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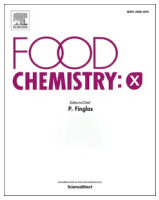

Mechanism study on the enhancement of bile acid-binding capacity in corn by-product juice via *Lactiplantibacillus plantarum* HY127 fermentation

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ABSTRACT

Hyperlipidemia is a common endocrine metabolic disease in humans. Long-term medications often have adverse effects, making the search for safer and more effective treatments crucial. This study aimed to explore the impacts and mechanisms of *Lactiplantibacillus plantarum* HY127 fermentation on enhancing bile acid-binding capacity (BABC). We fermented corn by-product juice (CBJ) by HY127 and investigated the BABC of HY127 bacterial cells and their metabolites. Our results indicated that HY127 cells (95.25 %) played a major role in enhancing BABC, with metabolites (31.50 %–66.41 %) also contributing. Compared to unfermented CBJ, the contents of phenolics, flavonoids, polysaccharides, and organic acids were significantly higher. Non-targeted metabolomics revealed upregulated amino acids, alkaloids, terpenoids, and other bioactive substances associated with BABC in the supernatant. This study confirmed that HY127 fermentation enhances the BABC of CBJ (increased by 32.02 %–78.76 %), providing a research foundation and technical reference for the development of LAB-fermented corn by-product beverages with hypolipidemic activities.

1. Introduction

Hyperlipidemia is a widespread endocrine metabolic disease in humans (Parhofer, 2015). Although relatively efficient drugs, such as statins, are available for the treatment of hyperlipidaemia, considering the side effects or high rates of secondary failure of medicine in long-term taking, the alternative natural products are still in urgent demand (Li et al., 2024; Ma et al., 2022). Bile acid-binding capacity (BABC) is usually used as an indicator to evaluate the hypolipidemic activities of raw materials in vitro (Li et al., 2017; Ma et al., 2022).

Bile acids, synthesized from cholesterol in the liver, play important roles in lipid metabolism (Ma et al., 2022; Shi et al., 2016). Certain bioactive constituents such as polysaccharides and polyphenols can bind to bile salts in the intestine, prevent the hepatic-intestinal circulation of bile acids, promote bile acid excretion, and accelerate the conversion of cholesterol to bile acids (Han et al., 2021; Naumann et al., 2020; Shi et al., 2016). Moreover, bile acid excretion affects cholesterol digestion and absorption in the intestine, thereby reducing cholesterol levels in the liver and plasma (Choi et al., 2015). Hence, BABC can be used to screen raw materials for potential blood lipid-lowering effects (Li et al., 2017).

Recently, Chinese herbs have gained attention for the prevention and treatment of chronic diseases. Corn, one of the three major grain crops in China, produces by-products such as corn silk (CS), corn husks (CHs), and corn cobs (CCs), which serve as Chinese herbal medicines and functional foods that are often discarded as waste. The Great Dictionary of Chinese Medicine states that corn by-products can lower blood lipid levels. For centuries, Chinese folk medicine has used boiled juices of CS

Abbreviations: BABC, Bile acid-binding capacity; HY127, *Lactiplantibacillus plantarum* HY127; BSH, Bile salt hydrolase; CBJ, Corn by-product juice; CC, Corn cobs; CH, Corn husks; CS, Corn silk; CSJ, Corn silk juice; DS, Decellularized supernatant; ESI, Electrospray ionization; FCBJ, Fermented corn by-product juice; TFC, Total flavonoid content; TPC, Total phenolic content; LAB, Lactic acid bacteria; PCA, Principal component analysis; QC, Quality control; HMDB, Human Metabolome Database.

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or CHs to relieve hyperlipidemia. Additionally, these by-products contain abundant natural compounds with BABC, such as flavonoids, polyphenols, and polysaccharides. For instance, CS contains 2.106 % flavonoids, CHs 1.013 %, CCs 0.662 %, and corn kernels 0.411 % (Xu, 2004). However, these complex chemical constituents are often glycosylated, methylated, esterified, or embedded in the cell wall, making them difficult to be released and absorbed into the human gastrointestinal tract, limiting their application and development (Wang, Li, et al., 2022).

Microbial transformation is an economically effective method for the conversion of complex compounds into bioactive metabolites. Lactic acid bacteria (LAB), members of the probiotic bacterial family, are widely used as fermentation strains to enhance the bioactivity of plant substrates (Wang, Feng, et al., 2022; Xiao et al., 2021). Previous studies have indicated that *Bifidobacterium animalis* subsp. *Lactis* HN-3 hydrolyzes flavonoid glycosides in *Elaeagnus angustifolia* var. *orientalis* (L.) kuntze fruit juice, thereby improving its biological activity and bioavailability (Wang, Li, et al., 2022). In addition, fermented ougan (*Citrus reticulata* cv. *Suavissima*) juice has shown inhibitory effects on high-fat diet-induced obesity in mice (Guo et al., 2021). LAB cells also exhibit various probiotic activities, such as binding to bile salts to reduce blood lipids (Yukimasa et al., 2009). Thus, we hypothesize that LAB fermentation can enhance the hypolipidemic properties of corn by-products.

In this study, *Lactiplantibacillus plantarum* HY127, known for its high BABC and screened from corn sourdough, was used to ferment CS, CH, and CC juices. To explore the effects and mechanisms of HY127 fermentation on enhancing BABC, we investigated the activity of HY127 bacterial cells and metabolites. We also examined the transformation of bioactive substances related to BABC using non-targeted metabolomic techniques. Our study provides a research basis for the development of functional beverages containing LAB-fermented corn by-products with hypolipidemic activity.

2. Materials and methods

2.1. Materials and reagents

Lactic, tartaric, acetic, malic, citric acids, gallic acid, rutin standards and phosphoric acid (HPLC) were acquired from Tanmo Quality Inspection Technology Co., Ltd. (Beijing, China). Corn by-products were obtained from an experimental farm at Jinzhou Medical University. Glucose standards and sodium cholate were acquired from Aokexing Biotechnology Co. Ltd. (Shanghai, China).

2.2. Strains and culture conditions

L. plantarum HY127 was isolated from corn sour dough. All stock cultures of HY127 was stored at −80 °C in MRS broth containing 25 % sterile glycerol. It can be used after two generations of activation when needed. The HY127 strains were cultured at 37 °C for 24 h to obtain cell concentrations of approximately 8 log CFU/mL.

2.3. Preparation of fermentation broth of CBJ

CHs, CS, and CCs were boiled in water for 20 min. The by-products were then removed, passed through a 60-mesh sieve, and both fermented and control samples were prepared. The control group was refrigerated after sterilization at 95 °C for 10 min. The fermentation group had its pH adjusted to 6.0, sterilized at 95 °C for 10 min, cooled, inoculated with 8 % inoculum, and incubated at 35 °C for 36 h. The samples were named cornhusk juice (CHJ), corn silk juice (CSJ), and corncob juice (CCJ), with the fermented versions labeled as FCHJ, FCSJ, and FCCJ, respectively.

2.4. Preparation of decellularized supernatant (DS) and cell suspensions

The fermented corn by-product juice (FCBJ) was centrifuged at 2960 ×*g* for 15 min at 4 °C, then filtered through 0.22-μm membrane filters to obtain DS. LAB cells were washed thrice with sterile water and resuspended to obtain a cell suspension.

2.5. Determination of BABC

BABC was determined as described previously (Ma et al., 2022; Yu et al., 2019), with sodium cholate as the bile acid. Briefly, 4 mL of 0.3 mmol/L sodium cholate in 0.1 M PBS buffer solution (pH 6.3) were mixed with the samples and incubated at 37 °C for 1 h. The mixture was centrifuged at 2960*g* for 10 min. Subsequently, 2.5 mL supernatant was mixed with 7.5 mL of 60 % sulfuric acid and incubated at 70 °C for 20 min. Absorbance was measured at 387 nm. BABC was measured using a sodium cholate standard curve and presented as percentage inhibition, calculated with the formula: BABC (%) = [(A – B) /A] × 100 %, where A is the initial amount of sodium cholate and B is the remaining amount.

2.6. Determination of total phenolic content (TPC) and total flavonoid content (TFC)

TPC measurements were performed using the method of Zheng et al. (2023), with slight modifications. In brief, 1 mL of samples was mixed with 1 mL of Folin–Ciocalteu reagent and 3 mL of 20 % Na_2CO_3 solution, incubated in a 50 °C water bath for 30 min, and absorbance was measured at 765 nm. Gallic acid was used as the standard.

The TFC measurements were conducted using the method of Li et al. (2023), with slight modifications. Briefly, 1 mL of the sample was mixed with anhydrous ethanol to a total volume of 15 mL, followed by 1 mL of 10 g/L $Al(NO_3)_3 \bullet 9H_2O$ solution and 1 mL of 9.8 g/L CH_3COOK solution. The mixture was topped off to 50 mL with water. After 1 h, absorbance was measured at 420 nm, using 30 % ethanol as the blank. Rutin was used as the standard.

2.7. Determination of polysaccharide content

Polysaccharide content was determined using a method adapted from Cai et al. (2022). Distilled water (1 mL) was added to 1 mL of the sample, followed by 1 mL of phenol. After shaking well, 5 mL of sulfuric acid was quickly added. The mixture was heated in a boiling water bath for 15 min, then cooled to approximately 25 °C, and absorbance was measured at 490 nm, with glucose as the standard.

2.8. Determination of organic acids

Organic acid content in CBJ and FCBJ was measured using HPLC. Samples were filtered through a 0.22 μm membrane before testing. The analysis conditions included a Cosmosil C18-PAQ chromatographic column (Nacalai Tesque Inc., kyoto, Japan) and a mobile phase of phosphoric acid, with detection at 210 nm.

2.9. Non-targeted metabolomic analysis

The relevant methods of metabolomics were based on our previous study (Zheng et al., 2023). Metabolites were chromatographically separated using a Thermo UHPLC system (Thermo Fisher Scientific, USA). MS data were collected using a Q Exactive HF-X MS instrument equipped with an electrospray-ionization (ESI) source operating in either ESI+ or ESI− mode. The raw data were input into Progenesis QI (version 2.3, Nonlinear Dynamics, Waters, USA) for peak identification and alignment following UPLC-MS analyses.

2.10. Statistical analysis

Data represented as mean ± standard deviation of three independent replicates. Statistical analyses were conducted using SPSS 26. A column chart was created using Origin 2021b. The data underwent analysis of variance using Duncan's test. Results were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. L. plantarum HY127 fermentation significantly enhanced the BABC of CBJ

As shown in Fig. 1A, HY127 fermentation significantly enhanced the BABC in CBJ. The BABC of FCHJ increased the most, with an increase of 78.76 %. FCCJ had the highest BABC (82.17 %). Within the intestinal lumen, bile acids interact with lipases to facilitate lipolysis and fat absorption (Kongo-Dia-Moukala et al., 2011). In addition, depletion of bile acids can promote cholesterol conversion into additional bile acids, thereby leading to reductions of liver and serum cholesterol levels (Kongo-Dia-Moukala et al., 2011; Prete et al., 2020). In summary, HY127 fermentation enhanced the BABC of CBJ. Moreover, it enhances the potential hypolipidemic activity of CBJ by reducing fat absorption and cholesterol content.

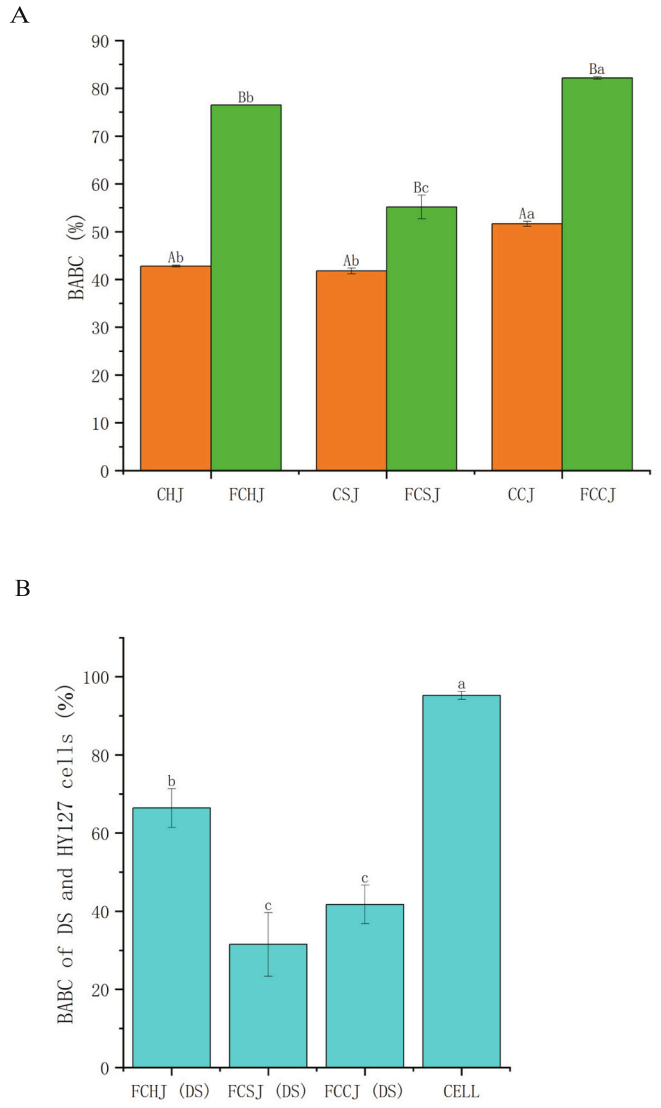

Fig. 1. (A) Bile acid-binding capacity (BABC) of CBJ before and after fermentation. (B) BABC of HY127 cells and decellularized supernatant (DS). Different uppercase letters indicate significant differences before and after fermentation, and different lowercase letters indicate significant differences among different corn by-product species ($P < 0.05$), the same below.

Some LAB cells, phenolics, and polysaccharides exhibit varying degrees of BABC (Gao et al., 2017; Ma et al., 2022; Yukimasa et al., 2009). Consequently, we hypothesize that the enhancement of BABC in FCBJ may be because of metabolites and/or HY127 cells.

3.2. Analysis of BABC of HY127 cells and DS in FCBJ

In order to explore the role of HY127 cells and their metabolites in enhancing the BABC of CBJ, the fermented juice was centrifuged, and the BABC of the DS and HY127 cells were measured separately. Notably, HY127 cells showed excellent BABC, reaching 95.25 % (Fig. 1B). Although the BABC of the decellularized FCHJ supernatant reached 66.41 %, it was still lower than that of the HY127 cells.

HY127 cells play a major role in the BABC of FCBJ. There may be two reasons for the outstanding BABC in HY127 cells. First, *L. plantarum* has been reported to exhibit bile salt hydrolase (BSH) activity (Gil-Rodríguez & Beresford, 2021; Prete et al., 2020). Furthermore, the administration of L. *plantarum* H6 markedly increased the abundance of BSH activity in the intestinal flora of mice (Qu et al., 2020). Thus, HY127 cells may secrete bile salt hydrolases to hydrolyze conjugated bile acids into deconjugated bile acids, which are more easily attached to the surface of HY127 cells (Gil-Rodríguez & Beresford, 2021; Lye et al., 2010). Second, the amino acids in the cell wall peptidoglycan structure of LAB can bind to cholesterol, and bile acids are steroid molecules synthesized from cholesterol (Lye et al., 2010). Therefore, we hypothesize that HY127 cells could also bind bile acids via this mechanism. Furthermore, a previous study clarified that probiotics can inhibit the formation of intestinal cholesterol micelles. The broken micelles cannot transport fatty acids to the surface of the intestinal mucosa for absorption, leading to a decrease in cholesterol levels (Qu et al., 2020). Moreover, administration of *L. plantarum* significantly reduces cholesterol levels in the serum and liver of mice (Qu et al., 2020). Additionally, studies have shown that L. *plantarum* significantly improves blood lipid levels in obese individuals, reducing non-High-density lipoprotein and Low-density lipoprotein cholesterol levels (Padro et al., 2024).

It was also found that DS exhibited BABC ranging from 31.5 % to 66.41 %. The enhancement of BABC in the supernatant could be attributed to metabolites produced by HY127. *L. plantarum* can utilize various carbohydrates in corn by-products to produce organic acids (Wang, Feng, et al., 2022). In addition, proteolytic enzymes secreted by LAB can hydrolyze proteins in corn by-products into amino acids (Li et al., 2023). More importantly, *L. plantarum* contains abundant carbohydrate enzyme (CAZys) genes, such as glycoside hydrolase and glycosyltransferase, providing molecular support for the complex metabolism of sugars in bacterial strains. This frees the bound glycosides in CBJ, producing more active substances such as polyphenols, flavonoids, and alkaloids (Mao et al., 2021; Wang, Li, et al., 2022). The generated organic acids, small peptides, amino acids, phenols, flavonoids, and other metabolic products provide a favorable basis for the enhancement of BABC in FCBJ (Kongo-Dia-Moukala et al., 2011; Ma et al., 2022; Xiao et al., 2023). In order to investigate the influence of HY127 fermentation on the active constituents of CBJ, the phytochemicals (TPC, TFC, and polysaccharides), organic acids, and small-molecule metabolites of CBJ and FCBJ were measured.

3.3. Effect of HY127 fermentation on TPC and TFC

Polyphenols and flavonoids are closely related to the BABC of raw plant materials. They can adsorb bile acids by forming hydrophobic spaces (Han et al., 2021; Naumann et al., 2020). Consequently, the micellar solubility of cholesterol decreases, which causes more

cholesterol to precipitate and be excreted in the feces (Choi et al., 2015). Clinical studies have shown that polyphenols can reduce total cholesterol, triglycerides and lipoprotein oxidation while increasing High-density lipoprotein cholesterol and enhancing the intestinal epithelial barrier (Tveter et al., 2023).

HY127 fermentation significantly increased the TPC and TFC in CBJ ($P < 0.05$). As shown in Fig. 2A and B, the TPC and TFC of FCSJ increased the most, by 2.74-fold and 4.35-fold, respectively, followed by FCHJ. The increase in TPC and TFC may be due to HY127 fermentation disrupting the cell wall of corn by-products and releasing phenolic and flavonoid compounds (Zheng et al., 2023). Moreover, the glycoside hydrolase secreted by HY127 hydrolyzes glycosylated phenols and flavonoids, releasing free phenols and flavonoids with high bioactivity and bioavailability (Wang, Li, et al., 2022). Consequently, the increase in TPC and TFC enhances the BABC of fermented CBJ.

3.4. Effect of HY127 fermentation on polysaccharides

Polysaccharides can reduce cholesterol in the blood as well as the digestion and absorption of fat in the gastrointestinal tract by binding to bile salts, thereby achieving a lipid-lowering effect (Choi et al., 2015; Gao et al., 2017). Although polysaccharides do not intermolecularly interact with bile acids, they rely on their viscosity to bind bile acids (Naumann et al., 2020). They can inhibit weight gain in obese mice, reduce serum, Low-density lipoprotein cholesterol and liver total cholesterol content, and adsorb bile acids in the intestine, reducing their reabsorption and promoting cholesterol catabolism (Shi et al., 2016).

The polysaccharide content of CBJ increased significantly after HY127 fermentation (Fig. 2C) ($P < 0.05$). Previous studies have reported that LAB can produce exopolysaccharides (Vivek et al., 2019; Zheng et al., 2023). The increase was most pronounced in FCSJ, which rose 1.96 times to 2.31 mg/mL ($P < 0.05$), indicating HY127's strong polysaccharide secretion ability in corn by-products, varying across different substrates. Therefore, an increase in polysaccharide content also enhanced the BABC of FCBJ.

3.5. Effect of HY127 fermentation on organic acids

It is a well-known fact that lactic acid bacteria ferment sugars to produce organic acids. Organic acids such as citric acid may have a regulating effect on hyperlipidemia (Duan et al., 2023; Feng et al., 2020; Yadikar et al., 2022). Therefore, we measured the organic acid changes in CBJ before and after fermentation. Table 1 shows that citric acid concentration increased significantly ($P < 0.05$), with the highest increase in FCCJ at 122.73 μg/mL, followed by FCSJ at 104.89 μg/mL. We observed a significant decrease in malic acid and tartaric acid concentrations simultaneously. This may be due to the conversion of tartaric and malic acids to citric acid during the tricarboxylic acid cycle (Duan et al., 2023). A previous study found that citric acid significantly decreases the levels of total cholesterol and triglycerides in hyperlipidemic rat models (Yadikar et al., 2022). Thus, citric acid produced by the fermentation of HY127 may also enhance BABC.

The production of lactic acid is generally recognized as a sign of successful fermentation (Huang et al., 2020; Markkinen et al., 2019). The lactic acid concentration in each sample increased significantly after fermentation ($P < 0.05$) (Duan et al., 2023; Feng et al., 2020). FCHJ showed the highest increase, from 80.7 to 25,371.46 μg/mL, followed by FCSJ, which increased from 737.98 to 25,992.72 μg/mL, much higher than previously reported in corn-based beverages fermented by LAB and yeast (3.7 g/L) (Freire et al., 2017). This may be because the xylan in CBJ is hydrolyzed into prebiotic xylooligosaccharides by enzymes produced by HY127 to promote the growth of HY127, thus promoting lactic acid production (Samanta et al., 2016). In conclusion, CBJ is a suitable substrate for HY127 cell growth, and the increase in the citric acid concentration may also have contributed to the enhancement of BABC. Of course, this requires further experiments to prove.

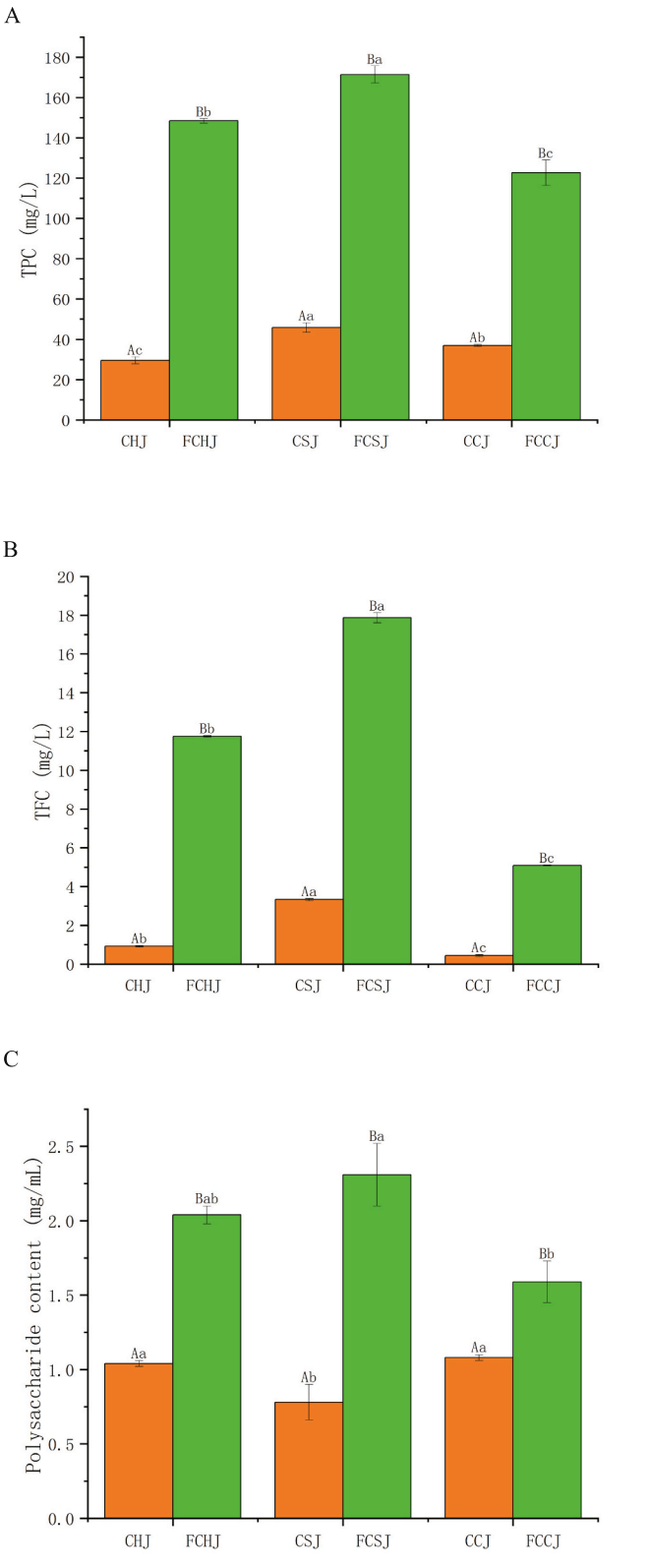

Fig. 2. (A) Total phenolic content (TPC) of CBJ before and after fermentation. (B) Total flavonoid content (TFC) of CBJ before and after fermentation. (C) Polysaccharide content of CBJ before and after fermentation.

Table 1
Effect of LAB fermentation on organic acid content.

Sample	Lactic Acid (μg/mL)	Acetic Acid (μg/mL)	Tartaric Acid (μg/mL)	Malic Acid (μg/mL)	Citric Acid (μg/mL)
CHJ	80.7 ± 6.52^{Ac}	ND	19.64 ± 0.54^{Ab}	24.56 ± 2.4^{Ac}	ND
FCHJ	$25{,}371.46 \pm 49.35^{Ba}$	ND	ND	ND	92.06 ± 0.22^{Ac}
CSJ	737.93 ± 38.46^{Aa}	ND	4.45 ± 0.74^{Ac}	339.65 ± 18.42^{Aa}	ND
FCSJ	$25{,}992.74 \pm 749.55^{Ba}$	ND	ND	ND	104.89 ± 0.01^{Ab}
CCJ	282.42 ± 3.17^{Ab}	ND	27.66 ± 0.42^{Aa}	91.16 ± 0.02^{Ab}	ND
FCCJ	$24{,}049.94 \pm 30.28^{Bb}$	ND	ND	ND	122.73 ± 1.34^{Aa}

Different uppercase letters indicate significant differences before and after fermentation, and different lowercase letters indicate significant differences among different corn by-product species ($P < 0.05$). ND: not detected.

3.6. Metabolic profile analysis of CBJ and FCBJ

To better understand the effect of HY127 fermentation on the BABC-related active constituents of CBJ, we performed a non-targeted metabolomics analysis. To the best of our knowledge, this is the first attempt at non-targeted metabolomic analysis of CHJ, CSJ, and CCJ before and after fermentation. There are 17 HMDB classifications (Fig. 3A), mainly including lipids and lipid-like molecules, organic acids and derivatives, organoheterocyclic compounds, organic oxygen compounds, benzenoids, phenylpropanoids, and polyketides. Principal component analysis (PCA) was used to evaluate the metabolite profiles of CBJ and FCBJ. As shown in Fig. 3B and C, the single sample points were highly aggregated, indicating that the repeatability of the experiment was good. Moreover, the separation of the samples before and after fermentation was considerable, indicating that HY127 fermentation substantially affected the small-molecule active constituents of CBJ. The total number of metabolite species increased significantly after fermentation: CSJ from 2292 to 2640, CHJ from 2314 to 2729, and CCJ from 2028 to 2434 (Fig. S1). These results indicate that the fermentation of HY127 increased the number of small-molecule metabolites in CBJ.

3.7. Differential metabolites analysis

To observe metabolite changes in CBJ more intuitively, differential volcano plots were created (Fig. 4). After fermentation, 734 metabolites were upregulated (red points) and 360 were downregulated (blue points) in CHJ, 676 were upregulated and 315 were downregulated in CSJ, and 786 were upregulated and 426 were downregulated in CCJ. The quantity of upregulated metabolites was considerablely higher than that of downregulated metabolites. These results indicated that HY127

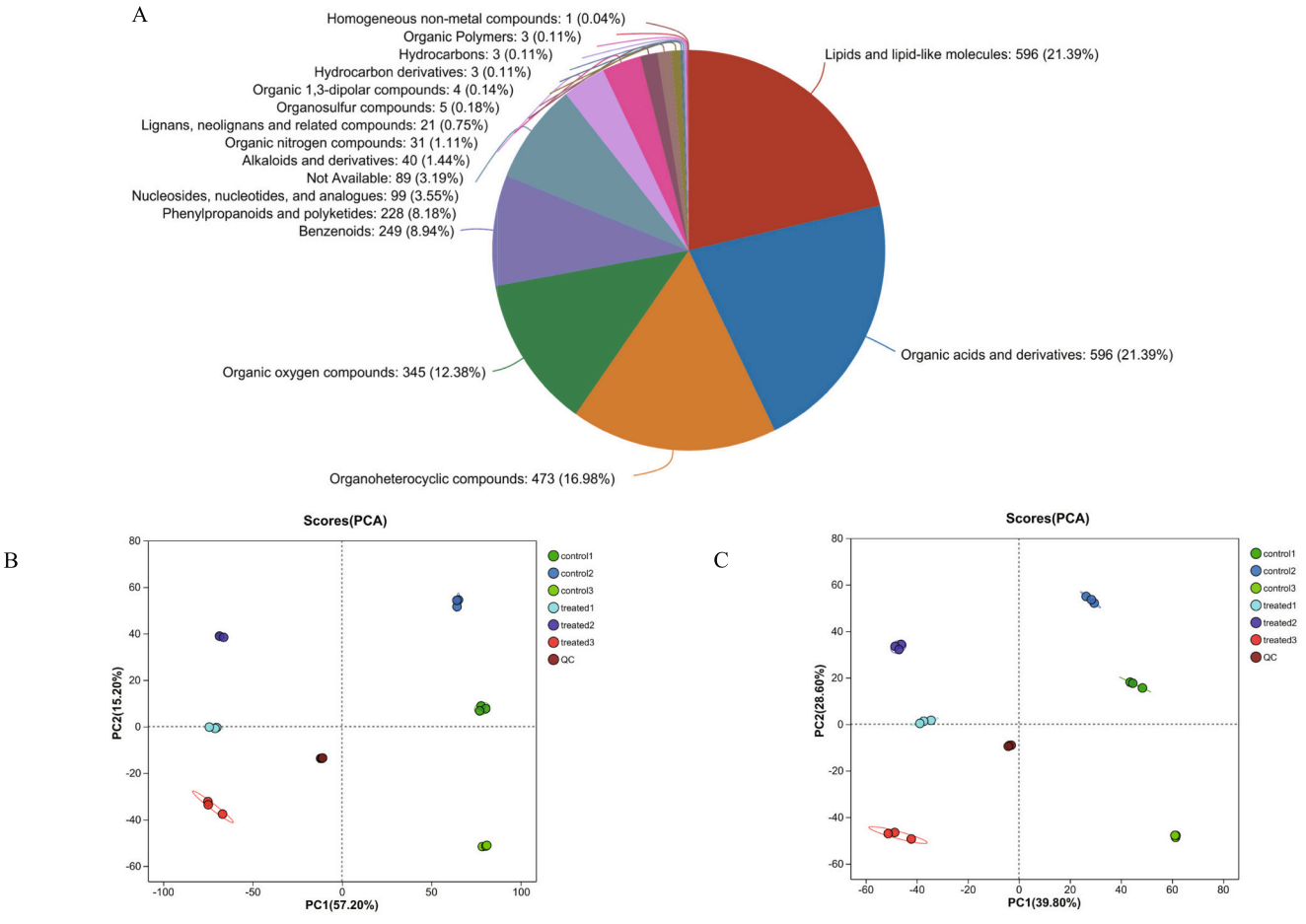

Fig. 3. Classification of total metabolites in corn by-products (A); PCA score plots of all metabolites in the ESI+ mode (B) and ESI− mode (C). Control 1: CHJ; Control 2: CSJ; Control 3: CCJ; Treated1: FCHJ; Treated2: FCSJ; Treated3: FCCJ; QC: quality control.

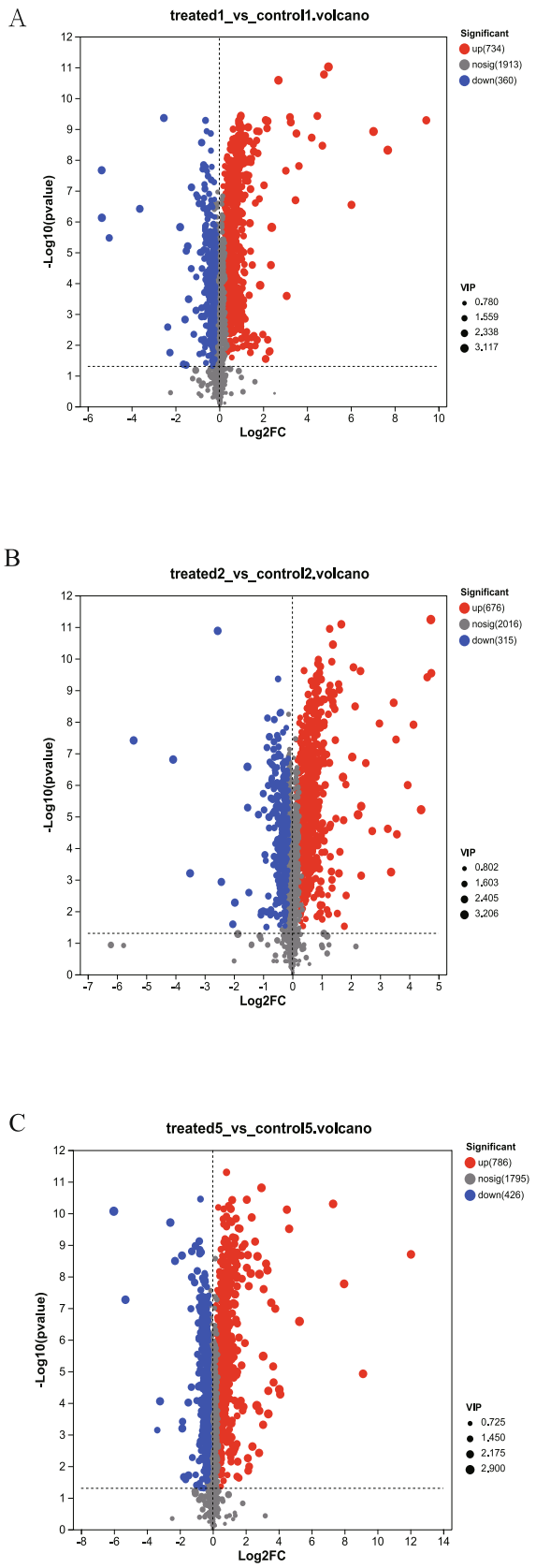

Fig. 4. Volcano plot of differential metabolites between CBJ and FCBJ. (A) CHJ and FCHJ. (B) CSJ and FCSJ. (C) CCJ and FCCJ.

fermentation increased the abundance of small-molecule metabolites in CBJ.

The upregulated bioactive metabolites primarily comprised amino acids, alkaloids, and terpenoids (Fig. 5). Amino acids accounted for the highest proportion of differential metabolites. Proteolytic enzymes secreted by LAB can hydrolyze proteins in corn by-products into amino acids (Li et al., 2023). For example, triple-bean soup fermented by *L. lactis* subsp. *lactis* YM313 and *Lactobacillus casei* YQ336 significantly increased amino acid content (Li et al., 2023). Similarly, *L. plantarum* fermentation of longan significantly increased phenylalanine, glycine, and isoleucine contents (Khan et al., 2018). Amino acids and their derivatives exhibit a variety of biological functions, including hypolipidemic and antioxidant activities (Kongo-Dia-Moukala et al., 2011; Li et al., 2021). Notably, hydrophobic amino acids can bind strongly to bile acids because of their strong interactions with lipids such as bile acids and cholesterol (Kongo-Dia-Moukala et al., 2011). Therefore, upregulated hydrophobic amino acids such as phenylalanine, glycine, isoleucine, lysine, and proline also promoted the enhancement of BABC in FCBJ.

The second category of differential metabolites comprised alkaloids. Corn by-products contain alkaloids that may be present in the form of glycosides (Feng et al., 2020). Glycoside hydrolase secreted by HY127 promotes the hydrolysis of alkaloid glycosides into alkaloids, and the organic acids produced by HY127 further promote hydrolysis. Alkaloids have been reported to have obvious therapeutic effects on hyperlipidemia and may regulate hyperlipidemia by participating in bile secretion and glyceride metabolism (Yang et al., 2022). Alkaloids such as yohimbine, lycorine, and baptifoline were upregulated in FCBJ. Therefore, the upregulated alkaloids may contribute to the enhancement of BABC in CBJ.

Terpenoids were the third category of differential metabolites. Corn by-products also contain terpenoids. Glycoside hydrolases produced by the fermentation of HY127 can also hydrolyze terpene glycosides into terpenes, and organic acids can further promote hydrolysis. Terpenoids have been shown to exhibit multiple biological activities, including BABC, antioxidant activities and anti-inflammatory (Wang, Liu, et al., 2022). Notably, terpenoids such as ginsenoside C—K, genipin, and valtrate were upregulated. Accordingly, these upregulated terpenoids may also contribute to the enhancement of the BABC of CBJ.

Compared with those in unfermented juice, downregulated proteins in the fermented juice were mainly nucleosides and monosaccharides, as HY127 utilizes compounds for growth and metabolism (Zheng et al., 2023).

Collectively, the increase in the abundance of numerous small-molecule active ingredients such as amino acids, alkaloids, and terpenes promoted an increase in the BABC of CBJ.

3.8. Key metabolic pathway analysis

A total of 116 KEGG pathways were identified via enrichment analysis of differentially expressed metabolites. Based on *p*-values, three pathways associated with BABC enhancement were screened from the top 20 KEGG pathways (Fig. S2), including the biosynthesis of various plant secondary metabolites, ABC transporters, and aminoacyl-tRNA.

Twenty-nine metabolites were found to be enriched in the biosynthesis of various plant secondary metabolites, mainly phenylpropanoids, amino acids, and terpenoids. Some substances are converted into active constituents that contain BABC; for example, prephenate is converted to the hydrophobic amino acid phenylalanine, and protopanaxadiol is converted to ginsenoside, which is upregulated during HY127 fermentation. The second KEGG pathway involved ABC transporters, with 26 metabolites mainly enriched in purine nucleosides and amino acids. Upregulated hydrophobic amino acids such as isoleucine, valine, and proline in this pathway suggest its importance in enhancing BABC activities in CBJ. In the 3rd KEGG pathway, 11 amino acids were enriched in aminoacyl-tRNA biosynthesis, and amino acids, such as isoleucine,

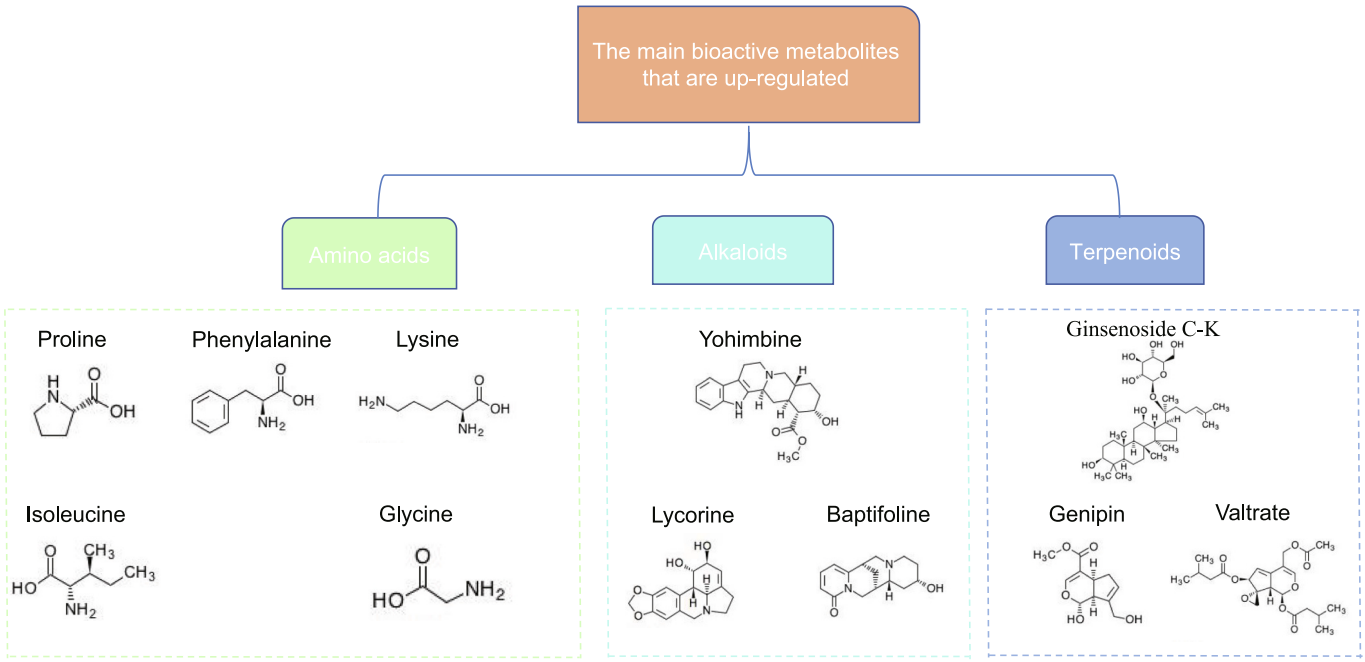

Fig. 5. Main bioactive metabolites that are upregulated.

lysine, and proline, were produced.

In addition, these metabolites were enriched in arginine and proline metabolism, lysine degradation, d-amino acid metabolism, cofactor biosynthesis, cyanoamino acid metabolism, and lysine biosynthesis. In summary, HY127 fermentation may produce bioactive constituents such as isoleucine, lysine, proline, yohimbine, and ginsenoside C—K through the above pathways, thereby enhancing the BABC of CBJ.

4. Conclusions

L. plantarum HY127 fermentation significantly enhanced the BABC of CBJ. Enhanced BABC activity was attributed to HY127 cells and their fermentation metabolites. The increased bioactive components from HY127 metabolism of corn by-products included polyphenols, flavonoids, polysaccharides, and organic acids. These changes in metabolic characteristics increased the number of small-molecule metabolites with BABC, such as hydrophobic amino acids, terpenoids, and alkaloids. Thus, HY127 cells played a major role in enhancing BABC. We hypothesize that the structure of the BSH and the cell wall peptidoglycan produced by HY127 promoted its binding to bile salts. Therefore, the enhancement of BABC in FCBJ was the result of the combined effect of HY127 cells and their bioactive metabolites. In summary, our study addresses a significant issue in human health, specifically hyperlipidemia, by exploring a novel approach to enhance BABC using L. *plantarum* HY127 fermentation of CBJ. These results not only contribute to advancing our understanding of LAB-fermented food products but also provide a research foundation for developing functional beverages aimed at managing hyperlipidemia.

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CRediT authorship contribution statement

Huanyong Lv: Writing – original draft, Validation, Project administration, Methodology, Data curation, Conceptualization. **Xiaohui Tang:** Writing – original draft, Methodology, Investigation. **Jian Zhang:** Writing – review & editing. **Menghan Ma:** Resources, Data curation. **Xinyi Li:** Visualization, Formal analysis. **Zhenjie Zheng:** Software, Formal analysis. **Yunhe Xu:** Writing – review & editing, Supervision, Funding acquisition. **Lili Zhang:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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