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Research Article

Prebiotic potential of enzymatically modified lingonberry polyphenols

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Abstract

In the present study, enzymatic glycosylation of polyphenolic compounds obtained from fruits of *Vaccinium vitis-idaea* L. (lingonberries) was performed. The individual components were identified by HPTLC and HPLC analysis. Fructosyltransferase – levansucrase L17 (FTF) was used for glycosylation purposes. To investigate the prebiotic potential of the glycosylated polyphenols, the metabolic profile of the probiotic strain *Lactobacillus plantarum* L10 was monitored in the presence of lyophilized lingonberry extract at concentrations ranging from 0.1 to 0.5%. An increase in lactate and acetate concentration was observed among the organic acids studied. The lactate values increased from 18% to 33% at lyophilizate concentrations of 0.1% and 0.5%, respectively. The same trend was observed for acetate, from 13% to 18%. Enzymatically modified polyphenols are very promising as prebiotics due to their ability to selectively modulate the gut microbiota and increase short-chain fatty acids (SCFAs) production, which is associated with improved human intestinal and systemic health.

Keywords

anthocyanins, enzymatic glycosylation, prebiotic potential, HPTLC, *Vaccinium vitis-idaea* L.

Introduction

The human intestinal tract is populated by a rich and dynamic bacterial ecosystem called the gut microbiota (GM), which plays a key role in host homeostasis. Multiple factors can affect this delicate balance, including genetics, age, antibiotics, and environmental factors, especially diet, causing a disturbance in the microbiota balance (dysbiosis) (Thursby and Juge 2017). Biotechnological advances made in recent years open good perspectives for improving the use of dietary polyphenols modulating GM in a wide range of diseases characterized by a dysbiotic phenotype (Kumar Singh et al. 2019; Yan et al. 2022).

Berries are dietary sources of polyphenols. The genus *Vaccinium* belongs to the family Ericaceae and includes over 450 species. In Bulgaria, four species are distributed in natural habitats. Lingonberry (*Vaccinium vitis-idaea* L.) is a small, evergreen shrub reaching a height of 5 to 25 cm. The plant species is associated with a spectrum of therapeutic properties such as antioxidant, antimicrobial, anti-inflammatory, anti-proteolytic, and anti-carcinogenic. These effects are based on the vitamins,

minerals, fiber, and phenolic compounds they contain (Pärnänen et al. 2024).

Phenolic compounds are one of the most abundant secondary metabolites in the plant kingdom. They are characterized by the presence of one or more aromatic rings and one or more hydroxyl groups in their chemical structure, ranging from that of a simple phenolic molecule to that of a complex high molecular weight polymer (gallotannins, catechin-type tannins, and lignins). Polyphenolic compounds have low bioavailability and extensive metabolism in the colon. This favors their interaction with intestinal microorganisms (Bian et al. 2020). There is a bidirectional interaction where polyphenols modulate the gut microbiota, and conversely, microorganisms can modulate the activity of phenolic compounds. This interaction can regulate the metabolism and bioavailability of polyphenols, converting them into metabolites that can affect host health (Kumar Singh et al. 2019).

The prebiotic potential of enzymatically modified polyphenols has become an intriguing area of research as polyphenols are known to influence the composition and function of the GM (Sayers et al. 2021). To overcome the low bioavailability, several different approaches have been developed aimed at improving the solubility and transport of polyphenols through the gastrointestinal tract and delivery to target intestinal regions. Intensive work on the glycosylation processes of various secondary metabolites has continued over the past decades. The glycosylation process combines sugar molecules with other low molecular weight biomolecules and natural small molecules, mainly related to secondary metabolites. The attachment of a sugar moiety to the corresponding aglycone results in a significant change in the activity of the molecules, which is crucial for their physicochemical properties as well as improving their pharmacokinetic parameters. Enzymatic modifications enhance the solubility, stability, and bioavailability of polyphenols (Thuan and Sohng 2013; Bié et al. 2023). Glycosides are more water-soluble than the corresponding aglycones, and increased hydrophilicity improves their activity, whereby they readily cross the cell membrane (Thuan and Sohng 2013). When enzymatically modified, polyphenols show altered structures that may boost their functional properties, including their ability to act as prebiotic agents. Polyphenols are metabolized by the gut microbiota into bioactive metabolites that can modulate the microbiome (Sejbuk et al. 2024). These metabolites often serve as substrates for beneficial bacteria, promoting their growth and activity.

Although polyphenols are currently recognized as modulators of gut microbiota composition, there is still no conclusive evidence for their prebiotic potential, and their mechanism of action has not been studied (Cueva et al. 2020). The ability of dietary polyphenolic compounds to stimulate the growth of probiotic microorganisms (*Lactobacillus* spp., *Bifidobacterium* spp., *Akkermansia* spp., *Roseburia* spp., and *Faecalibacterium* spp.) has been demonstrated based on some preclinical studies, but these studies are limited.

The present study aims to investigate the influence of enzymatically glycosylated polyphenols via levanzacharase L17 on the metabolic profile of probiotic lactobacilli strains.

Materials and methods

Plant material

The fruits of *Vaccinium vitis-idaea* L. (*Vitis idaeae fructus*) were collected after their full ripening from a floristic region: the Rhodope Mountains of Bulgaria – Yundola (42.0630°N, 23.8546°E) at 1400 m altitude. The fruits were gathered from the natural habitat in September during the vegetative season in 2022. The species was identified, and the herbarium specimen of the species is stored in the Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv. The collected fruits were cleaned of impurities before being frozen and stored at –79 °C (Lexicon II Ultra-low Temperature Freezer, Esco Lifesciences Group, Republic of Singapore, Malay Peninsula) until the time of analysis.

Chemicals and reagents

HPLC-grade methyl alcohol, acetonitrile, lactic, formic, acetic, propionic, butyric, isovaleric, and valeric acids were obtained from Merck (Darmstadt, Germany). Folin–Ciocalteu's phenol reagent, gallic acid, potassium chloride, sodium acetate, sodium carbonate anhydrous, potassium phosphate monobasic, potassium phosphate dibasic, ethanol, ethyl acetate, sulfuric acid, hydrochloric acid, formic acid, glacial acetic acid, 2-aminoethyl diphenylborinate, MRS (de Man-Rogosa-Sharpe) medium, lactose, and sucrose were purchased from Merck (Darmstadt, Germany). Standards: cyanidin chloride, kuromanin chloride (cyanidin-3-*O*-glucoside chloride), cyanidin-3-*O*-arabinoside, cyanine chloride (cyanidin-3,5-di-*O*-glucoside chloride), delphinidin chloride, myrtillin chloride (delphinidin-3-*O*-glucoside chloride), delphine chloride (delphinidin-3,5-di-*O*-glucoside chloride), malvidin chloride, oenin (malvidin-3-*O*-glucoside), malvidin-3-*O*-galactoside, malvin chloride (malvidin-3,5-di-*O*-glucoside chloride), peonidin chloride, peonidin-3-*O*-arabinoside, and petunidin-3-*O*-glucoside for HPLC were purchased from Extrasynthese (Lyon, France). Flavonol standards: rutin, myricetin, quercetin, and kaempferol were purchased from Merck (Darmstadt, Germany).

The enzyme (*β*-galactosidase) and substrate (*o*-nitrophenyl-*β*-D-galactopyranoside) were purchased from Megazyme.

Preparation of *Vaccinium vitis-idaea* L. extracts

Water extraction

The whole frozen fruits were blended with distilled water – a liquid/solid ratio of 8:1 (mL/g). The extraction process was

carried out using a bioreactor and an ultrasonic bath (Bandelin Sonorex, Berlin, Germany) at 35 °C for 20 min and filtered through nylon cloth. This procedure was repeated twice under the same conditions. Both extracts obtained were combined and concentrated using a Buchi/R-100 rotary evaporator at a temperature not exceeding 50 °C.

Organic solvent extraction

The organic solvent (ethanol): water: hydrochloric acid (70/30/1 v/v/v) mixture was used as the extraction solvent mixture. The ultrasonic bath extraction conditions and preparation of the stock solution were applied as in the water extraction section above.

Total phenolic content (TPC) assay

The Folin-Ciocalteu method was used to determine the total phenolic content, as described by Singleton and Rossi (Singleton and Rossi 1965). Measurements were performed on a UV–Vis Biochrom Libra S80PC Double Beam Spectrophotometer at 760 nm after incubation for 5 min at 50 °C. Gallic acid was used as the reference standard. The results were expressed as milligrams of gallic acid equivalent per 100 g of fresh fruits (mg GAE/100 g fw).

Total anthocyanin content (TAC) assay

The total anthocyanin content of the extracts was determined according to the pH differential method (Lee et al. 2005). The absorbance values were measured with a UV–Vis Biochrom Libra S80PC spectrophotometer at 520 and 700 nm. The results were expressed as mg cyanidin-3-glucoside equivalents per 100 g of fresh fruits (mg C3GE/100 g fw) using a molar extinction coefficient of 29 600.

High-performance thin-layer chromatographic (HPTLC) identification of anthocyanins

The analysis was carried out using a CAMAG system. To identify anthocyanins in *Vitis idaeae fructus* by HPTLC, the technique described by Bernal-Gallardo et al. (2021) with some modifications was applied (Bernal-Gallardo et al. 2021). Reference solutions were prepared by dissolving 1.1 mg of the standards of the anthocyanins tested in 1 mL methanol. A 10:1.1:1.1:2.7 (v/v/v/v) mixture of ethyl acetate, formic acid, glacial acetic acid, and water was used as the mobile phase for the separation of anthocyanins in lingonberry extracts. HPTLC Si 60 F_{254}, 20 cm × 10 cm (Merck, Darmstadt, Germany), was employed and developed in a CAMAG Automatic Developing Chamber ADC 2 (Muttenz, Switzerland). Standard solutions and extracts were applied to the plate using the CAMAG Linomat 5 semi-automatic module (Muttenz, Switzerland). Application volumes were as follows: 6.0 µL of the extracts and 2.0 µL of the standards were applied by injection to the plate at a constant application rate of 150 nl/s. The application was in the form of bands 9 mm long, with a distance between individual bands of 13 mm, a distance from the bottom edge of the plate of 8 mm, and a distance from the left edge of the plate of 20 mm. The development distance of the solvent front was 70 mm from the bottom edge of the plate and took 20 min. After development, the plate was air-dried for 5 min. The plate was heated on a TLC plate heater (CAMAG TLC Plate Heater 3) at 120 °C for 5 min and immediately derivatized. For derivatization, 2-aminoethyl diphenylborinate (2-APB) reagent was used (1 g 2-APB in 200 mL ethyl acetate). Images were documented using CAMAG TLC Visualizer 2, and the resulting data were processed with VisionCATS software under visible light, UV 366 nm.

High-performance liquid chromatography (HPLC) analysis of flavonols

The flavonol composition of the extracts was assessed by HPLC analysis using the chromatographic system (Shimadzu, Japan), which consists of an autosampler (Nexera X2, SIL-30AC), a column oven CTO-20AC, and an SPD-20A UV-Vis detector (Shimadzu, Japan). Analysis was carried out using a column Mediterranea Sea RP-18e (150 mm × 4.6 mm × 5 µm) (Teknokrom, Spain). The HPLC analysis was performed using an isocratic program as follows: 0–15 min, 46% A (methanol) to 12% B (acetonitrile) to 42% C (ultrapure-grade water), the flow rate of 0.5 mL/min, and the detection wavelength was 360 nm. The column temperature was maintained at 40 °C. Results were analyzed using Lab-Solution Nexera-XR-RF software. The samples were determined by the retention time of rutin, myricetin, quercetin, and kaempferol standards. The flavonol content was calculated using a standard curve constructed with their solutions at concentrations ranging from 300 µg/mL to 2.5 µg/mL of the corresponding flavonols with a correlation coefficient r2 > 0.9991.

High-performance liquid chromatography (HPLC) analysis of anthocyanins

Anthocyanins were identified and quantified using the chromatography system from the section above. Analysis was carried out using column Chromolith Performance RP-18e (100 mm × 4.6 mm × 2 µm) (Supelco). The program used was isocratic: A/B – 60/40 (v/v), with mobile phase A – formic acid: water in a ratio of 10:90 (v/v); mobile phase B – formic acid: methanol: water in a ratio of 10:40:50 (v/v/v); column temperature: 40 °C; mobile phase flow rate: 0.7 mL/min; analytical wavelength: 520 nm. The identification of the peaks was conducted by the retention times towards the standards of anthocyanins.

Glycosylation of isolated polyphenols by fructosyltransferase

Enzymatic glycosylation of phenolic components of *V. vitis-idaea* L. was performed under the following conditions: a quantitative ratio of solvent and raw plant material 1:1 (v/v), pH – 5.2, in the presence of 5% to 10% sucrose

and fructosyltransferase – levansucrase L17 (FTF) with activity in the final volume of the reaction mixture of at least 0.1 U/mL, enzyme reaction duration 24 h, T = 30 °C, and shaking 100 rpm/min (Iliev et al. 2018). After the expiration of the enzyme reaction, the latter was inactivated and the product was lyophilized.

Study of the prebiotic potential of lyophilized polyphenolic extracts after FTF reaction

To study the prebiotic potential of lyophilized polyphenolic products in concentrations of 10 ÷ 50 mg, we used the bacterial strain *Lactobacillus plantarum* L10 from the collection of the Department of Biochemistry and Microbiology at Plovdiv University, Bulgaria. The strain was incubated for 24 hours on MRS medium at 37 °C in the presence of *V. vitis-idaea* L. lyophilized extract (0.1%; 0.25%; 0.5%).

After the end of the fermentation process, at the end of the 24th hour and subsequent centrifugation at 9000 rpm for 15 min at 4 °C, the metabolic profile was monitored by elucidating the supernatants for short-chain fatty acids.

Short-chain fatty acids (SCFAs) assay

The SCFAs were determined on a high-performance liquid chromatography system, Kopik-Tech, UV Detector (Kopik-tech, λ = 210 nm). An Aminex HPX-87H 5 μm (250 mm × 4.6 mm) column was used, and an isocratic mobile phase containing 0.005 M H_2SO_4, a flow rate of 0.6 mL/min, and a column temperature of 40 °C. Standard curves were fitted with linear sections between concentrations (1.56 mmol/L–25 mmol/L) and with a correlation coefficient r2 > 0.999.

Enzymatic β-gaclactosidase (EC 3.2.1.23) activity of probiotic lactobacilli strains

The β-galactosidase activity was performed according to the method of Lim and Chae (Lim and Chae 1989), based on the amount of *o*-nitrophenol (oNP) released by the degradation of oNP-β-D-galactopyranoside substrate. The reaction mixture contained 250 μL of 5.5 mmol/L oNP-β-D-galactopyranoside substrate in 50 mmol/L KH_2PO_4 buffer (pH 6.8), 100 μL of the bacterial lysate, and 100 μL of distilled water. The mixture was incubated for 20 min at 37 °C. The reaction was stopped by the addition of 2 mL of 1 mol/L Na_2CO_3. The released oNP was measured spectrophotometrically at 405 nm.

Results and discussion

Total phenols and total anthocyanins content

The accumulation of secondary metabolites in plants is highly dependent on several factors, such as climate and other environmental conditions, and the ontogenesis of the plant species. The measured values of total polyphenols for wild lingonberry from Bulgaria were relatively close in water and ethanol extraction, 262.4 mg GAE/100 g fw and 207.5 mg GAE/100 g fw, respectively (Table 1). However, the values of total anthocyanins in water extraction were more than 2 times higher than those in organic extraction, 14.5 mg C3GE/100 g fw and 6.5 mg C3GE/100 g fw, respectively. Water appears to be a better extractant for total monomeric anthocyanins as water-soluble pigments. Some authors report much higher amounts for the TPC and TAC contents in the range as follows: phenolic compounds (477–775 mg/100 g fw), anthocyanins (20–57 mg/100 g fw) (Urbonaviciene et al. 2023). The data are for four regions in Northern Europe (Norway, Finland, Latvia, and Lithuania).

Table 1. Total polyphenols and total monomeric anthocyanins in fruits of *V. vitis-idaea* L.

	Vitis idaeae fructus	
Type of extractant	**Total Phenolic Content (mg GAE/100 g fw)**	**Total Anthocyanin Content (mg C3GE/100 g fw)**
Water	262.4 ± 19.6	14.5 ± 1.4
Ethanol	207.5 ± 14.7	6.5 ± 0.6

Data are presented as means of three analyses ± SD.

HPTLC identification of anthocyanins

Concerning the standards used for anthocyanins: cyanidin-3-glucoside chloride (kuromanin chloride) had an Rf ~ 0.43; cyanidin-3-arabinoside, an Rf ~ 0.50; and delphine chloride, an Rf ~ 1.1. Cyanidin-3-arabinoside and delphinidin-3,5-diglucoside chloride (delphine chloride) were identified in aqueous ultrasonic extract. Cyanidin-3-glucoside chloride and delphinidin-3,5-diglucoside chloride were identified for the aqueous extract obtained using a bioreactor. The same anthocyanins determined in the aqueous ultrasonic extract were found in the ethanolic extract. In all tested extracts, delphine chloride was present as a diglucoside of delphinidin as well as cyanidin glycosides, arabinoside, and glucoside, respectively. Lee and Finn identified for each of the five samples examined the presence of three anthocyanins: cyanidin-3-galactoside, cyanidin-3-glucoside, and cyanidin-3-arabinoside (Lee and Finn 2012). Lingonberries contain predominantly cyanidin-based anthocyanins, as found in other studies (Ek et al. 2006; Lätti et al. 2011). Fig. 1 presents profiles of the three detected anthocyanins and the tested extracts.

Glycosylation of isolated polyphenols by fructosyltransferase

Enzymes such as glycosyltransferases and fructosyltransferases are used to modify polyphenols (Andreu et al. 2023; Tarasov et al. 2023). According to the carbohydrate-active enzyme classification system, bacterial FTFs belong to the glycoside hydrolase family 68 (GH68) (Moulis et al. 2021). The enzyme fructosyltransferase (FTF, EC 2.4.1.10) cleaves the glycosidic bond of the fructosyl donor molecule (sub-

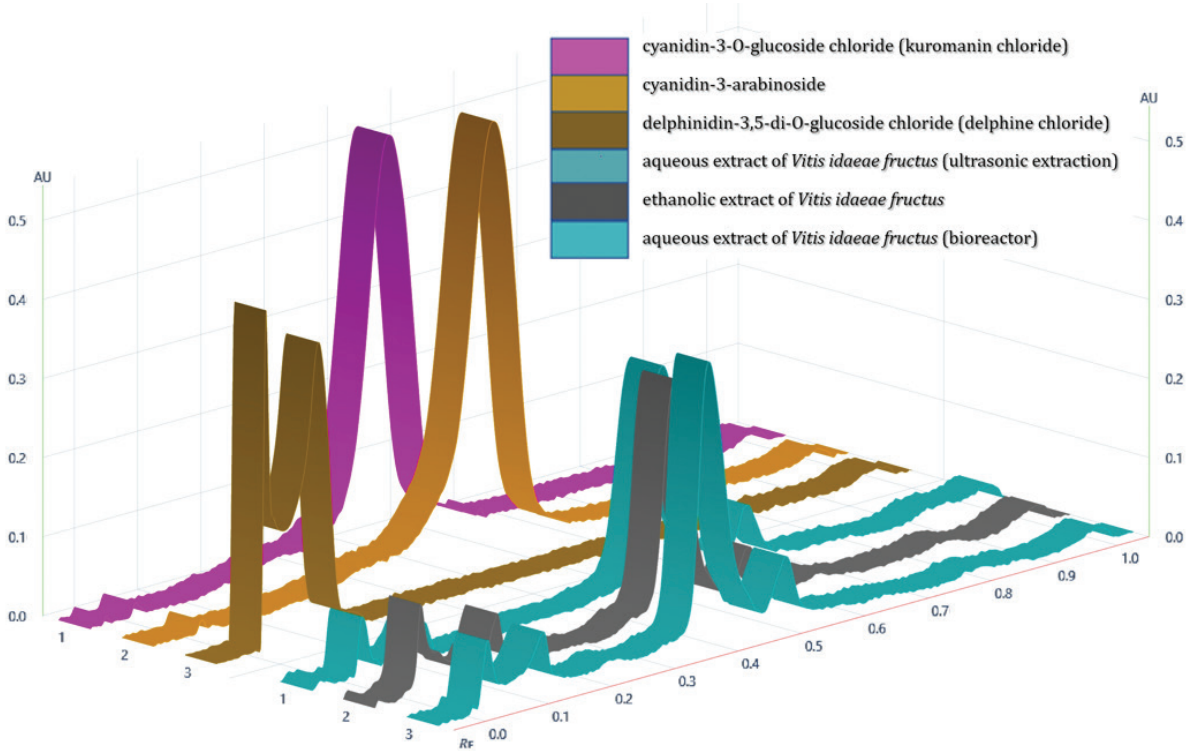

Figure 1. HPTLC profiles of standard solutions of anthocyanins (cyanidin-3-glucoside chloride, cyanidin-3-arabinoside, and delphinidin-3,5-diglucoside chloride) and test solutions – lingonberry extracts (aqueous – ultrasound, ethanol – ultrasound, and aqueous – bioreactor) under UV 366 nm after derivatization with 2-aminoethyl diphenylborinate.

strate, i.e., sucrose, raffinose, stachyose, verbascose) and uses the released energy to bind the fructose moiety to the growing fructan chain, but also to sucrose or to another acceptor molecule (Meng and Fütterer 2003; Tieking et al. 2003).

In our study on enzymatic modification of polyphenols in an aqueous extract of *V. vitis-idaea* L., we used fructosyltransferase (Bivolarski et al. 2013; Iliev et al. 2018). FTF catalyzes three distinct reactions depending on the fructosyl acceptor molecule: polymerization (using the growing fructan chain as an acceptor), transfructosylation (using monosaccharides, disaccharides, or oligosaccharides as acceptors), and hydrolysis (using water as an acceptor) (Li et al. 2015). Optimal conditions for glycosylation of isolated polyphenols of the genus *Vaccinium* using FTF were established. Our results show that when we monitored the concentration of the three anthocyanins with the highest concentration in the aqueous extract, only delphinidin-3,5-diglycoside increased after enzymatic reaction in the presence of FTF (Fig. 2).

The formation of a triglycoside with the attachment of an additional fructose residue can be assumed. The ratio of delphinidin-3,5-diglucoside, cyanidin-3-glucoside, and cyanidin-3-arabinoside before and after the fructosyltransferase reaction was 10.6:1:1.4 and 11.5:1:1.5, respectively. On monitoring the change in concentration of rutin, myricetin, and quercetin under the conditions of the fructosyltransferase reaction studied, an increase in rutin concentration was found (Table 2). This may be the result of the glycosylation of quercetin.

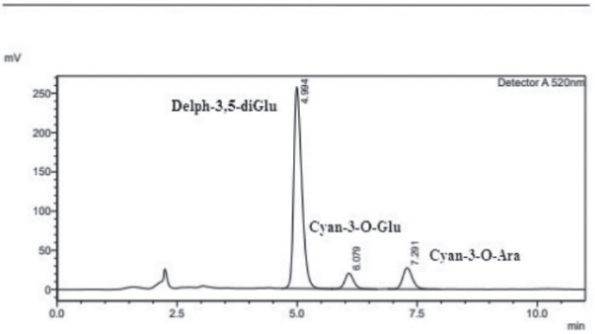

Figure 2. HPLC chromatogram of anthocyanins in aqueous extract of *Vitis idaeae fructus* after enzymatic reaction with FTF.

Metabolic profile of probiotic strain *Lactobacillus plantarum* L10 cultured in the presence of lyophilized extract of *V. vitis-idaea* L. after enzymatic reaction with FTF

A bacterial strain *Lactobacillus plantarum* L10, incubated for 24 h on modified MRS with 4% lactose and lyophilized extract of *V. vitis-idaea* L. in a concentration of 0.1% ÷ 0.5% at 30 °C with agitation, was used to investigate the prebiotic potential of lingonberry extract. The control contained no extract but only *L. plantarum* L10 in mMRS medium and 4% lactose. The metabolic products in the resulting supernatant were determined and analyzed by HPLC. The data are presented in Table 3.

Table 2. Chromatographic data of flavonols in aqueous extract of *Vitis idaeae fructus* before and after enzymatic reaction with FTF.

	Rutin	Myricetin	Quercetin	Kaempferol
Ret. Time (min.)	4.013/4.153	4.720/5.094	6.175/6.187	8.244/8.323
Area (%)	3.131/15.029	21.188/28.951	0.060/0.390	0.054/0.580

*The first data are obtained before the enzymatic reaction with FTF.

Table 3. Content of short-chain fatty acids at 24-hour fermentation of *L. plantarum* L10 in the presence of lyophilized *V. vitis-idaea* L. extract.

Short-chain fatty acids (SCFAs)	Concentrations, mmol/L			
	Control	0.1%	0.25%	0.5%
Lactic	129.0	153.0	164.2	172.0
Formic	4.9	5.1	4.0	4.4
Acetic	49.5	56.7	63.2	64.4
Propionic	41.0	0	0	0
Isovaleric	3.1	3.1	0	0
Valeric	11.8	12.2	4.3	3.7

Among the organic acids examined during cultivation in the presence of the lyophilized extract of *V. vitis-idaea* L., an increase in lactate and acetate concentration was observed. The lactate values increased from 18% to 33% at lyophilisate concentrations of 0.1% and 0.5%, respectively. The same trend was observed for acetate, but from 13% to 18%.

It is important to note that the ratio of lactate to acetate does not change with the increasing percentage of lyophilized extract added and is in the range of 2.5:1.0 to 3.0:1.0. The cell growth of the tested strain *L. plantarum* L10 in the presence of the lyophilized extract of *V. vitis-idaea* L. was not inhibited, and, regardless of the lyophilization concentration, the cell concentration reached 5×10^8 after 24 hours of fermentation (data not shown). This proves that enzymatically modified polyphenols have no inhibitory effect on cell growth. Since 4% lactose was present in the culture medium of the probiotic strain *L. plantarum* L10, we examined to what extent the lyophilized *V. vitis-idaea* L. extract inhibited β-galactosidase activity (Table 4). The data showed that enzyme activity was not affected regardless of the concentration of lyophilized extract of *V. vitis-idaea* L., which correlated with the cell biomass data.

Table 4. Enzyme activity profile of probiotic strain *L. plantarum* L10 during the fermentation process in the presence of the lyophilized extract of *V. vitis-idaea* L.

	β-Galactosidase activity (U/mL)			
	Lyophilized *V. vitis-idaea* L. extract			
	Control	0.1%	0.25%	0.5%
6h	0.50	0.46	0.43	0.43
12h	0.38	0.41	0.38	0.40
24h	0.30	0.26	0.23	0.23

Although the exact mechanisms need further elucidation, preclinical and clinical data suggest that dietary polyphenols have prebiotic properties and exert antimicrobial effects against pathogenic microorganisms inhabiting the intestinal tract. The mechanisms of prebiotic action are obviated by the fact that modified polyphenols are more readily fermented into SCFAs such as acetate and butyrate, which improves gut health. In addition, enzymatic modification can create structures that selectively enhance the growth of beneficial bacteria, inhibiting pathogenic ones. Enhanced bioactive metabolites from enzymatic processes reduce intestinal inflammation, indirectly maintaining the balance of the microflora. Increased diversity of the gut microbiome, improved intestinal barrier function, reduced inflammation, and oxidative stress in the gut can be highlighted as health benefits (Zhang 2022). All of this also refers to systemic health benefits, including improved metabolic and cardiovascular health. Examples of positive enzymatic modification of particular classes of phenolic compounds include, for example, the enzymatically glycosylated flavonoids, which have shown superior prebiotic potential compared to their native forms. Anthocyanins, as a subclass of flavonoids, have shown improved modulation of gut bacteria, enhancing the production of SCFAs. Enzymatic esterification of phenolic acids ameliorates their solubility and microbiota-accessible fractions (Andreu et al. 2023). The prebiotic effect of any phenolic compound may be influenced by the food source and chemical structure of the compound, along with individual differences in the composition of the gut microbiota (Serreli and Deiana 2019).

Conclusion

In conclusion, enzymatically modified polyphenols are very promising as prebiotics due to their ability to selectively modulate the gut microbiota and increase SCFAs production, which is associated with improved human intestinal and systemic health. However, further studies are needed to confirm their effects in different populations.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Author contributions

Conceptualization, D.K.-B., I.I. and M.N.; methodology, D.K.-B., M.N., I.I., and T.V.; software, D.K.-B.; investigation, D.K.-B., N.B. and K.I.; data curation, D.K.-B., K.I. and N.B; writing – original draft preparation, D.K.-B. and I.I.; writing – review and editing, K.I., N.B. and I.I.; visualization, D.K.-B. and M.N.; supervision, K.I. and I.I. All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.

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