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Research article

Effects of different enzyme extraction methods on the properties and prebiotic activity of soybean hull polysaccharides

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ABSTRACT

In this study, five different processes, including hot water (HW-ASP), single enzyme (cellulase, pectinase and papain; C-ASP, PE-ASP, and P-ASP), and compound-enzyme (cellulose: pectinase: papain = 3:3:1; CE-ASP) for the extraction of soybean hull polysaccharides (ASPs) were employed, and the characterization and prebiotics activity of five polysaccharides were analyzed. These polysaccharides possessed different primary structural characteristics, including molecular weight distribution, monosaccharide composition, chemical composition, surface morphology, potential particle size, etc., while similar functional groups. *In vitro* digestibility assay indicated that C-ASP had strong resistance to gastric juice hydrolysis and α-amylase as compared with HW-ASP. Furthermore, C-ASP elevated the acidifying activity and promoted the growth of probiotics (*Lactobacillus paracasei, Lactobacillus rhamnosus*, and *Lactobacillus acidophilus*) during the fermentation ($p < 0.05$). C-ASP improved the levels of total short-chain fatty acids (SCFAs) and had better prebiotic activity than HW-ASP ($p < 0.05$). These findings denote that enzyme-assisted polysaccharides extracted from soybean hulls have the potential to be served as novel probiotics.

1. Introduction

As a major economic crop in the Northeast of China, soybean production and processing are characteristic industries (Cabezudo et al., 2021). However, the processing of soybeans also faces the problem that a large number of processing by-products cannot be effectively utilized (Gao et al., 2020). At present, a part of the soybean bean hull is used as animal feed, and the rest of the hull is treated as agricultural waste (Zhao et al., 2018). Therefore, the polysaccharide derived from the soybean hull has great value and significance for the utilization and processing of the soybean hull, which can not only improve its utilization rate but also enhance its practical value (Yuan et al., 2015). Soybean hull polysaccharide has shown potent biological activities as compared to other plant polysaccharides, such as liver protection ability and anti-cancer activity, etc. (Chen et al., 2021; Lin et al., 2022; Yang et al., 2021).

A large number of experiments have shown that many natural polysaccharides of plant sources cannot be completely digested by the upper gastrointestinal digestion system of the human body, and reach the colon. Polysaccharides as potential prebiotics, promote intestinal flora by gut microbial fermentation, such as the growth of *Lactobacilli* and *Bifidobacteria*, and produce SCFAs, thereby improving human intestinal health (Jiang et al., 2022; Mahdhi et al., 2017; Tarique et al., 2022). Based on this, the development of natural polysaccharides with probiotic activity have become one of the hot spots in the research area of polysaccharides to evaluate the probiotic activity of new plant-derived polysaccharides (Gao et al., 2020). A great number of studies have shown that the molecular weight, monosaccharide composition, and glycosidic bond configuration of polysaccharides affect their probiotic activity (Chen et al., 2020a; Chengxiao et al., 2021; Wan et al., 2021). Therefore, we speculated that different enzymatic extraction methods might affect the probiotic activity of polysaccharides. However, some studies on the impacts of diverse enzyme extraction methods on the bioactivity of polysaccharides were mainly focused on the comparison of their antioxidant capacity (Dong et al., 2016; Kungel et al., 2018; Yuan et al., 2015), while

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studies on the impacts of diverse enzyme extraction methods on the probiotic activity of polysaccharides have not been reported so far.

Therefore, different methods were used to prepare ASPs in this study. These polysaccharides were certified and evaluated about the extraction rate, structural configuration and molecular characteristics. Through fermentation experiments, the *in vitro* proliferation effect and acidifying activity of different polysaccharides on probiotics were compared. Finally, the polysaccharide component with better probiotic activity was selected for the measurement of SCFAs.

2. Materials and methods

2.1. Materials and reagents

Soy hulls of Hei he 43 soybeans were purchased from the Yu Wang Group in Harbin Province of China. The standards for fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc), xylose (Xyl), mannose (Man), fructose (Fru), Ribose (Rib), guluronic acid (GulA), and the dextran MW standards were obtained from Sigma-Aldrich. The chemicals and reagents utilized were analytically pure.

The *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* were obtained by activating and cultivating bacterial powder in the laboratory. *Lactobacillus paracasei* (CICC 6244) was purchased from the China Industrial Microbial Culture Collection. Each strain was activated prior to use in MRS broth (Chen et al., 2020b).

2.2. Extraction of polysaccharides using different methods

2.2.1. Hot water extraction

The method for extraction of HW-ASP was according to the reported study with minor modifications (Wang et al., 2022a, 2022b). The soybean hulls (50 g) were broken into powdered and discolored using ethanol solution (1%) in a ratio of 1:10 (m/v). The dried soybean bean hull fractions were extracted with a ratio of soybean powder of 1:20 (m/v), at 85 °C for 4 h. After cooling and filtering, the extract was centrifuged at 4500×g for 10 min, the supernatant was collected and concentrated, and the pH of the supernatant was adapted to 4.0 with 6 mol/L HCl. The soybean polysaccharide was precipitated by the addition of 2 volumes of pure ethanol, and dried at 65 °C for 6 h, thus obtaining HW-ASP. Protein removal from polysaccharides was performed using Savage's method (chloroform/n-butanol, 4:1, v/v) (Li et al., 2011). The deproteinized polysaccharide was dialyzed and lyophilized.

2.2.2. Single enzyme extraction

The polysaccharides were extracted with cellulase, papain, and pectinase, respectively, the addition amount was 2%, and the ratio of solid to liquid was 1:20 (w/v), extracted at 65 °C for 1.5 h. And then quickly heated up for enzyme inactivation treatment, and the subsequent operations are the same as the hot water extraction method to attain C-ASP, P-ASP, and PE-ASP.

2.2.3. Compound enzyme extraction

50 g soybean powder was dissolved in 1 L distilled water and mixed with 2% complex enzymes (cellulase: pectinase: papain, v: v: v = 3:3:1). The subsequent operations were the same as the single-enzyme extraction method to gain CE-ASP.

2.3. Characterization of ASPs

2.3.1. Chemical analysis

The total sugar content of ASPs was determined by the phenol-sulfuric acid method (Wang et al., 2010). The content of protein was calculated according to the Bradford method (Bradford, 1976). The content of uronic acid was determined by m-hydroxybiphenyl method (Alyassin et al., 2020).

2.3.2. Analysis of monosaccharide composition and molecular weight

The monosaccharide composition of ASPs was estimated as previously reported by Dong et al. (2016). The ASP was hydrolyzed using trifluoroacetic acid (TFA, 4 M) in a sealed tube, followed by removal of excess TFA. The residue was then dissolved in methanol and evaporated to dryness, and the operation was repeated four times. The residue was subsequently dispersed for analysis in ultrapure water. The processed samples were investigated by a Shimadzu liquid chromatograph LC-20A provided with a C_{18} column (4.6 × 150 mm × 5 μm).

The molecular weight of ASP fractions was measured by high-performance gel permeation chromatography (HPGPC) technique with dextran standards (PSS, Mainz, Germany) (Chen et al., 2019).

2.3.3. Determination of zeta potential and particle size

The zeta potential (Zp) and the mean hydrodynamic diameter (Z-average) of ASPs solutions were measured by NanoBrook 90Plus Zeta instrument at 25 °C.

2.3.4. Analysis of Fourier transform-infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM)

The ASPs were blended with KBr powder and pressed into flakes. The spectra of the components were recorded by FT-IR (Scimitar 2000, Agilent) with a scan range of 4000–400 cm^{-1}.

The morphology of ASPs were assessed by SEM (Phenom pro, Phenom-World, The Netherlands). Adhere 5 ASPs to the sample stage and perform gold spraying, choosing 1 kV as the accelerating voltage.

2.4. Hydrolysis resistance of polysaccharides to artificial gastric juice

The hydrolysis resistance of ASPs in artificial gastric juice was calculated by referring to the method of He et al. (2016). Fructooligosaccharides (FOS) was used as a positive control. The pH of artificial human gastric juice (8.00 g NaCl, 0.20 g KCl, 0.18 g $MgCl_2{\cdot}6H_2O$, 0.10 g $CaCl_2{\cdot}2H_2O$, 8.25 g $Na_2HPO_4{\cdot}2H_2O$ and 14.35 g $NaHPO_4$ were dissolved in 1 L distilled water) was adjusted with 1 M HCl. Subsequently, ASPs and FOS were dissolved in buffers of pH 1.0, 2.0, 3.0, 4.0, and 5.0 to a volume concentration of 1.0 % (w/v), respectively, and then incubated at 37 °C for 6 h. The reducing sugar and total sugar contents of the reaction solutions were measured at specific time points (0, 1, 2, 4, and 6 h). The degree of hydrolysis of the ASPs fractions was analyzed as follows (He et al., 2016):

$$\text{Hydrolysis degree } (\%) = \frac{\text{Reducing sugar released}}{\text{Total sugar} - \text{Initial reducing sugar}} \times 100\% \quad (1)$$

where the reducing sugar released is the difference between its final and initial content.

2.5. Effect of α-amylase on polysaccharide hydrolysis

The hydrolytic resistance of five ASP fractions to α-amylase was confirmed following the method cited by Chen et al. (2020a). The ASPs were dissolved in sodium phosphate buffer (20 mM), and the pH of the solution was adjusted to 5.0, 6.0 and 7.0. Then, 2 U of α-amylase was added and the reaction was carried out at 37 °C for 6 h. Fractions were withdrawn at specific time points to measure the degree of hydrolysis, as described in Section 2.5.

2.6. Probiotic activities of ASPs

2.6.1. Medium preparation

To assess whether the ASPs fractions could be used as carbon source substitutes for probiotic proliferation. 20 g/L (2.0% w/v) of sterile ASPs were subjoined to 10 mL of basal MRS medium. FOS served as a prebiotic control, MRS basal medium without glucose was the blank control, and

the positive control was glucose. MRS basal medium was autoclaved while all carbon sources were irradiated with ultraviolet (UV) light for 60 min.

2.6.2. Effects of ASPs on probiotics proliferation

The promotion of probiotic proliferation by five ASPs was assessed using the method reported by Huang et al. (2019). In this study, we used *L. paracasei, L. rhamnosus*, and *L. acidophilus* as the reference bacterial strains. Continuous dilution of the activated strain with sterile sodium chloride solution (1% w/v). Then 150 μL carbon source media were added to the 96-well plate followed by inoculation with 50 μL microlux diluted cultures and the plate was then incubated in a biochemical incubator at 37 °C for 48 h. The cell density of probiotics was determined by the value of OD_{600}.

2.6.3. Analysis of medium pH and SCFAs

The pH of the culture medium was measured by pH meter at the same time interval. Gas chromatography (GC) was used to determine the contents of SCFAs (Chen et al., 2019). 20 μL of the sample solution was precisely pipetted into an ampoule bottle, diluted using 980 μL of ultrapure water, and mixed by vortexing the solution. The collected medium was centrifuged to remove cells for IC analysis. The analysis was implemented on an Agilent IC system equipped with a Dionex Ion Pac AS11-HC-4 um and conductivity detector. Mobile phase: A: H_2O; B: 50 mM KOH; flow velocity: 1.5 mL/min; injection amount: 25 μL; column temperature: 30 °C; Suppressor: Dionex Anion Self-Regenerating Suppressor.

2.7. Statistical analysis

All experiments were repeated three times, and the experimental data were represented by the mean ± standard deviation (SD). Origin 8.0 software was used for graphing, and SPSS 26.0 software was used for variance analysis, with $p < 0.05$ as the significance test standard.

3. Results and discussion

3.1. Effects of different extraction methods on the yield of ASPs

The extraction rates of ASPs obtained by different extraction methods were shown in Table 1. The extraction yield of CE-ASP was the highest (10.02 ± 0.22%), followed by C-ASP (9.60 ± 0.35%), PE-ASP (9.22 ± 0.34%), P-ASP (8.55 ± 0.09%), and HW-ASP (6.90 ± 0.11%), indicating that the extraction methods had a evident influence on the extraction rate of ASPs (Dong et al., 2016). Compared with other methods for extracting polysaccharides from soybean hull, the extraction rate of polysaccharides obtained by enzymatic method was slightly higher than that by ammonium oxalate and microwave-assisted extraction (Yang et al., 2020). These findings speculated that the compound enzyme method may promote the rupture of the cell wall and the hydrolysis of glycoprotein complexes to release more polysaccharides (Song et al., 2020). These results demonstrated that the enzymatic extraction of polysaccharides could be used as a promising replacement technology for the production of ASPs.

3.2. Characterization of polysaccharides

3.2.1. Chemical component of ASPs

The highest total sugar content CE-ASP (51.00%), followed by C-ASP (48.94%), P-ASP (46.55%), PE-ASP (46.22%), and HW-ASP (45.90%), which shown in Table 1. The contents of uronic acid of ASPs were determined to be 19.21% (HW-ASP), 20.35% (P-ASP), 26.91% (PE-ASP), 22.70% (C-ASP), and 25.38% (CE-ASP). The differences in the chemical composition may be interrelated to the methods of extracting polysaccharides (Feng et al., 2021; Song et al., 2020). After deproteinization of ASPs by the savage method, no protein was detected.

3.2.2. Monosaccharide composition and molecular weight of ASPs

The results of the monosaccharide composition of the five ASPs are graphically presented in Table 1 and Fig. S1. As shown in Table 1, The monosaccharide composition of HW-ASP and PE-ASP contained Man, GluA, Rha, Glc, Gla, and Ara, with the mole ratio of 1.854:0.112:0.269:0.031:0.716:0.284, and 0.921:0.079:0.125:1.070:0.531:0.250, respectively. The monosaccharide composition of C-ASP and P-ASP contained Man, GluA, Glc, Gla, and Ara. The monosaccharide mole ratio of C-ASP was 0.944:0.026:0.866:0.294:0.140, and the monosaccharide mole ratio of P-ASP was 0.783:0.026:0.611:0.343:0.179. The monosaccharide composition of CE-ASP contained Man, GluA, Rha, Glc, Gla, Ara, and Xyl with the mole ratio of 0.955:0.090:0.197:0.655:0.508:0.378:0.055. The results showed that the distinct extraction processes did not affect the monosaccharide composition of the polysaccharide, but changed the monosaccharide content, and these results were consistent with previous research results (Ji et al., 2022; Song et al., 2020).

The molecular weights distributions of polysaccharides extracted by the enzyme extraction method were less than 1000 Da (Table S1), indicating that the enzyme extraction degrades the polysaccharide. These results indicated that polysaccharides can be degraded by enzymes to a certain extent, converting a part of high molecular weight components into low molecular weight components, thus producing more low molecular weight components (Kaczmarska et al., 2022). It is reported that

Table 1. Chemical and monosaccharides compositions of ASPs extracted by different methods.

Items	HW-ASP	P-ASP	PE-ASP	C-ASP	CE-ASP
Yield (%)[1]	6.90 ± 0.11^{d}	8.55 ± 0.09^{c}	9.22 ± 0.34^{b}	9.60 ± 0.35^{ab}	10.02 ± 0.22^{a}
Carbohydrate (%)[1]	45.90 ± 0.99^{b}	46.55 ± 1.03^{b}	46.22 ± 1.52^{b}	48.94 ± 1.01^{a}	51.00 ± 1.08^{a}
Protein (%)[1]	ND	ND	ND	ND	ND
uronic acid (%)[1]	19.21 ± 0.56^{d}	20.35 ± 0.88^{d}	26.91 ± 0.45^{a}	22.70 ± 0.94^{c}	25.38 ± 0.56^{b}
Monosaccharide composition molar ratios					
mannose	1.854	0.783	0.921	0.944	0.955
Glucuronic acid	0.112	0.026	0.079	0.026	0.090
Rhamnose	0.269	ND	0.125	ND	0.197
glucose	0.031	0.611	1.070	0.866	0.655
galactose	0.716	0.343	0.531	0.294	0.508
arabinose	0.284	0.179	0.250	0.140	0.378
xylose	ND	ND	ND	ND	0.055

ND is not detected.

[1] Results are expressed as the mean ± standard deviation of three independent experiments (n = 3), with different mean superscripts within a column significantly different ($p < 0.05$).

Table 2. Particle size and Zeta potential of ASPs extracted by different methods.

Samples	HW-ASP	P-ASP	PE-ASP	C-ASP	CE-ASP
Particle size (nm)	-28.13 ± 1.35[d]	-30.43 ± 0.94[b]	-29.55 ± 1.63[c]	-31.05 ± 1.79[a]	-30.93 ± 1.56[a]
Zeta potential (mV)	598.22 ± 1.22[a]	370.10 ± 1.05[b]	373.49 ± 1.45[b]	340.38 ± 1.69[c]	302.69 ± 1.35[d]

[a]Value with no letters in common are significant difference ($p < 0.05$).
Results are expressed as the mean ± standard deviation of three independent experiments (n = 3).

the bioactivity of polysaccharides is related to molecular weight, polysaccharides with lower molecular weight more likely to have higher water solubility and bioavailability (Kaczmarska et al., 2022).

Compared with the microwave-assisted extraction of ammonium oxalate, the monosaccharide composition and molecular weight of soybean hull polysaccharides were also different (Yang et al., 2020; Wang et al., 2022a, 2022b). Therefore, different extractants can affect the basic composition of polysaccharides.

3.2.3. Zeta potential and particle size of ASPs

The characterization of the particle size and charge of the polysaccharide can indicate the stability of the solution (Wang et al., 2022a, 2022b). As can be seen from Table 2, polysaccharides obtained in the aqueous solution by different extraction methods have different particle size, and the effective particle sizes of C-ASP (340.38 ± 1.69 nm) and CE-ASP (302.69 ± 1.35 nm) were significantly smaller than PE-ASP (373.49 ± 1.45 nm), P-ASP (370.10 ± 1.05 nm), and HW-ASP (598.22 ± 1.22 nm), which may be related to their different molecular weight distributions. The zeta potentials of C-ASP, CE-ASP, and P-ASP all reached |±30|. C-ASP had the most negative charge. Due to the polarity of the charge, the particles in C-ASP showed no obvious aggregation. The larger the negative charge of C-ASP, and smaller the diameter, indicating its better stability (Chen et al., 2019).

3.2.4. FT-IR spectroscopy of ASPs

As shown in Figure 1, the five ASPs had comparable typical infrared absorption spectra with the characteristic absorption peak of hydroxyl at 3427 cm^{-1}. (Ren and Liu, 2020). The absorption peak at 2943 cm^{-1} was owing to C–H stretching vibration (Ji et al., 2021). The absorption peak at 1730 cm^{-1} was ascribed to the C=O stretching vibration, indicating that the sample still contained uronic acid (Kungel et al., 2018). Absorbance peaks at 1625 cm^{-1} and 1410 cm^{-1} indicate possible carbonyl groups (Gao et al., 2020). The peak at 1249 cm^{-1}suggested that the sugar was in the O–H variable-angle vibration. The absorption peaks at 1141 cm^{-1} and 1016 cm^{-1} were attributed to C–O stretching vibration (Tian et al., 2020). The uptake absorption peaks of α-conformation and β-conformation of polysaccharides are at 844 cm^{-1} and 891 cm^{-1}, respectively (Chen et al., 2008). Characteristic absorption peaks were observed at 850 cm^{-1} and 893 cm^{-1}, demonstrating that ASPs were attached by α-glycosidic and β-glycosidic bonds.

3.2.5. SEM of ASPs

The apparent morphology of five ASPs was shown in Figure 2. The consequence showed that the appearance of HW-ASP was rough and flaky, while the surface of PE-ASP, P-ASP, and C-ASP was relatively complete. CE-ASP had a fragile texture and rough loose surface, which was relatively loose. In addition, the loose and irregular surface structure made it possible to have a larger surface area (Song et al., 2022). SEM analysis provides strong evidence for the reason why the enzymatic method improves the extraction efficiency of polysaccharides (Chen et al., 2008).

3.3. Hydrolysis resistance of ASPs by artificial human gastric fluid

The digestibility of FOS, HW-ASP, P-ASP, PE-ASP, C-ASP, and CE-ASP in artificial gastric juice with different pH levels were shown in Figure 3. The hydrolysis degree of carbohydrates in artificial gastric fluid significantly increased with the enhancement of the acidic conditions ($p < 0.05$). After 6 h of incubation, the polysaccharides obtained by enzymatic hydrolysis were lower in pH than those obtained by hot water ($p < 0.05$), indicating that polysaccharides obtained by enzymatic hydrolysis were resistant to gastric juice hydrolysis but still higher in hydrolysis than the positive control group. After 6 h of reaction at pH 5.0, the degree of

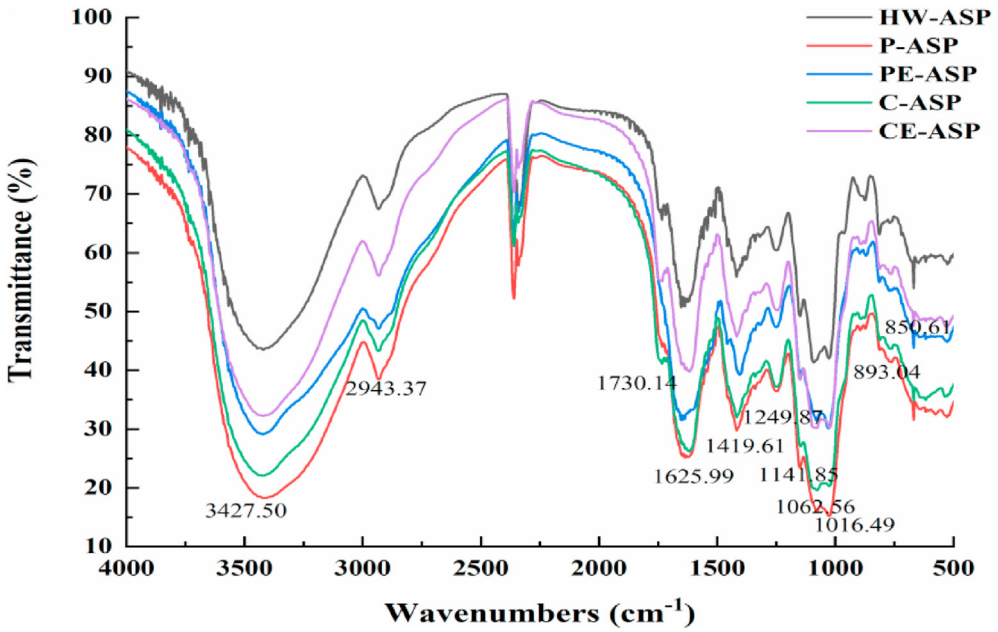

Figure 1. FT-IR spectrum of five ASP fractions.

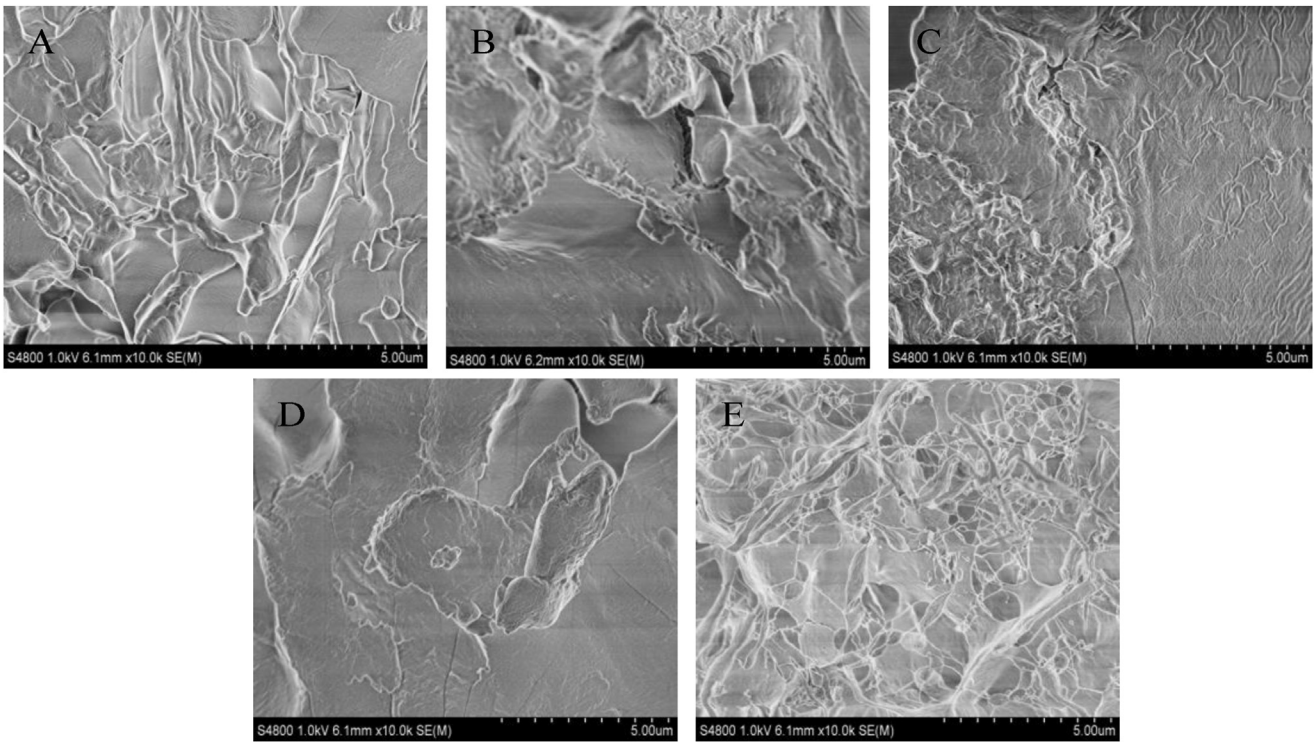

Figure 2. SEM of five ASP fractions. A, HW-ASP; B, P-ASP; C, PE-ASP; D, C-ASP; E, CE-ASP.

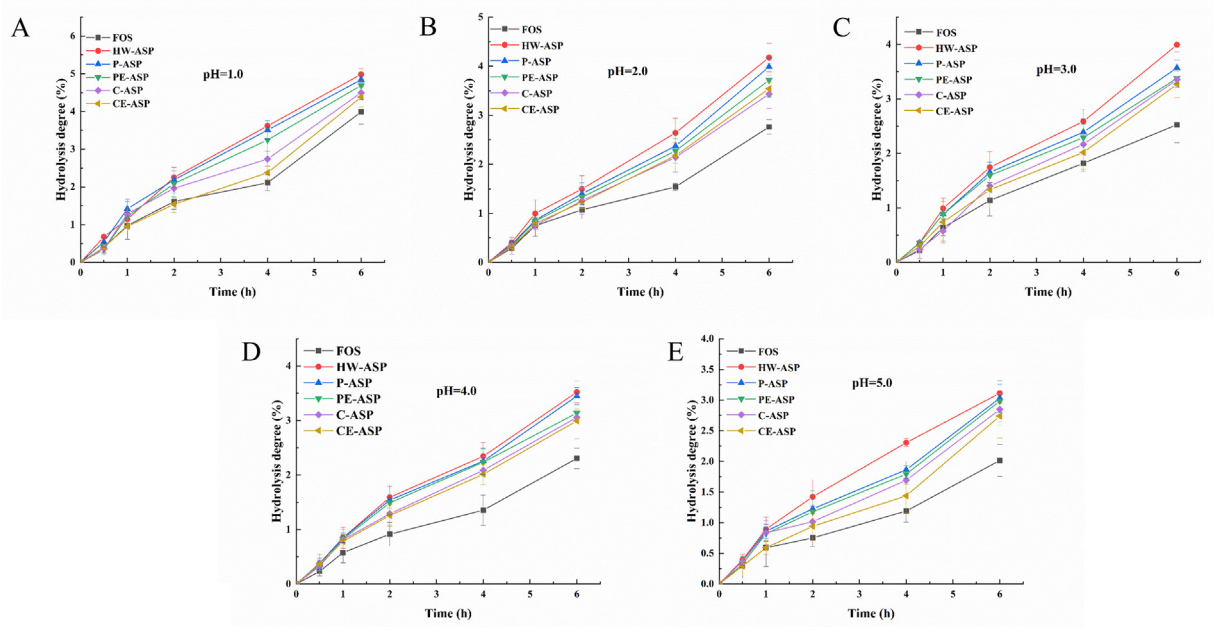

Figure 3. Resistance of FOS and the five ASP fractions to artificial gastric juice. A, pH = 1.0; B, pH = 2.0; C, pH = 3.0; D, pH = 4.0; E, pH = 5.0. Data were mean ± standard error (n = 3).

hydrolysis of FOS, HW-ASP, P-ASP, PE-ASP, C-ASP, and CE-ASP were 2.01%, 3.41%, 3.03%, 2.98%, 2.85%, and 2.74%, respectively. Studies have shown that the composition of monosaccharides and the type of glycosidic bonds may affect the resistance of polysaccharides in artificial gastric juice (Xu et al., 2021). In general, β-bonds are more steady than α-bonds (Chen et al., 2019). The monosaccharide compositions and β-type glycosidic bonds help us to understand why the ASPs were resistant to acid hydrolysis. Compared with the polysaccharides obtained by enzymatic extraction, the complex enzyme breaks the long-chain structure of the original polysaccharides, thus releasing more active units, keeping the relative molecular weight at a suitable low level, thus improving the overall activity of the polysaccharides (Anwar et al., 2021).

3.4. The resistance of ASPs to α-amylase

Only polysaccharides with strong resistance to α-amylase can be used as prebiotics (Chen et al., 2020b). With the increase of incubation time

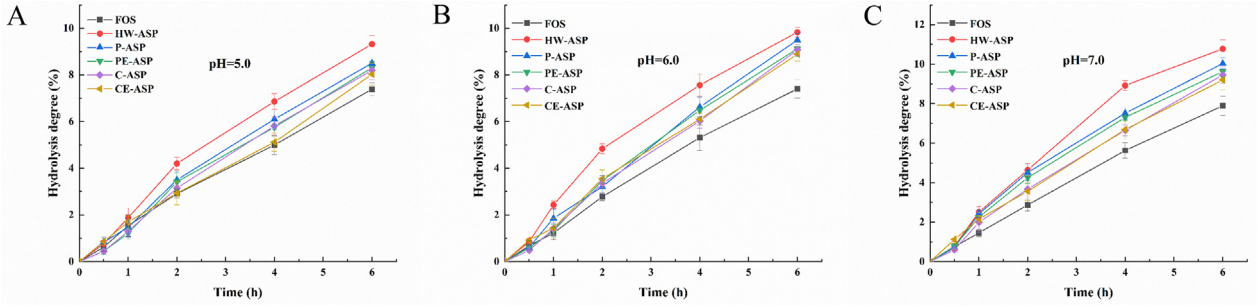

Figure 4. Resistance of FOS and the five ASP fractions to α-amylase. A, pH = 5.0; B, pH = 6.0; C, pH = 7.0. Data were mean ± standard error (n = 3).

and pH value (Figure 4), the hydrolysis degrees of five ASPs and FOS treated with α-amylase at pH 5.0–7.0 enhanced markedly ($p < 0.05$). It is worth noting that the pH had an important impact on the degree of α-amylase hydrolysis of ASPs. The hydrolysis degrees of five ASPs at different pH values was as follows: 5.0 < 6.0 < 7.0. For example, the maximum degree of hydrolysis of HW-ASP, P-ASP, PE-ASP, C-ASP, CE-ASP, and FOS after 6 h of reaction at pH 7.0 was 10.77%, 10.03%, 9.62%, 9.45%, 9.20%, and 7.89%, respectively, which may be attributed to their structural configurations. All five ASPs fractions contained β-type glycosidic bonds, which associated with the lower hydrolysis degree of α-amylase (Wichienchot et al., 2010). Considering the high stability to α-amylase and artificial gastric juice hydrolysis, the five ASPs can be used as prebiotic preparations.

3.5. Probiotic activity of ASPs

3.5.1. Effect of ASPs on the growth of probiotics

As shown in Figure 5, the growth of three probiotics in media containing ASPs, FOS, and Glc were notably higher than that of the blank controls after 24 h ($p < 0.05$), suggesting that all probiotics were able to utilize ASP, FOS, and Glc. Probiotics can rapidly metabolize carbon sources, resulting in the accumulation of SCFAs, so the pH of the medium is not suitable for the growth of probiotics (Chen et al., 2020a). The overall results of five ASP fractions obviously revealed that these ASPs were not lethal to the tested probiotics. They have proven to be great growth substrates, and the addition of PE-ASP, C-ASP, and CE-ASP was better than other ASPs.

Studies have shown that monosaccharide compositions such as polysaccharides containing glucose, galactose, and xylose have better probiotic activity (Zhang et al., 2018). ASPs prepared by different enzymatic methods have prebiotic activity, probably because they are all constituted of glucose and galactose. In addition, differences in the monosaccharide content of the five ASPs may also affect their probiotic activities. On the other hand, low molecular weight polysaccharides are more readily utilized by probiotics. (Yeung et al., 2021). And the water solubility of polysaccharides may also influence the growth of probiotics (Wang et al., 2021).

3.5.2. SCFAs production

The pH of the three probiotics in different medias were measured and the results were displayed in Figure 5. After 48 h of culture with *L. paracasei*, the pH decreased significantly ($p < 0.05$), while the pH of all tested samples reached the minimum value in the following order (HW-ASP > P-ASP > PE-ASP > CE-ASP > C-ASP > FOS > Glc) at the concentration of 2.0%. Similar to *L. paracasei*, C-ASP and CE-ASP also caused a distinct decrease ($p < 0.05$) of pH after 48 h when cultivated with

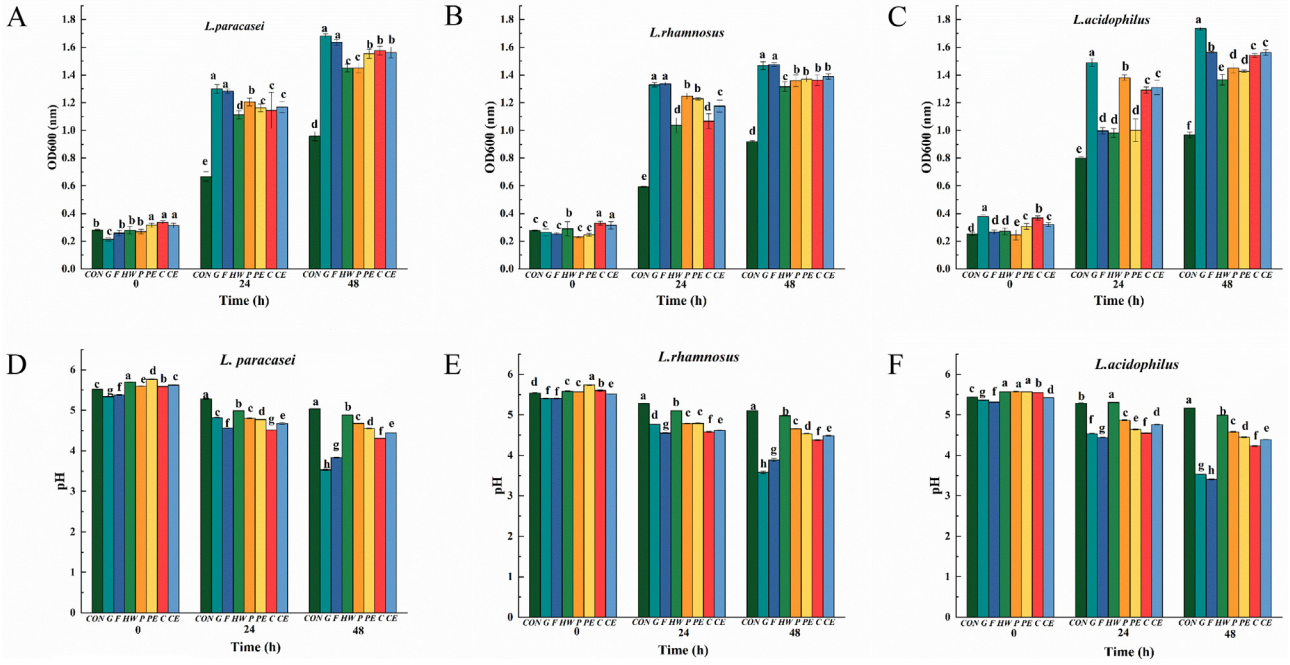

Figure 5. Effect of five ASPs, Glc and FOS on acidifying activity and proliferation after 48 h incubation of probiotics. A, B, C was the growth curve graph; D, E, F was the pH graph. Data were mean ± standard error (n = 3). Different letters mean a significant difference at the same incubation time. CON group: glucose-free MRS base medium (Control); G group: glucose MRS base medium; F group: FOS medium; HW group: HW-ASP medium; P group: P-ASP medium; PE group: PE-ASP medium; C group: C-ASP medium; CE group: CE-ASP medium.

Table 3. Short-chain fatty acids profile in liquid cultures of three probiotic strains after fermentation for 48 h with different carbon sources.

Bacteria	Carbon source	AA (mg/L)	PA (mg/L)	Total SCFAs (mg/L)
L.paracasei	FOS	47.52 ± 1.77^{a}	1.77 ± 0.83^{b}	49.29 ± 0.95^{ab}
	HW-ASP	37.79 ± 5.00^{b}	4.50 ± 0.42^{a}	42.29 ± 4.70^{b}
	C-ASP	47.89 ± 2.76^{a}	2.40 ± 2.08^{ab}	50.29 ± 4.59^{a}
L.rhamnosus	FOS	53.18 ± 1.72^{a}	1.04 ± 0.13^{b}	54.22 ± 0.41^{b}
	HW-ASP	46.89 ± 3.81^{b}	2.60 ± 2.36^{ab}	49.49 ± 3.06^{b}
	C-ASP	47.47 ± 2.41^{b}	3.87 ± 0.10^{a}	51.34 ± 2.33^{ab}
L.acidophilus	FOS	48.44 ± 0.84^{a}	2.31 ± 0.14^{b}	50.75 ± 0.54^{b}
	HW-ASP	46.22 ± 1.44^{b}	3.64 ± 0.96^{ab}	49.86 ± 0.48^{b}
	C-ASP	49.37 ± 0.84^{a}	5.06 ± 1.44^{a}	54.42 ± 1.94^{a}

AA: Acetic acid; PA: Propionic acid;
All results are presented as the mean ± standard deviation of three independent experiments. Means in the same column with different letters are significantly different for each probiotic strain ($p < 0.05$).

L. rhamnosus and *L. acidophilus*. All three probiotics metabolized the five ASPs with different efficiencies, resulting in varying degrees of pH reduction. The pH values of five ASPs were also significantly higher than those of G or F groups.

SCFAs are the main end-products of probiotic metabolism of carbon sources and maintain the balance of intestinal redox (Higashi et al., 2020). The three probiotics metabolized by different carbon sources during the growth process mainly produced acetic acid and propionic acid, the contents of which are shown in Table 3. After 48 h of fermentation of all probiotics, the concentrations of acetic acid and propionic acid in the C-ASP group were notably higher than those in HW-ASP ($p < 0.05$), but there was no obvious difference in acetic acid content between FOS and C-ASP ($p > 0.05$). Compared with FOS as a carbon source medium, propionic acid produced by the three probiotics in the carbon source medium containing HW-ASP and C-ASP was significantly increased ($p < 0.05$). The total SCFAs concentration of *L. paracasei,* using different carbon sources, remained in the following order: C-ASP > FOS > HW-ASP. Whereas for *L. rhamnosus* and *L. acidophilus*, it remained as FOS > C-ASP > HW-ASP and C-ASP > FOS > HW-ASP, respectively. According to the experiment, different enzyme extraction methods had an evident impact on the prebiotic activity of ASPs. Previous studies on *in vitro* digestion and probiotic proliferation showed that C-ASP had better prebiotic activity ($p < 0.05$).

The results indicated that the fermentation speed and utilization degree of polysaccharides by probiotics in a fermentation medium are associated with the complex structure and the features of polysaccharides, including monosaccharide composition, molecular weight, particle size, and their morphological characteristics etc. (Chen et al., 2020b). Polysaccharides with higher sugar content and lower molecular weight are more likely to be utilized by probiotics as a carbon source, and significantly affected the content of SCFAs (Wu et al., 2022). In the present study, the higher prebiotic activities observed in C-ASP might be partially attributed to its higher content of sugar and lower molecular weight. In addition, the smaller particle size of C-ASP promoted the contact surface area increasing between C-ASP and probiotics. C-ASP was more easily used or fermented by probiotics, and its total content of SCFAs was higher than that of HW-ASP. The production of acid causes a decrease in the pH of the fermentation liquid (Yeung et al., 2021). With the growth of culture time, the bacteria produce more and more acids and the pH of the fermentation liquid decreases continuously.

4. Conclusions

Five polysaccharides (ASPs) were extracted from soybean bean hulls using different methods (hot water method, single enzyme method, complex enzyme method), all of which had high digestibility *in vitro*. ASPs can promote the proliferation of probiotics and activate their acidifying activity. In addition, C-ASP with elevated total sugar content, lower molecular weight distribution, and smaller particle size was used for *in vitro* fermentation, it would promote the proliferation of probiotics and induce the production of total SCFAs. Regarding their *in vitro* digestive properties and probiotic activity, ASPs may be used as novel probiotics.

Declarations

Author contribution statement

Hong Song, Zunqin Zhang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yixue Li, Ying Zhang, Lina Yang, Shengnan Wang, Yutang He, Jun Liu: Contributed reagents, materials, analysis tools or data.

Danshi Zhu, He Liu: Conceived and designed the experiments.

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Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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