments. Phosphoric acid pretreatment showed greater potential for biomass breakdown and oligosaccharide release. FTIR analysis detected xylan and pectin in the pellets produced by precipitating the hydrolysates of mango peel (MP), pineapple peel (PP), rice bran (RB) and sugarcane leaf (SCL). The hydrolysate and the precipitate presented different XOS (xylobiose and xylotetraose) profiles and were more than twofold greater in the PP, RB, and SCL pellets (71.28, 109.55, and 188.48 mg/mL, respectively) than in the MP pellets (0.29 mg/mL). SCL hydrolysate, as a carbon source, promotes probiotic growth but is unsuitable for pathogen growth. Furthermore, fermenting the spray-dried SCL hydrolysate powder with probiotics (*Bacillus subtilis* and lactic acid bacteria) significantly increased its phenolic (236.07 μg GAE/mL) and flavonoid (2.75 mg QE/mL) contents and antioxidant activity (75.77 %). This study highlights the potential for a synergistic interaction between XOS and bioactive compounds, which may considerably benefit probiotics and their hosts. This research demonstrates an efficient and straightforward method for producing XOS, yielding prebiotics at 189.72 g/kg of biomass. This approach provides a viable alternative for the development of plant-based, value-added food products.

1. Introduction

Xylooligosaccharides (XOS) are prebiotic materials composed of xylose units bonded by a β-(1,4)-linked backbone. Xylose residuals vary from 2 to 10 and are known as xylobiose, xylotriose, etc. (Qaseem et al., 2021). XOS are produced from xylan, which is extracted from lignocellulosic materials by hydrolyzing xylan with steam, water, a diluted solution of mineral acids, or alkaline solutions (Liu et al., 2018; Nair et al., 2017; Poletto et al., 2020; Zhou et al., 2019). When XOS are produced, understanding the structural components of lignocellulosic materials, namely, cellulose, lignin, and especially hemicellulose, which are sources of xylan, is necessary (Zhang et al., 2020a). A suitable starting material for XOS production contains a large quantity of hemicellulose.

As the world's 6th largest producer of rice and 3rd largest producer of sugarcane (FAO, 2025), Thailand faces considerable challenges in managing its agricultural residues. With increasing crop production, the volume of residual biomass continues to grow, and traditional disposal methods, such as burning, remain prevalent. Approximately 5.6 million tons or 83 % of all agricultural residue burned originates from rice and sugarcane, contributing to 64 % of the total PM10, 60 % of the total PM2.5, 86 % of the total NOx, and 84 % of the total SO_2 emissions from agricultural and forest fires in Thailand (Kumar et al., 2020). To address these environmental concerns, various technological approaches for adding value to agricultural waste, such as food production, energy generation, fertilizer development, and agro-processing, have been explored, with a particular focus on utilizing agricultural residues from the sugarcane industry (Fukuda, 2020). However, as the production of sugarcane and rice biomass continues to increase (Kanchanapiyaa and Tantisattayakul, 2024), current waste management strategies may

Abbreviations: ABTS, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; FTIR, fourier transform infrared spectrometer; GA, gluconic acid; HMF, hydroxymethylfurfural; HPLC, high-performance liquid chromatography; LAB, lactic acid bacteria; MP, mango peel; MSS, mango seed shell; PA, phosphoric acid; PP, pineapple peel; RB, rice bran; RH, rice husk; RS, rice straw; SCB, sugarcane bagasse; SCL, sugarcane leaf; TFC, total flavonoid content; TPC, total phenolic content; RSC, reducing sugar content; XOS, xylooligosaccharides; $ZnCl_2$, zinc chloride.

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become insufficient.

In addition to sugarcane and rice, Thailand exports processed fruits, such as mangoes and pineapples, which are highly popular worldwide. The processing of these fruits generates substantial amounts of waste, including peels and seeds (Hemung et al., 2022; Sriprom et al., 2024). These biomasses contain hemicellulose, which plays a crucial role in the production of prebiotic XOS. By combining multiple processes, XOS production can be optimized for higher yields and targeted product specificity. For the production of XOS from sugarcane leaves, Mensah et al. (2024) developed and optimized a two-step process of hydrothermal pretreatment and enzymatic hydrolysis to achieve high conversion yields and high product specificity, which has considerable potential for supplying the nutraceutical industry with materials. Further optimization of these production steps could reduce costs and increase efficiency for large-scale applications.

The selection of biomass raw materials in this study was primarily based on their hemicellulose content, as xylan is the key precursor for producing XOS with potential prebiotic activity. This approach was important for identifying agricultural residues with high xylan contents that, upon hydrothermal and acid pretreatment, could yield considerable amounts of XOS. Although hydrothermal pretreatment, which combines heat and pressure, efficiently produces XOS, this process can release high amounts of monosaccharides and sugar derivatives such as furfural and hydroxymethylfurfural (HMF) (Zhang et al., 2020b). Lignocellulose can be cleaved into low-molecular-weight oligomers via hydrothermal pretreatment and acid hydrolysis (Cocero et al., 2018). Acid catalysis further enhances the decomposition of hemicellulose, particularly when it is performed under short reaction times and at low temperatures (Santibáñez et al., 2021).

Although many types of acids are used for pretreatment, the reaction mechanism of each catalyst in breaking down the structure of lignocellulosic material is different. Three catalytic agents, including gluconic acid (GA), phosphoric acid (PA), and zinc chloride ($ZnCl_2$), have been utilized to dissociate lignocellulosic materials, as they effectively facilitate XOS production using Lewis and Brønsted acid mechanisms (Hoang et al., 2021; You et al., 2020; Zhou et al., 2019). Gluconic acid (GA) is a green, sustainable solvent commonly used for polysaccharide recovery from biomass materials (Contreras et al., 2014). Zhou et al. (2019) reported that sugarcane biomass pretreatment with GA at concentrations below 5 % resulted in the highest XOS yield, which was primarily composed of X2–X6. Similarly, phosphoric acid is recommended for use in dilute form due to its effectiveness in pretreating lignocellulosic biomass while minimizing the formation of inhibitory compounds (Hoang et al., 2021).

Dilute acid pretreatment primarily affects hemicellulose, which partially solubilizes cellulose and lignin to produce oligomers and other carbohydrates (Jönsson and Martín, 2016). Acid concentrations between 0.1 % and 2 % are commonly used for biomass pretreatment (Hoang et al., 2021). For example, Nair et al. (2017) reported maximum xylan and arabinan hydrolysis in wheat straw pretreated with 1.75 % acid for 15 min. Among the most effective inorganic catalysts for biomass swelling, $ZnCl_2$ selectively solubilizes hemicellulose into oligomeric and monomeric sugars (Kamireddy et al., 2013). Hemicellulose is more easily degraded than cellulose when pretreated with zinc chloride at concentrations ranging from 0.2 to 0.8 % (w/w) (You et al., 2020).

The reaction time also plays a critical role in XOS production. An excessive reaction time of 30 min can lead to a decrease in XOS yield and an increase in byproducts (Hoang et al., 2021; You et al., 2020; Zhou et al., 2019). Therefore, to maximize the XOS oligomer yield, the reaction time should be minimized. In addition, moisture retention in the final XOS product is a challenge, potentially leading to fungal contamination. Cost efficiency is also crucial, with studies exploring the conversion of prebiotics into powders for fiber-rich food applications (Vilas et al., 2024). Compared with other techniques, spray drying is a preferred method because it effectively reduces moisture, concentrates the product, and lowers production costs (Iaconelli et al., 2015). The aim of this study was to add value to agricultural biomass and address waste management challenges by employing a hydrothermal method combined with dilute acid pretreatment for XOS production. The analysis focused on the oligosaccharide content, xylan functional groups, and prebiotic potential of the final product, particularly its ability to support the growth of *Bacillus subtilis* and lactic acid bacteria. Furthermore, the bioactive compounds and antioxidant activities generated through bacterial fermentation were assessed.

2. Materials and methods

2.1. Agricultural biomass

Eight agricultural biomasses were used in this study: rice bran (RB), rice husk (RH), rice straw (RS), mango peel (MP), mango seed shell (MSS), pineapple peel (PP), sugarcane bagasse (SCB), and sugarcane leaf (SCL). These biomasses were oven-dried at 60 °C until dry and ground to 60 μm mesh powder. The levels of various agricultural biomass components, including cellulose, hemicellulose, and lignin, were determined via the AOCC method (Van Soest et al., 1991). The moisture content of the biomass after the hot air-drying process was assessed using a method adapted from Zheng et al. (2024). This method involved determining the water content through a heating and drying process. One gram of sample was dried at 60 °C in a dry oven until a constant weight was achieved. The water content was then calculated using the following equation:

$$\textit{Moisture content}\ (\%) = [(W_1 - W_2)/W_1] \times 100 \quad (1)$$

where W_1 and W_2 are the weights of the samples at the start and end of the drying process, respectively.

2.2. Pretreatment of agricultural biomass

The catalysts used for the pretreatment of the biomass in this study included gluconic acid (GA; $C_6H_{11}NaO_7$, Carlo Erba, Milan, Italy), phosphoric acid (PA; H_3PO_4, RCI Labscan Ltd., Ireland), and zinc chloride ($ZnCl_2$, Carlo Erba, Milan, Italy). Each catalyst was used at a concentration effective for biomass conditioning while preventing the formation of byproducts (Liu et al., 2018; Nair et al., 2017; Zhou et al., 2019). Therefore, the concentrations of GA were 1 %, 3 %, and 5 % (w/v); the concentrations of PA were 0.5 %, 1.0 %, and 1.5 % (v/v); and the concentrations of $ZnCl_2$ were 0.2, 0.4, and 0.6 % (w/v). Briefly, 10 g of dried biomass was placed in 100 mL of catalyst solution at each concentration and pretreated by autoclaving at 121 °C and 15 psi for 15 or 30 min. The experiment was conducted with triplicate replicates for each condition. After the samples cooled, the liquids were collected using vacuum filtration, and the reducing sugar content (RSC) was determined.

2.3. Carbohydrate precipitation

To precipitate carbohydrates, the pH of the liquor sample was adjusted to 5, and the reaction was carried out by using 95 % ice-cold ethanol at a liquor-to-ethanol ratio of 1:2. The precipitate was allowed to rest overnight to settle and separated by centrifugation, followed by washing with ethanol. The washed precipitate pellet was subsequently dried at 50 °C and stored in desiccator containers until the weight was constant. The carbohydrate recovery was calculated (Samanta et al., 2012) using the following equation:

$$Carbohydrate\ recovery\ (\%) = \frac{(dry\ weight\ of\ extracted\ carbohydrate\ (g) \times 100)}{sample\ weight\ (g)} \quad (2)$$

2.4. Analysis of the hydrolysate and precipitate composition

The compositions of the monosaccharides (xylose, glucose, and arabinose), oligosaccharides (xylobiose, xylotriose, and xylotetraose), furfural and HMF (hydroxymethylfurfural) in the hydrolysates and precipitates dissolved in deionized water were determined via high-performance liquid chromatography (HPLC). The samples were filtered through a 0.22 μm filter. One hundred microliters of the filtered sample were injected into an HPLC system (Agilent HPLC; Model-1260) under chromatographic conditions using a Hi-Plex H 7.7 × 300 mm, 8-μm column (PL1170–6830) with a mobile phase of 0.005 M H_2SO_4, a flow rate of 0.55 mL/min, a column temperature of 60 °C, and a refractive index detector (RID) at 50 °C. Standards for XOS determination, β-(1–4)-xylobiose (Cat. No: O-XBI, MW: 282.24, >95 %), β-(1–4)-xylotriose (Cat. No: O-XTR, MW: 414.36, >95 %), and β-(1–4)-xylotetraose (Cat. No: O-XTE, MW: 546.47, > 95 %), were purchased from Megazyme (Bray, Ireland). The standards for xylose, glucose, HMF, and furfural were purchased from Sigma–Aldrich (St. Louis, MO, USA), and arabinose was purchased from TCI (Japan).

2.5. Evaluation of functional groups on the precipitate

Changes in the functional groups of the precipitate were analyzed via Fourier transform infrared (FTIR) spectroscopy (Bruker model: Tensor 27). The ATR spectra were recorded from 4000 to 600 cm^{-1}. The sample holder was first cleaned with absolute ethanol, and a prior spectrum was taken from the empty holder to ensure that no contaminants were present that could interfere with the results. Data processing was performed to transform percent transmittance into absorbance, and the resulting data were plotted against wavenumber. Insoluble wheat arabinoxylan (Megazyme, Ireland) and pectin (TCI, Japan) were used as standards.

2.6. Prebiotic properties of the hydrolysates

The microorganisms used in this study were isolated from different sources, as shown in Table 1. Hydrolysates and precipitates were used as carbon sources for assessing the growth and prebiotic properties of all the tested bacteria. A hydrolysate solution (0.1 % v/v), precipitate (0.1 % w/v), and glucose (0.1 % w/v) as a control were added to the minimal medium. Rich media were used for inoculum preparation; specifically, De Man, Rogosa and Sharpe (MRS) and Luria–Bertani (LB) media (Difco & BBL, USA) were used for LAB and *B. subtilis* and pathogens, respectively. The inoculum was grown for 24 h, and the optical density at 600 nm was measured before the inoculum was added to the broth medium. All the samples were incubated at 37 °C under static conditions, and the optical density was measured at 24 h via a spectrophotometer. The prebiotic activity score was calculated by comparing the optical density values determined immediately after bacterial inoculation and after 24 h of incubation according to the following equation (Reque et al., 2019):

$$Prebiotic\ activity\ score = OD\ (24h) - OD\ (initial) \quad (3)$$

Table 1
Bacterial species and strains used in this study.

Bacteria	Source
Bacillus subtilis	
B. subtilis B312	Plant
B. subtilis CE330	Chicken cecum
B. subtilis CO13	Chicken colon
B. subtilis D13	Chicken duodenum
B. subtilis I9	Chicken ileum
B. subtilis PB22	Pig feces
Lactic acid bacteria	
Enterococcus durans CH33	Chicken intestine
Enterococcus faecium CA4	Chicken intestine
Enterococcus faecalis PL4	Pig feces
Enterococcus faecalis PL41	Pig feces
Lacticaseibacillus paracasei OR1	Orange
Lacticaseibacillus rhamnosus MA3	Flower
Ligilactobacillus salivarius CH24	Chicken intestines
Pediococcus acidilactici SH8	Fermented shrimp
Pathogens	
Staphylococcus aureus ATCC 25923	Reference strain
E. coli ATCC 25922	Reference strain
S. Typhimurium ATCC 13311	Reference strain

2.7. Evaluation of spray-dried powder hydrolysate

The spray-dried hydrolysate was prepared by adding gum arabic to the hydrolysate, which was adjusted to a sugar content of 10 % via a reflectometer before being injected into a spray-dryer system (Spray Dryer TP-S15, Topition) with a nozzle diameter of 2.5 mm and inlet and outlet temperatures of 170 °C and 89 °C, respectively. The feed pump rate and aspirator blower capacity were controlled at 150 mL/h and 100 %, respectively. After the spray drying process, the spray-dried powder (1 % w/v) was used as a carbon source in minimal medium. Inocula prepared as described above were added and incubated at 37 °C for 48 h. Samples were taken to measure the optical density at 600 nm to determine the specific growth rate (μ) using the following equation:

$$Specific\ growth\ rate\ (\mu) = (ln(X/X_0)/t \quad (4)$$

where X is the optical density at a specific time and X_0 is the initial optical density (Divyashree et al., 2009). The supernatants were collected to determine the reducing sugar content (RSC), total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity.

2.8. Determination of the reducing sugar content (RSC)

The reducing sugar content (RSC) was determined via a dinitrosalicylic acid (DNS) assay (Miller, 1959). Briefly, 0.1 mL of a suitable dilution of the sample was mixed with 0.1 mL of DNS reagent, and the tube contents were mixed immediately. The samples were placed in a boiling water bath and incubated for 10 min, after which 1 mL of distilled water was added. The samples were cooled, and their absorbances were measured at 540 nm. A standard graph was generated using standard glucose (Sigma, USA).

2.9. Determination of the total phenolic content (TPC)

The total phenolic content (TPC) was determined by the Folin–Ciocalteu colorimetric method (Phuyal et al., 2020). For each sample, 0.5 mL of sample, 2.5 mL of 10 % Folin–Ciocalteu reagent and 2 mL of 7.5 % Na_2CO_3 were mixed well and incubated for 30 min at 40 °C in a water bath. The absorbance was then measured at 760 nm. Gallic acid solutions were prepared and used as standards. The TPC of the sample is presented as micrograms of gallic acid equivalents per milliliter of sample (μg GAE/mL).

2.10. Determination of the total flavonoid content (TFC)

The TFC was determined by a colorimetric aluminum chloride assay, as described by Kwaw et al. (2017). A mixture of 1 mL of the sample, 4 mL of distilled water and 0.3 mL of 5 % (w/v) $NaNO_2$ was mixed and allowed to stand for 5 min. Afterward, 0.3 mL of 10 % $AlCl_3$ (w/v) was added, mixed, and allowed to stand for 5 min. Two milliliters of 1 M NaOH were added, followed by 2.4 mL of distilled water to adjust the volume to 10 mL. After the solution was allowed to stand at room temperature for 10 min, its absorbance was measured at 415 nm. Quercetin solutions were prepared and used as standards. The TFC was calculated based on the milligrams of quercetin equivalents per milliliter

of sample (mg QE/mL).

2.11. Determination of antioxidant activity

The antioxidant activity was determined via the ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay method described by Laophongphit et al. (2023). Briefly, 7.4 mM ABTS and 2.6 mM potassium persulfate ($K_2S_2O_8$) were mixed at a ratio of 1:0.5 (v:v) and left in the dark for 12–16 h at room temperature. The ABTS working solution was prepared by diluting the ABTS stock solution in phosphate buffer solution (PBS, pH 7.2) to obtain an ABTS working solution with an initial absorbance of ~0.7 ± 0.02 at 734 nm. A 10 μL aliquot of sample was mixed with 990 mL of ABTS working solution and left in the dark for 30 min before the absorbance was measured at 734 nm. The antioxidant activity was calculated and expressed as the ABTS radical scavenging activity (ABTS RSA %) using Trolox solution as a standard according to the following equation:

$$ABTS\ RSA\% = [(A_{control} - A_{measure})/A_{control}] \times 100 \quad (5)$$

2.12. Statistical analysis

All the experiments were conducted at least in triplicate, and the results are presented as the mean ± standard deviation (SD). One-way ANOVA followed by Duncan's new multiple range tests was used to determine the differences in the means at significance levels of 0.05 and 0.01. All the statistical data were analyzed using the IBM SPSS Statistics 27 software program.

3. Results and discussion

3.1. Determination of agricultural biomass components

Lignocellulosic materials such as mango peels, mango seed shells, pineapple peels, rice bran, rice husks, rice straw, sugarcane bagasse, and sugarcane leaves are recognized as agricultural wastes in Thailand. The moisture content of biomass is a critical factor influencing its suitability as a feedstock for bioenergy production (Liu et al., 2017) and the efficiency of biomass pretreatment (Saadon and Osman, 2023). All the agricultural biomass samples were dried, and the final moisture content ranged from 2.99 % to 10.74 %, as shown in Table 2. Significant differences in moisture content were observed among the various types of agricultural waste biomass. For sugarcane biomass, bagasse retained more moisture than did leaves, which aligns with the findings of Saadon and Osman (2023), who reported a higher moisture content in the stems. A moisture content below 10 % is necessary for effective pretreatment and enzyme hydrolysis to achieve high yields of sugar released from lignocellulosic biomass (Tucker et al., 2003; Saadon and Osman, 2023). The moisture content of biomass can influence lignocellulose fractionation, as a relatively high moisture content interferes with the extraction and isolation process. Furthermore, a higher moisture content in biomass lowers the lignocellulose concentration per unit weight, as a significant portion of the weight is contributed by water molecules, which in turn affects the overall yield of the bioconversion product (Saadon and Osman, 2023). Optimizing the moisture content makes bioconversion processes more consistent and predictable, leading to reliable and high bioconversion product yields.

Table 2
Moisture content of the agricultural biomass.

Group	Agricultural biomass	Moisture content (%)
Fruit	Mango peel (MP)	4.35 ± 0.08^{cG}
	Mango seed shell (MSS)	6.63 ± 0.17^{bD}
	Pineapple peel (PP)	10.74 ± 0.14^{aA}
Rice	Rice bran (RB)	6.17 ± 0.27^{bE}
	Rice husk (RH)	5.92 ± 0.05^{cF}
	Rice straw (RS)	6.80 ± 0.06^{aC}
Sugarcane	Sugarcane bagasse (SCB)	7.34 ± 0.12^{aB}
	Sugarcane leaf (SCL)	2.99 ± 0.21^{bH}

The values are presented as the means ± standard deviations (SD) of three replicates.
Lowercase letters indicate a significant difference between biomasses for each group ($p < 0.05$).
The uppercase letters indicate significant differences among all biomasses ($p < 0.05$).

All agricultural waste biomass comprises cellulose, hemicellulose, lignin, and other components in varying ratios (Fig. 1). Among these constituents, the top three with the highest contents of cellulose were RH (49.77 %), followed by MSS (44.15 %) and RS (41.58 %). Moreover, the hemicellulose content was highest in SCL (33.44 %), followed by RS (29.90 %) and PP (29.14 %). The hemicellulose content in SCL is consistent at 20–35 %, as reported by Moodley and Kana (2018). A key component of XOS production is hemicellulose, which contains xylan, the main precursor for XOS production; however, its release from hemicellulose–lignin complexes poses challenges (Zhang et al., 2020b). Increasing the amount of lignin in raw materials could increase prebiotic production costs (Amorim et al., 2019). A greater proportion of lignin was found in MSS (31.02 %) than in the other lignocellulosic biomasses. Consequently, all the materials except MSS were chosen for the pretreatment process. Prebiotics derived from plants, such as XOS, are attractive because they resist acidity and temperature. Another important method of this recent study was the preparation of selected raw materials by grinding or milling to increase the contact area between the reagents and the complex structure and to disrupt cell wall interactions. By interacting with hemicellulose, cellulose crystallinity decreases, and polysaccharide solubility increases (Broxterman and Schols, 2018). This step is important for sample preparation before pretreatment.

3.2. Determination of products from pretreatment and precipitation

In this study, gluconic acid, phosphoric acid, and zinc chloride were selected for the pretreatment of agricultural materials. Compared with pretreatment for 15 min, pretreatment with 3 % GA for 30 min significantly increased the RSC released from the MP (35.71 mg/mL) and significantly increased carbohydrate recovery to 99.60 % ($p < 0.01$). Additionally, pretreatment with PA and $ZnCl_2$ resulted in high RSC of approximately 29.17 mg/mL and 29.64 mg/mL, with carbohydrate recoveries of 23.10 % and 18.17 %, respectively (Fig. S1A). A large amount of reducing sugars, approximately 22–37 mg/mL, was released from PP after pretreatment for 30 min compared with 15 min, while carbohydrate recovery was not significantly different (Fig. S1B). In the rice group, pretreatment with PA prominently increased the RSC in the RB and RS groups to 13.89 mg/mL and 15.98 mg/mL, respectively, after pretreatment with 1.5 % PA, whereas the RH released a sugar content of 1.83 mg/mL after pretreatment with 0.5 % PA. Moreover, high carbohydrate recoveries of 15.67 %, 3.47 %, and 9.10 % were detected for RB, RH, and RS, respectively, after PA pretreatment (Fig. S2). Pretreatment with 1.5 % PA significantly induced the release of reducing sugars from SCB (35.28 mg/mL) and SCL (11.60 mg/mL), including high carbohydrate recoveries (7.30 % and 11.60 %, respectively) (Fig. S3).

Pretreatment is usually performed to solubilize the physical and chemical barriers that make native biomasses recalcitrant. In this study, water and high pressure were used as the main ingredients for material pretreatment. During the high-pressure process, water penetrates the biomass and hydrated cellulose to remove most of the hemicellulose and some of the lignin (Singh et al., 2021). Then, hemicelluloses are soluble when water autoionizes, resulting in hydronium ions. In addition to thermal treatment, chemicals are used to improve the process of releasing the complex structure of the material, including a high sugar content. In this study, the measured RSC revealed the structure of the biomass and the dissolution of sugars of different types. According to Dada et al. (2021), acid pretreatment of RB can release glucose, fructose, sucrose, and xylose from the hydrolysate. By disrupting the

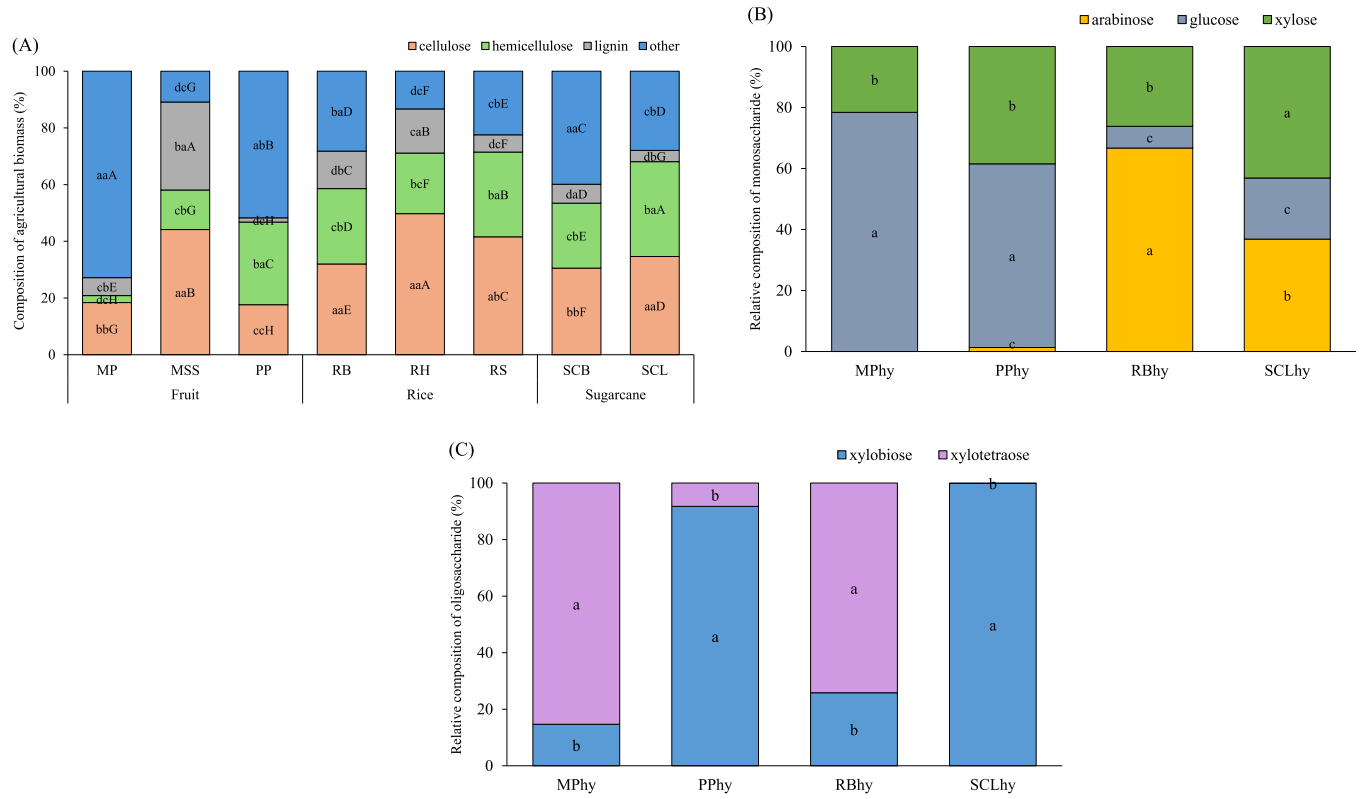

Fig. 1. (A) Analysis of the agricultural biomass composition. The first lowercase letter indicates significant differences in all components ($p < 0.01$) within the same biomass. The second lowercase letter indicates significant differences in each component ($p < 0.01$) between biomass groups, and the uppercase letter indicates significant differences in each component ($p < 0.01$) among all types of biomasses. Relative percentages of (B) monosaccharides and (C) oligosaccharides in the hydrolysates, as determined by HPLC. Lowercase letters indicate significant differences in the sugar contents ($p < 0.05$) within the same biomass. MP, mango peel; PP, pineapple peel; RB, rice bran; RH, rice husk; RS, rice straw; SCB, sugarcane bagasse; SCL, sugarcane leaf; hydrolysates of mango peel (MPhy), pineapple peel (PPhy), rice bran (RBhy), and sugarcane leaf (SCLhy).

polymerization and crystallinity indices of cellulose, boiling and acid treatments result in a greater release of sugars. However, hemicellulose was recovered as xylan precipitated with the ethanol precipitation process (Wang et al., 2018).

Since cost-effectiveness and environmental friendliness are the key criteria for XOS production, this study utilized organic acids such as GA, which is recognized as an inexpensive and biobased organic acid (Zhou et al., 2019). Interestingly, the high RSC and carbohydrate recovery from MP indicate that GA was suitable for breaking down the structure of MP. However, the recovery of harvested carbohydrates must be considered in terms of their properties, including the type of material, as the hemicellulose content detected in MP was lower than that in other biomasses. Thus, the type of carbohydrates harvested in pellets might contain other components in addition to xylan, and this hypothesis must be proven in the next step. As Haykiri-Acma and Yaman (2019) reported, various biomasses are usually pretreated with 0.5–5 % PA to prevent the dissolution of hemicellulose, extractable unsteady constituents, and mineral compounds. In this study, PA was the most effective pretreatment agent, and the carbohydrates collected yielded the greatest amount of released reducing sugars among all the samples studied; the optimal concentration and time of pretreatment differed among the biomasses (Table 3). According to Amarasekara and Ebede (2009), the zinc chloride concentration affects degradation but does not affect the pretreatment of RH or SCL samples in this study, whereas some materials, such as MP, PP, and SCB, can be pretreated with zinc chloride to obtain reducing sugars at a level similar to that obtained with phosphoric acid. However, to break glycosidic bonds, zinc chloride coordinates glycosidic oxygen with zinc, which acts as a Lewis acid. The subsequent hydrolysis of cellulose to D-glucose can yield furfural and HMF as secondary products of the reaction (Shapla et al., 2018).

Table 3
Hemicellulose content, pellet yield and ratio of the pellet yield to hemicellulose content under suitable conditions for the pretreatment of agricultural biomass samples.

Agricultural biomass	Pretreatment		Hemicellulose content	Pellet yield	Relative Pellet yield/Hemicellulose (%)
	Chemical	Time (min)	(% w/w)	(mg/g biomass)	
Mango peel (MP)	3.0 % GA	30	2.47 ± 0.22	995.94 ± 173.89	40.87 ± 10.08
Pineapple peel (PP)	0.5 % PA	30	29.14 ± 0.10 ^{c}	106.10 ± 91.86 ^{abc}	0.36 ± 0.31 ^{ab}
Rice bran (RB)	0.5 % PA	15	26.58 ± 0.07 ^{d}	156.80 ± 3.70 ^{a}	0.59 ± 0.02 ^{a}
Rice husk (RH)	1.5 % PA	30	21.36 ± 0.18 ^{f}	34.70 ± 5.14 ^{c}	0.16 ± 0.03 ^{b}
Rice straw (RS)	1.5 % PA	30	29.90 ± 0.03 ^{b}	91.40 ± 0.00 ^{abc}	0.31 ± 0.00 ^{b}
Sugarcane bagasse (SCB)	0.5 % PA	15	22.94 ± 0.12 ^{e}	72.50 ± 7.09 ^{bc}	0.31 ± 0.03 ^{b}
Sugarcane leaf (SCL)	1.5 % PA	30	33.44 ± 0.17 ^{a}	115.90 ± 3.67 ^{ab}	0.35 ± 0.02 ^{ab}

GA, gluconic acid; PA, phosphoric acid.
The values are presented as the means ± standard deviations (SD) of three replicates.
Lowercase letters indicate a significant difference among all biomasses ($p < 0.05$), except the mango peel (MP) biomass.

Nevertheless, considering the high carbohydrate pellet yield for each biomass under suitable conditions (Table 3), the yields of MP, PP, RB, and SCL increased significantly. Among the biomasses, RB presented a significantly greater pellet yield and percentage relative to the hemicellulose content, excluding the MP sample. However, preliminary analysis of the functional groups within pellets warrants consideration.

3.3. Determination of the hydrolysate and precipitate composition

HPLC analysis revealed that the sugar content varied depending on the biomass and the quantity of the major product (Table 4). The contents of monosaccharides (arabinose, glucose, and xylose) and oligosaccharides (xylobiose and xylotetraose) were significantly greater in the pellet than in the hydrolysate for PP (ranging from 41.28 to 61.53 mg/mL for the monomer and 21.35 to 71.28 mg/mL for the oligomer), RB (ranging from 2.16 to 4.56 mg/mL for the monomer and 52.26 to 109.55 mg/mL for the oligomer), and SCL (ranging from 7.16 to 115.17 mg/mL for the monomer and 18.97 to 188.48 mg/mL for the oligomer), whereas the MP had a high content in the hydrolysate rather than in the pellet (ranging from 30.45 to 13.92 mg/mL for the monomer and 2.15 to 0.29 mg/mL for the oligomer). Additionally, all the samples were free of toxic microbial chemicals such as HMF and furfural, and xylotriose was not detected in any of the samples. The use of suitable conditions for the pretreatment of each material results in the effective solubilization of hemicellulose. After a precipitation step, the monosaccharides and oligosaccharides were collected, increasing the sugar concentration. This finding correlated with the hemicellulose content of the xylan backbone for biomass, especially for SCL, which had the highest hemicellulose content among all the material samples.

According to the relative composition percentages, glucose was the predominant product in the MP and PP hydrolysates at approximately 78.42 % and 60.21 %, respectively. Moreover, RB and SCL had arabinose and xylose as the major products at 66.72 % and 43.10 %, respectively (Fig. 1B). Two major XOS products were detected in the hydrolysates: xylobiose and xylotetraose. Although the PP and SCL hydrolysates predominantly contained xylobiose, the MP and RB hydrolysates contained xylotetraose (Fig. 1C). The production of sugar from lignocellulosic materials still relies strongly on hemicellulose. However, the nature of XOS depends on the pretreatment method and type of lignocellulosic biomass used (Mehta et al., 2022). Complex bonds and branching in xylan create a physical barrier that limits the production of XOS. Based on the side group, substitution, and linkage type, the degree of polymerization (DP) ranges from two to ten units, and XOS prebiotics with fewer than four monomeric units can promote beneficial bacterial proliferation in the human gut (Amorim et al., 2019). Furthermore, some studies have shown that the hydroxyl groups in XOS also contribute to their resistance to acid hydrolysis and their ability to reach the colon intact, where they can be metabolized by probiotic microbes to produce beneficial short-chain fatty acids (SCFAs) (Kathiresan et al., 2024).

The analysis of sugar composition revealed that oligosaccharides were more abundant than monosaccharides in all the samples, except for MP. The XOS yield from SCL, PP, and RB with low concentrations of GA and PA ranged from 189.72 to 522.64 g/kg biomass, whereas the XOS yield from MP was 21.53 g/kg biomass (Table S1). This comparison indicates that suitable conditions destabilized the structure of the biomass, and different amounts of sugar were released according to its structural characteristics, while the MP contains sugars that are quite different from those of other materials, especially oligosaccharides, which are less abundant. These observations, together with the pellets obtained in previous experiments, show that the precipitates from the MP hydrolysate may contain components other than saccharides. This hypothesis must be tested in further experiments. Additionally, heat treatment can release phenolic compounds from agricultural biomass (Arampath and Dekker, 2019). In this study, the phenolic content in the hydrolysates of the four agrowastes ranged from 230.00 to 416.70 μg GAE/g biomass, with the MP hydrolysate exhibiting the highest phenolic content (Table S1). Solvent extraction, particularly under acidic conditions, promotes the release of polyphenols by disrupting hydrogen bonding and other interactions between the polyhydroxyl groups and the plant matrix, leading to their dissolution (Huang et al., 2024). Along with XOS and other sugars, these phenolic compounds contribute to bacterial growth regulation in hydrolysates. According to Zhang et al. (2023), phenolic compounds function as antioxidants, protecting organelles, proteins, lipids, DNA, and RNA from oxidative damage. Consequently, these compounds may support the proliferation of intestinal microorganisms, such as *Lactobacillus/Enterococcus* spp. and *Bifidobacterium* spp.

Although the same materials are used, the addition of a few steps affects the final product. The evidence is clear that the hydrolysate and precipitate of monosaccharides and oligosaccharides differ (Gautério et al., 2022). This difference means that, although the precipitation process yields a relatively high concentration of XOS, some monosaccharides are lost in the process. Concentrated XOS are more suitable for functional foods aimed at modulating the gut microbiota and improving host health (Saville and Saville, 2020). The utilization of XOS, which requires specific enzymes and transport systems, provides a selective advantage in fermentation for certain beneficial bacteria (Mäkeläinen et al., 2010). A mixture of monosaccharides and oligosaccharides in pretreatment hydrolysate is more appropriate for microbial use and industrial fermentation processes, as it supports a wide range of

Table 4
Contents of monosaccharides and oligosaccharides in the hydrolysates and pellets of mango peels, pineapple peels, rice bran, and sugarcane leaves, as determined by HPLC.

Sugar types	Sugar concentration (mg/mL)							
	Mango peel (MP)		Pineapple peel (PP)		Rice bran (RB)		Sugarcane leaf (SCL)	
	Hydrolysate	Pellet	Hydrolysate	Pellet	Hydrolysate	Pellet	Hydrolysate	Pellet
Total monosaccharide	30.45 ± 0.22	13.92 ± 0.82	41.28 ± 0.80	61.53 ± 4.47	2.16 ± 0.28	4.56 ± 1.49	7.16 ± 0.32	115.17 ± 12.08
Arabinose	ND	ND	0.54 ± 0.14	1.92 ± 0.55	1.44 ± 0.20	2.44 ± 1.62	2.64 ± 0.32	6.63 ± 1.05
Glucose	23.88 ± 0.17	11.15 ± 0.71	24.85 ± 0.34	22.33 ± 2.29	0.15 ± 0.02	2.12 ± 0.14	1.44 ± 0.10	100.97 ± 10.08
Xylose	6.57 ± 0.05	2.77 ± 0.14	15.88 ± 0.57	37.29 ± 1.80	0.57 ± 0.07	ND	3.09 ± 0.18	7.57 ± 1.09
Total oligosaccharide	2.15 ± 0.36	0.29 ± 0.05	21.35 ± 0.97	71.28 ± 2.12	52.26 ± 6.13	109.55 ± 31.54	18.97 ± 0.55	188.48 ± 29.39
Xylobiose	0.32 ± 0.07	0.29 ± 0.05	19.58 ± 0.25	55.80 ± 3.18	13.49 ± 0.96	5.16 ± 0.17	18.94 ± 0.51	26.41 ± 0.70
Xylotriose	ND	ND	ND	ND	ND	ND	ND	ND
Xylotetraose	1.84 ± 0.40	ND	1.76 ± 1.04	15.48 ± 1.09	38.77 ± 5.17	104.39 ± 31.70	ND	162.07 ± 29.67
Comparison between the types of sugars (total monosaccharides vs. total oligosaccharides)								
	***	***	***	*	**	*	***	**
Comparison between the types of samples (hydrolysates vs. pellets)								
Monosaccharide	***		**		ns		**	
Oligosaccharide	***		***		*		**	

*, **, and *** indicate $p < 0.05$, < 0.01, and < 0.001, respectively. ns indicates no significant difference. ND indicates not detected.

microbial growth, and high product yields are desired (Wu and Xu, 2025). Thus, these conclusions have important implications for the use of monosaccharides and oligosaccharides as a nutrient source for bacteria. Therefore, the presence of XOS (xylobiose and xylotetraose) and phenolic compounds in the hydrolysate makes them an interesting candidate for testing as an efficacious prebiotic to support the growth of probiotic microorganisms.

3.4. Evaluation of the functional groups of the precipitates

FTIR is an effective tool for studying the physicochemical and conformational properties of carbohydrate pellets, and it can also be used to determine the functional groups present in a sample corresponding to a signature molecule. In this study, after FTIR analysis of xylan (Fig. 2), it was revealed that all the materials had spectra of arabinoxylan, an oligomer, and a polymer with a low degree of branching between 1200 and 900 cm^{-1} (Bian et al., 2013). The specificity and spectral pattern of xylan differ depending on the material. A band between 1036 and 1039 cm^{-1} was observed, which is characteristic of xylan produced by the binding of C—O, C—C, and C—OH within sugar molecules in PP and SCL (Chaikumpollert et al., 2004). The peak between 1043 and 1045 cm^{-1} was attributed to MP, which is characteristic of xylan produced by the binding of C—O—C bonds within glycosidic bonds. The peak between 1233 and 1241 cm^{-1}, which is attributed to C–O bonding, was found in the spectra of the RB, MP, and PP samples. The peak between 1364 and 1378 cm^{-1} corresponds to the binding of C—CH_3 in the RB, PP, and SCL samples. The peak between 1408 and 1420 cm^{-1}, which was attributed to uronic acid ions, was found in the spectra of all the samples (Gullón et al., 2011).

The peak between 1628 and 1647 cm^{-1} was characteristic of water molecules confined to xylan in RB (Kačuráková et al., 1999). For all the samples, a spectral band at 1730 cm^{-1} corresponding to the -O stretching vibration of the acetyl group in hemicellulose was not detected, indicating that the pretreatment process initiates the breakdown of hemicellulose through the release of sugars or the deacetylation of hemicellulose (Mensah et al., 2024). Interestingly, compared with those of standard pectin compounds, the spectra of the pellets from MP presented the following characteristic peaks of pectin: 1016 cm^{-1}, 1226 cm^{-1}, 1597 cm^{-1}, and 1741 cm^{-1}. Pectin is the primary plant cell wall of fruits and interacts with hemicellulose (Broxterman and Schols, 2018). It was suggested that xylan might act as a covalent connection between pectin and the hemicellulose–cellulose network or between pectin and cell wall proteins. Thus, xylan can coprecipitate with pectin because both polymers are covalently, nonester linked to each other (Broxterman and Schols, 2018). Additionally, orthodox lignin bands at 1244 cm^{-1} corresponding to the guaiacyl unit were not detected. The availability of guaiacyl in lignin prevents fiber swelling, thus impeding enzyme accessibility (Lai and Idris, 2016). The disappearance of this band suggested the structural modification of lignin. Hydrothermal pretreatment was shown to be effective for delignification according to Mensah et al. (2024).

FTIR is a convenient method for examining xylan, including XOS, in sediments. An analysis of the bonds, including functional groups, in precipitate samples can reveal prebiotic structures. Precipitates may be analyzed to determine the functional groups and chemical bonds present in xylan and XOS from the peaks present in the FTIR spectra of the precipitates. According to Pramasari et al. (2024) and Liang et al. (2015), the FTIR spectrum of XOS is very similar to that of pure xylan, indicating that XOS contain xylan molecules. The spectra of XOS also contain the peaks of the -OH (hydroxyl) and CH groups that are commonly found in xylan, the peak indicating the presence of water, the peak corresponding to the hemicellulose structure, the peak that identifies the unique arrangement found in xylan (called the β−1,4 linkage) or even the peak indicating CH groups and special sugar structures. The backbone of the oligosaccharide structure used by probiotic bacteria is composed of these functional groups (Kathiresan et al., 2024). Following a precipitation investigation, the results obtained in this study suggest that hydrothermal pretreatment releases hemicellulose and xylan, including XOS structures, from the material. Further research is needed to determine whether the XOS structure has prebiotic properties.

3.5. Prebiotic properties of the hydrolysates and precipitates

The hydrolysates and precipitates from each agrowaste, including MP, PP, RB, and SCL, were used as the sole carbon source in minimal medium to evaluate their ability to support the growth of *B. subtilis*, LAB and pathogens, which are reported as prebiotic activity scores. The key property of prebiotics is their ability to be recognized by probiotic enzymes; additionally, they contribute positively to the intestinal microbiota by metabolizing probiotics. However, functional groups are involved in these activities, particularly in the interaction between probiotics and prebiotic oligosaccharides. Probiotic bacteria recognize, transport, and ferment sugar molecules based on the hydroxyl group in their structure (Kathiresan et al., 2024). Thus, prebiotic activity reflects the ability of a certain substrate to improve the growth of a beneficial microorganism (Reque et al., 2019). The presence of xylan and pectin detected in the hydrolysate derived from the agrowaste suggests a potential impact on the prebiotic activity of the hydrolysate and pellet

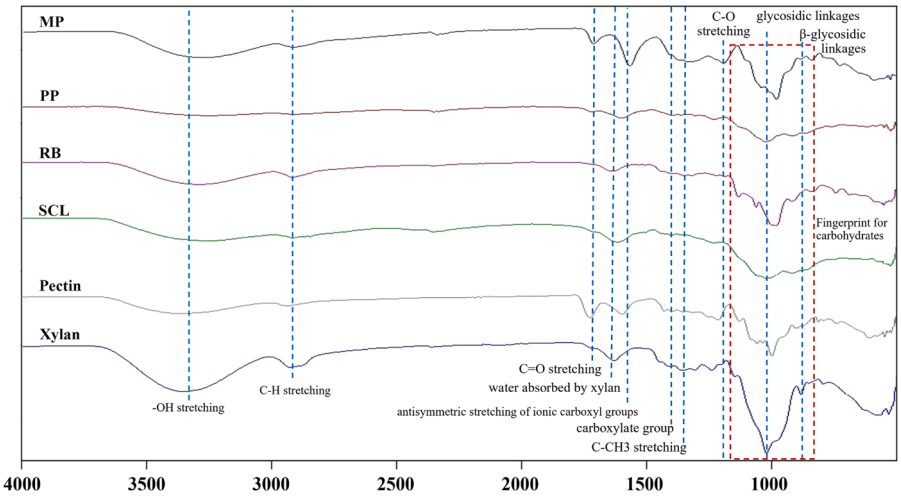

Fig. 2. FTIR spectra of carbohydrate pellets from MP (mango peel), PP (pineapple peel), RB (rice bran), and SCL (sugarcane leaf) compared with those of xylan and pectin standards.

(Figs. 1 and 2). As a hemicellulose component, xylan can be converted into XOS, which are well known for their prebiotic properties, particularly in promoting the growth of beneficial gut bacteria such as LAB (Smith and Melrose, 2022). Pectin also has prebiotic effects by stimulating the growth of beneficial microbiota (de Oliveira et al., 2024).

The analysis of the correlation between the type of carbon source (hydrolysate and precipitate) and prebiotic activity (Fig. 3) revealed that bacillus growth and pathogen growth were positively correlated with the precipitates but negatively correlated with the hydrolysates. In contrast, LAB growth was positively correlated with hydrolysates and precipitates. Considering these correlations, the growth of all isolates with a nutrient source from SCL was greater than that from other sources, which was attributed to the content of sugars, including monosaccharides and XOS (xylobiose and xylotetraose). An analysis of XOS properties by Yang and Xu (2018) revealed that xylobiose and xylotriose from XOS hydrolysate were consumed by *Bifidobacterium adolescentis, Weissella cibaria*, and *Lactobacillus brevis*, which presented increased cell density on XOS. The formation of lactic acid and acetic acid was observed after 48 h of in vitro incubation. This finding is similar to that of Zidan et al. (2021), who reported that the XOS produced from sugarcane residues supported the growth of *Lactobacillus casei* and *Bifidobacterium animalis*. Similarly, Kathiresan et al. (2024) reported that probiotics such as *Lactobacillus plantarum* and *Lactobacillus fermentum* selectively utilize XOS rich in hydroxyl groups, as evidenced by their high prebiotic indices and growth scores. Bacteria ferment XOS by utilizing enzyme systems such as xylanases and glycosidases that target specific glycosidic linkages and recognize -OH configurations for efficient binding and hydrolysis.

Although the MP hydrolysate had a positive relationship with growth, the MP precipitate had the opposite correlation. By combining the HPLC results with those from the FTIR analysis, it was determined that the MP precipitates were composed of pectin. Although pectin has been investigated for its prebiotic potential, its high molecular weight often results in inconsistent outcomes due to the significant microbial activity required for its breakdown and utilization (de Oliveira et al., 2024). Consequently, the high pectin content or certain compounds present in the MP precipitate may contribute to low bacterial growth. Additionally, pectin exhibits significant antibacterial activity, causing cell membrane damage and structural deformation when hydrolyzed into oligosaccharides (Gao et al., 2023). Moreover, the reduction in sugar content in the precipitate, which results from the precipitation process, has a direct effect on growth. When the hydrolysate and precipitate were compared, the hydrolysate demonstrated a higher prebiotic activity score, indicating greater prebiotic potential for LAB. This potential may be attributed to its more diverse sugar composition following the precipitation step, as supported by the HPLC and FTIR results.

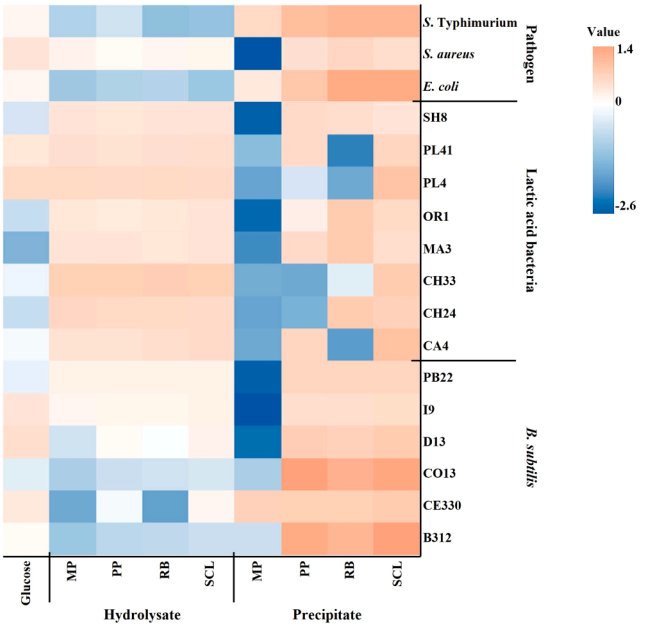

Fig. 3. Heatmap of prebiotic activity scores for the hydrolysates and carbohydrate precipitates of agricultural biomasses toward lactic acid bacteria, *B. subtilis* and pathogens. MP, mango peel; PP, pineapple peel; RB, rice bran; RH, rice husk; RS, rice straw; SCB, sugarcane bagasse; SCL, sugarcane leaf; and glucose (control).

However, although the hydrolysates have a more diverse sugar composition, they were ineffective at promoting pathogen growth. The low growth of the pathogens may indicate an unsuitable environment for the agrowaste hydrolysates, suggesting that the pathogens cannot effectively utilize the carbon source in the hydrolysates or that the hydrolysates contain inhibitory agents. Consequently, hydrolysates are more suitable than precipitates for primary microbial cultivation and further development, as they are rich in nutrients and compounds that are beneficial to probiotic bacteria while limiting pathogen growth. The phenolic compounds, such as polyphenols, in the hydrolysates are considered prebiotic substances that promote the growth of commensal bacteria. They can positively influence LAB growth, enabling these bacteria to metabolize phenolic compounds along with food, which significantly reduces the toxicity levels of the primary compounds (Piekarska and Klewicka, 2021). In contrast, pathogens, in particular, often cannot grow in environments containing phenolic compounds because they cannot adapt to or metabolize these substances effectively. This inability makes phenolic compounds effective at limiting pathogen growth while potentially supporting the proliferation of beneficial microorganisms. Thus, hydrolysates are more suitable than precipitates for primary microbial cultivation and further development, as they are rich in nutrients and compounds beneficial to probiotic bacteria while limiting pathogen growth. However, the limitation of pathogen growth remains an interesting area for further investigation to understand the underlying causes more fully.

Since bacteria can grow well in hydrolysate and a high sugar content can easily cause contamination, especially fungal contamination, a drying process is used to maintain the quality of the hydrolysate. In the next step, bacterial isolates were selected to test the properties of the dry powder. Therefore, CA4, OR2, and SH8 were used for the LAB, whereas B312, CE330, and CO13 were used for the bacillus. It has been reported that bacterial cultures can release beneficial compounds during fermentation, even if the fermentation product is mango juice. As all 6 isolates exhibited potential growth characteristics when SCL was supplied as a nutrient, they were selected to study their growth characteristics in media supplemented with spray-dried powder from SCL hydrolysate.

3.6. Evaluation of spray-dried powder products

To exploit the dried SCL–XOS hydrolysate powder, its properties must be considered in terms of promoting bacterial growth as well as releasing bioactive compounds produced by fermentation. An analysis revealed that antioxidant activity (ABTS RSA %) was increased from 40.80 % to 66.90 % after 24 h by fermentation with *B. subtilis* (Fig. 4) but from 40.80 % to approximately 75.77 % within 48 h by LAB fermentation (Fig. 5). Additionally, *B. subtilis* fermentation increased the TFC from an initial concentration of 1.04 mg QE/mL to 2.75 mg QE/mL, and the TPC increased from 111.37 μg GAE/mL to 167.10 μg GAE/mL within 48 h (Fig. 4). For the LAB isolate OR1, the TFC peaked at 1.84 mg QE/mL at 24 h before gradually decreasing. The TPC of the LAB isolate CA4 was notably high at 207.47 μg GAE/mL after 24 h and further increased to 236.07 μg GAE/mL after 48 h (Fig. 5).

In contrast, for the pathogens (Fig. 6), no significant change in the ABTS was observed, except for *Salmonella Typhimurium*, which

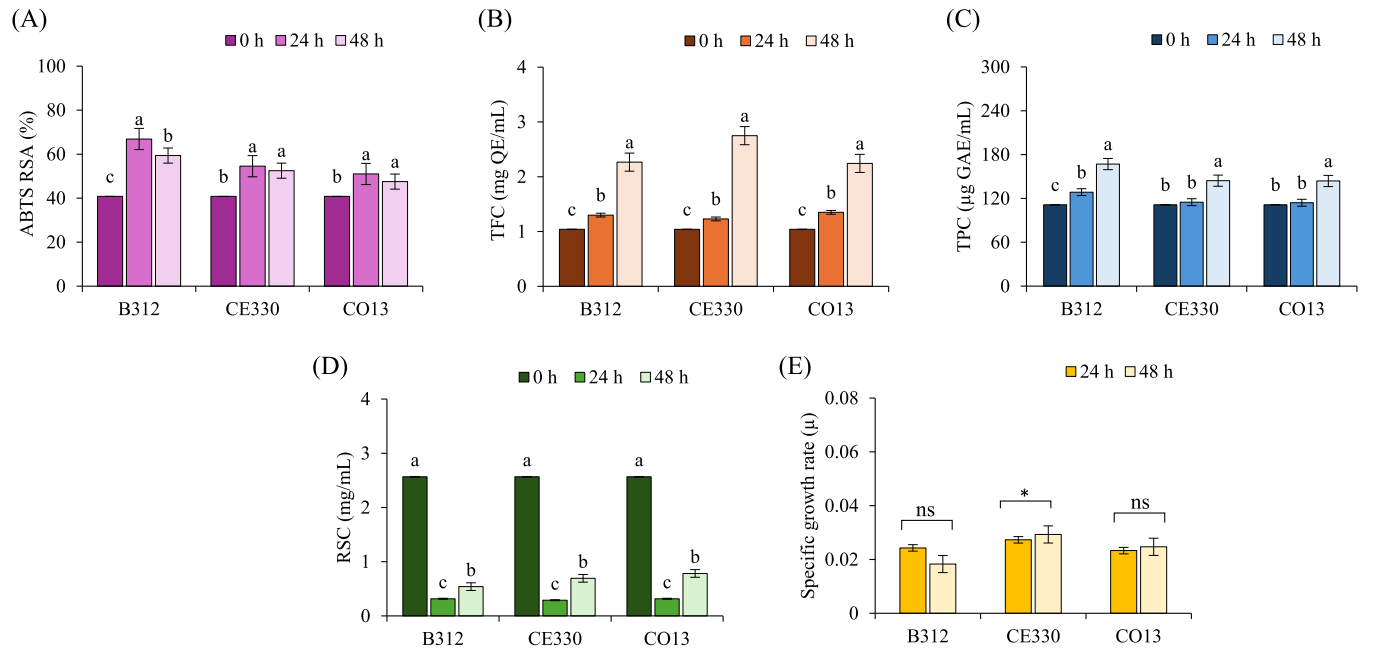

Fig. 4. Effects of the replacement of the carbon source with sugarcane leaf dry powder on the growth of *B. subtilis* at 0 h, 24 h and 48 h, including the (A) ABTS RSA (%), (B) TFC, (C) TPC, (D) RSC, and (E) specific growth rate (µ). The values are presented as the means ± standard deviations (SD) of three replicates. Lowercase letters indicate significant differences between time points for each strain ($p < 0.05$). * p values of 0.05. ns indicates no significant differences.

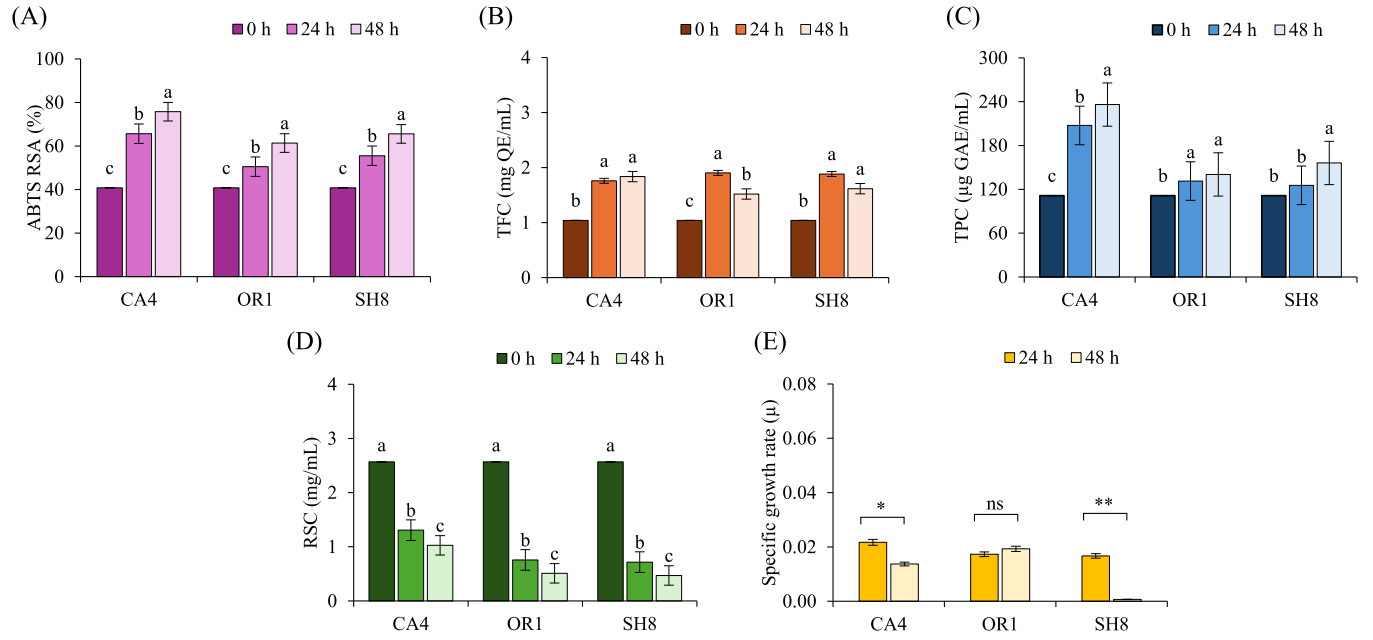

Fig. 5. Effects of the replacement of the carbon source with sugarcane leaf dry powder on the growth of lactic acid bacteria (LAB) at 0 h, 24 h, and 48 h, including the (A) ABTS RSA (%), (B) TFC, (C) TPC, (D) RSC, and (E) specific growth rate (µ). The values are presented as the means ± standard deviations (SD) of three replicates. Lowercase letters are defined as significant differences between time points for each strain ($p < 0.05$). * and ** indicate $p < 0.05$ and <0.01, respectively. ns indicates no significant differences.

presented a high RSA of 53.73 % at 24 h. The TFC slightly increased to approximately 2.15 mg QE/mL at 48 h. The TPC values were not significantly different for *Escherichia coli* and *S. Typhimurium*, whereas *S. aureus* presented a decrease in the TPC to 98.07 µg GAE/mL. During fermentation with *B. subtilis*, LAB and pathogens, the RSC decreased as the specific growth rate increased. The growth of LAB was similar to that of *B. subtilis,* which had a specific growth rate of approximately 0.02 within 24 h and then slightly declined at 48 h (Figs. 4 and 5). In contrast, the specific growth rate of the pathogens within 24 h was approximately 0.05, which decreased to 0.02 within 48 h (Fig. 6).

In our study, gum arabic was incorporated during the spray-drying process of SCL–XOS to increase the stability and bioavailability of the prebiotic compounds, as it is widely recognized for its excellent emulsifying and stabilizing properties, which facilitate the formation of microcapsules with high encapsulation efficiency and enable the controlled release of bioactive compounds (Mutavski et al., 2025). Furthermore, gum arabic is a natural fiber-rich substance that contains amino acids, vitamins, and sugars such as galactose, rhamnose, and arabinose. In

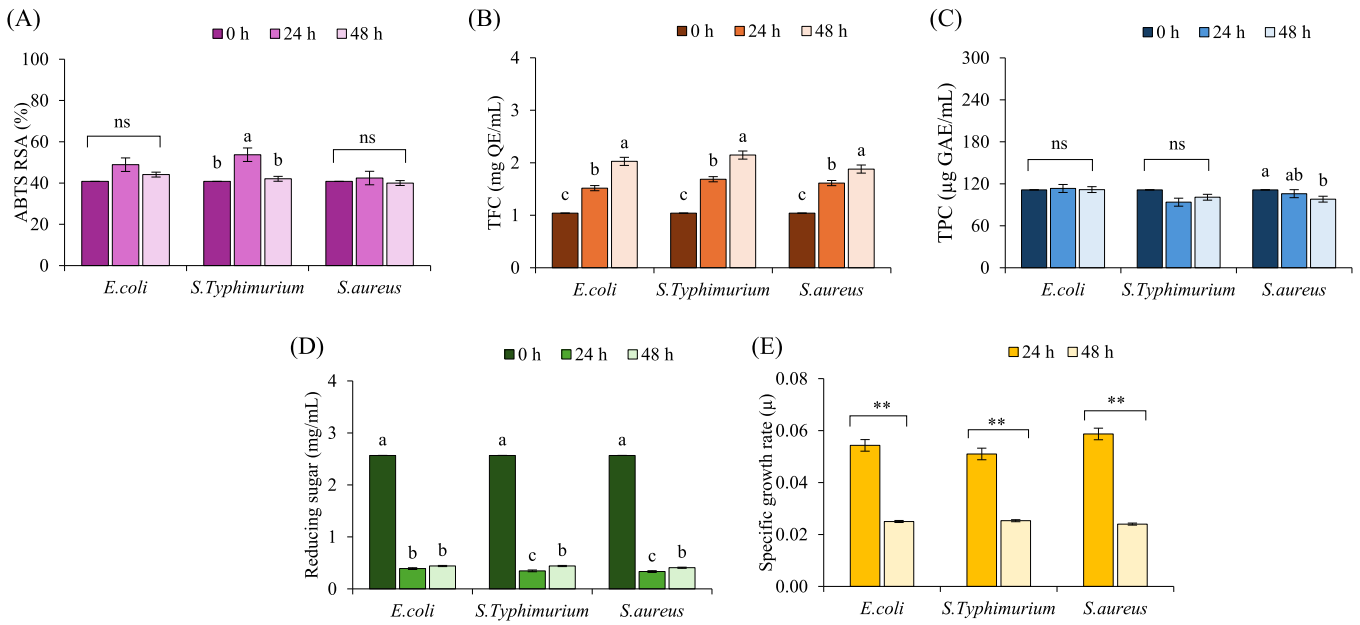

Fig. 6. Effects of the replacement of the carbon source with sugarcane leaf dry powder on the growth of pathogens at 0 h, 24 h, and 48 h, including the (A) ABTS RSA (%), (B) TFC, (C) TPC, (D) RSC, and (E) specific growth rate (µ). The values are presented as the means ± standard deviations (SD) of three replicates. Lowercase letters are defined as significant differences between time points for each strain ($p < 0.05$). ** p value of 0.01. ns indicates no significant differences.

some studies, gum arabic was demonstrated to promote the growth of beneficial bacteria and the production of SCFAs, resulting in prebiotic properties (Al-Baadani et al., 2022). Compared with dry powder of gum arabic, dry powder of SCL retained higher levels of ABTS RSA, TFC, and TPC (Tables S2–S4). Thus, the presence of reducing sugars and the specific growth rate of the bacteria could be measured (Tables S5, S6). Accordingly, the dry powder produced with SCL hydrolysate, as well as its properties, may also be strengthened with gum arabic. With respect to the prebiotic contribution of gum arabic, its presence in the SCL–XOS powder may have further supported the growth of beneficial gut bacteria and SCFA production. However, to assess the combined prebiotic effects accurately, future studies should include a control group with gum arabic alone and evaluate its impact on probiotic activity.

The LAB and *B. subtilis* fermentation of a medium with dried SCL hydrolysate containing XOS prebiotic produced an increase in the content of the antioxidant activities and phenolic compounds. Increases in the ABTS RSA, TFC, and TPC were positively correlated with the growth of LAB and *B. subtilis* in the first 24 h (Table 5), which is consistent with the results reported by Laophongphit et al. (2023). These results indicate that XOS derived from SCL not only promote the growth of beneficial probiotics but also contribute to improved antioxidant properties. Similarly, Liu et al. (2017) reported increased antioxidant activity in rice

Table 5
Pearson correlation coefficients for the reducing sugar content (RSC), antioxidant activity (ABTS), total flavonoid content (TFC), total phenolic content (TPC), and specific growth rates of *B. subtilis*, lactic acid bacteria (LAB), and pathogens cultured on spray-dried SCL powder.

Bacteria	Incubation (h)		ABTS	TFC	TPC	Growth
B. subtilis	0–24	RSC	−0.844**	−0.910**	−0.618**	−0.990**
		ABTS		0.741**	0.866**	0.832**
		TFC			0.534*	0.896**
		TPC				0.604**
	24–48	RSC	−0.489*	0.868**	0.668**	0.927**
		ABTS		−0.335	0.072	−0.400
		TFC			0.752**	0.962**
		TPC				0.722**
Lactic acid bacteria	0–24	RSC	−0.726**	−0.983**	−0.444	−0.948**
		ABTS		0.778**	0.860**	0.815**
		TFC			0.532*	0.952**
		TPC				0.605**
	24–48	RSC	0.377	0.391	0.706**	−0.075
		ABTS		−0.204	0.830**	0.007
		TFC			0.067	−0.316
		TPC				−0.032
Pathogen	0–24	RSC	−0.618**	−0.963**	0.385	−0.995**
		ABTS		0.671**	−0.414	0.579*
		TFC			−0.402	0.958**
		TPC				−0.338
	24–48	RSC	−0.246	−0.751**	0.249	−0.985**
		ABTS		−0.003	−0.048	0.238
		TFC			−0.208	0.699**
		TPC				−0.190

* p value < 0.05.
** p value < 0.01 indicate significant differences.

bran hydrolysate, possibly due to microbial hydrolysis and biotransformation during fermentation, which release phenolic compounds and free hydroxyl groups. Bioactive compounds such as flavonoids and phenolics can be found in a wide variety of fruits and vegetables. During fermentation, microbial enzymes such as β-glucosidase cleave glycosidic bonds that link flavonoids and phenolics to sugar molecules. This enzymatic action releases aglycones, which are antioxidant-rich bioactive forms that were originally bound to sugar molecules (Molina et al., 2023). Additionally, the positive correlation between the RSC and the growth of *B. subtilis* observed from 24 to 48 h may be caused by bacteria switching to utilize oligosaccharides (xylobiose and xylotetraose) after initially using monosaccharides (glucose, arabinose, and xylose) during the first 24 h. The increase in RSC and antioxidant activity may be attributed to β-glucosidases, which can specifically hydrolyze β-glucosidic linkages, releasing monosaccharides and mobilizing phenolic compounds and flavonoids (Johnson et al., 2021; Ozturk et al., 2024; Zhao et al., 2021).

These antioxidant effects are further complemented by the biological activities of flavonoids, which not only act as antioxidants but also exhibit anti-inflammatory properties and beneficial effects on the gut microflora by inhibiting pathogenic bacteria while promoting beneficial bacteria. Huang et al. (2025a) reported that flavonoids obtained from *Penthorum chinense* protected beneficial Bacteroidota bacteria and inhibited harmful Firmicutes and Verrucomicrobiota bacteria, which led to the alleviation of dextran-sulfate sodium (DSS)-induced colitis in mice. Therefore, the dry SCL powder could serve as an enhanced prebiotic through synergistic effects with XOS, TPC, and TFC. When probiotics are combined, they may improve microbial growth and provide beneficial effects. This study showed that the correlations among ABTS RSA, TFC, and pathogenic growth were positive, whereas TPC was negatively correlated (Table 5). The mechanism by which flavonoids increase pathogen activity remains unclear, but it may involve the metabolism of pathogens associated with the release of flavonoids. However, the pathogen did not present a significant increase in antioxidant activity or compounds. These findings indicate that probiotic bacteria are more likely to produce more bioactive compounds than pathogens when dried SCL–XOS hydrolysate powder is used as a nutrient source. Consequently, the antioxidant enhancement observed during fermentation suggested the potential of dried SCL–XOS hydrolysate as a prebiotic for developing functional foods aimed at promoting health. According to a previous study, the addition of prebiotic oligosaccharides, including XOS, resulted in increased antioxidant activity in milk fermented by probiotics (Guo et al., 2017).

The prebiotic potential of this system can be attributed to the enzymatic activity of probiotic bacteria, which produce a wide array of enzymes, including those capable of metabolizing XOS. In the colon, xylan can be selectively degraded by enteric bacterial enzymes, including xylanase and arabinofuranosidase, to form low-MW XOS (Yang and Xu, 2018). Similarly, endo-1,4-beta-xylanase 11, a family 11 xylanase from *Bacillus firmus* K-1, can polymerize xylo-substrates to a degree of polymerization of seven (Jommuengbout et al., 2009). *In silico* modeling has further revealed that the enzymes endo-1,4-beta-xylanase B and beta-D-xylosidase produced by L. *brevis* and *B. adolescentis* interact with xylose via three simple hydrogen bonds, a carbon−hydrogen bond, a van der Waals bond, a donor-unfavorable donor−donor bond, and an acceptor-unfavorable acceptor bond (Khangwal et al., 2022). Moreover, the changes in TPC and TFC are closely associated with bacterial metabolism. Under acidic conditions generated by bacterial fermentation, hydrogen bonding reactions can occur between hydronium ions and polyhydroxyl flavonoids, increasing their extraction from plant material (Huang et al., 2024). Flavonoids such as anthocyanins extracted from plant materials not only support gut microbial diversity but also affect digestion systems, including translation, structure and ribosome formation; amino acid transport and metabolism; carbohydrate transport and metabolism; cell wall/membrane/enzyme formation; replication; recombination and repair; transcription; inorganic ion transport and metabolism; and energy production and conversion (Huang et al., 2025b). Overall, this study demonstrated that the probiotic activity of XOS as a carbon source can not only produce organic acids but also provide ideal conditions for the extraction of flavonoids and other phenolic compounds.

The production of XOS using abundant lignocellulosic biomass is cost-effective and renewable (Amorim et al., 2019). Sugarcane leaf, an agricultural waste material, has minimal costs and can be utilized for XOS production. This technology not only increases the value of biomass but also plays a role in addressing the increasing waste challenge. In addition to the low cost of SCL biomass, the extraction method and final product quality play crucial roles in determining the economic feasibility of large-scale XOS production. In this study, considering the hemicellulose content of sugarcane leaves (33.44 % w/w) and the XOS yield obtained from hydrolysate (189.72 g/kg biomass), >56.74 % (w/w) of the sugarcane leaves were successfully extracted. Additionally, the dry powder production process retained the prebiotic and bioactive properties of the XOS contained in the hydrolysate (Table S7). Thus, as revealed in this study, dilute acid and hydrothermal pretreatment processes are fast methods of XOS production with minimal byproduct formation, whereas the dry powder process effectively maintains the functional properties of the final product. Hydrolysates, when used as dry powders, are beneficial for preventing fungal growth because their low moisture and sugar contents promote LAB and *Bacillus* growth and increase their antioxidant activity. The drying process, which is performed under optimal conditions, is associated with the persistence of XOS, similar to findings where spray-dried XOS powder still retains high levels of bioactive compounds (Zhang et al., 2019). Furthermore, XOS reportedly encourage the growth of beneficial bacteria in the mammalian gut, especially *Bifidobacterium* and *Lactobacillus*, which increase SCFA production levels. These metabolic byproducts enhance a wide range of health benefits, including improved mineral absorption; the modulation of intestinal function; lipid and glucose metabolism; the regulation of immune modulatory activities; the reduction of colon cancer risk; the enhancement of antioxidant capacity; and antimicrobial and anti-inflammatory effects (Aachary and Prapulla, 2011). Consequently, dried SCL–XOS hydrolysate powder can promote probiotic growth and is suitable for application as a promising prebiotic in the animal feed industry due to its cost-effectiveness. The demand for XOS in the health food sector is growing, and the increasing use of XOS as an ingredient in animal feed is expanding market opportunities (Mensah et al., 2023).

Thus, the scalability of the pretreatment method is crucial for further economic development, although considerations such as the cost-effectiveness of the chemicals and equipment used should be evaluated. Additional studies on the effects of dry hydrolysate powder on animal health are also important. Nevertheless, this study demonstrated the prebiotic potential of dried SCL powder and emphasized the benefits of utilizing agricultural biomass in this manner. This approach is considered one of the most innovative methods for reducing waste and solving environmental problems through the use of biomass.

4. Conclusions

The combination of phosphoric acid with hydrothermal pretreatment of PP, RB, and SCL effectively releases a range of sugars, including xylooligosaccharides (XOS), such as xylobiose and xylotetraose. This finding indicates that the process not only enhances biomass swelling and structural disruption but also facilitates XOS liberation. Among the tested biomasses, the SCL hydrolysate presented the highest concentration of XOS, which is likely to promote probiotic growth while being unsuitable for pathogenic bacteria. Furthermore, spray-dried SCL powder promoted the production of probiotics and bioactive compounds, with synergistic effects among XOS, phenolics, and flavonoids. These findings emphasize the efficiency of the reaction, which can benefit the health of the host. Importantly, no toxic byproducts, such as furfural or

hydroxymethylfurfural (HMF), were detected. Thus, this study presents a simple, safe, and cost-effective method for producing prebiotic XOS from SCL, offering health benefits and a sustainable solution for agricultural biomass utilization and environmental impact reduction.

Ethical statement - studies in humans and animals

The current study does not involve the use of animal or human subjects.

CRediT authorship contribution statement

Nipaporn Chadathong: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Surasak Siripornadulsil:** Writing – review & editing, Resources, Conceptualization. **Wilailak Siripornadulsil:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100707.

Data availability

Data will be made available on request.

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