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OPTIMIZATION OF PREBIOTIC RATIO FOR IMPROVED BSH ACTIVITY OF *ENTEROCOCCUS FAECALIS* CGZ3

Koushik Koujalagi[1], *Dr Alok Malviya*[2*]

Address(es): *Dr Alok Malviya*, Associate professor,
[1] Christ (Deemed to be University), Research scholar, Department of Life Sciences, Bangalore, Karnataka, India, 560029.
[2] Christ (Deemed to be University), Associate professor, Department of Life Sciences, Bangalore, Karnataka, India, 560029.

*Corresponding author: alokmalviya@gmail.com

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ABSTRACT

This study aimed to optimize prebiotic formulations to enhance bile salt hydrolase (BSH) activity in *Enterococcus faecalis* CGz3, a promising probiotic strain, using a one-factor-at-a-time (OFAT) approach followed by response surface methodology (RSM). Prebiotics such as *Taraxacum officinale* (dandelion) root extract, inulin, fructooligosaccharides, sorbitol, and maltitol were tested for their effects on BSH activity. A Box-Behnken design identified an optimal blend of sorbitol (0.4 mg/mL, v/v), maltitol (0.2 mg/mL), and dandelion root extract (0.5 mg/mL), achieving a BSH activity of 182.5 mg/mL. Dandelion root extract was prepared via Soxhlet extraction at 80°C using polar and non-polar solvents and analyzed for total phenolic content (TPC), total carotenoid content (TCC), and antioxidant capacity via the DPPH assay. Gas chromatography-mass spectrometry (GC-MS) detected 25 bioactive compounds, and high-performance liquid chromatography (HPLC) quantified chlorogenic acid, a potent polyphenol. These findings highlight the potential of plant-based prebiotics in synbiotic development and provide a novel approach for optimizing prebiotic ratios to support cholesterol metabolism through BSH activity.

Keywords: Bile Salt Hydrolase (BSH), chlorogenic acid, optimization, prebiotics, and Response Surface Methodology (RSM)

INTRODUCTION

Prebiotics boost probiotic viability and efficiency, enhancing gut health (established health benefits). Prebiotics boost BSH enzyme activity, which boosts probiotic efficiency. To be effective, prebiotics need to be selectively utilised by beneficial microbes once they reach the large intestine, since they are not hydrolysed or absorbed in the upper GI tract (stomach and small intestine) **(Al-Sheraji et al., 2013; Slavin, 2013)**. Inulin, maltitol, sorbitol, and fructooligosaccharides are some of the most popular prebiotics; however, there are many more options. The functional benefits of probiotics can be maximised by optimising the ratio of combined prebiotics, which efficiently support the growth and metabolic activity of probiotics. Synbiotic formulations are necessary to optimise gastrointestinal function and gut health **(Markowiak & Śliżewska, 2017)**. Prebiotics significantly impact gut microbiota composition and metabolism. Trillions of gut microbiota microbes—including yeast and bacteria—are essential for digestive health and function.

This study optimizes prebiotic formulations to boost bile salt hydrolase (BSH) activity in *Enterococcus faecalis* CGz3, a potential probiotic, to reduce serum cholesterol. BSH enzymes hydrolyze conjugated bile salts (e.g., glycocholate, taurocholate) into free bile acids and amino acids, reducing their reabsorption in the gut. This increases fecal bile acid excretion, prompting hepatic cholesterol conversion to bile acids via 7α-hydroxylase (CYP7A1), thereby lowering circulating cholesterol **(Jones et al., 2012)**. Inulin and fructooligosaccharides (FOS), long- and short-chain β-fructans, are fermented by gut bacteria like *Bifidobacterium* and *Lactobacillus*, producing short-chain fatty acids (SCFAs) such as acetate and butyrate. These SCFAs upregulate BSH gene expression and enhance bile acid deconjugation, reducing cholesterol absorption **(Slavin, 2013)**. Maltitol and sorbitol, polyol prebiotics, are fermented by gut microbiota into SCFAs, which modulate gut pH and stimulate BSH-producing bacteria, indirectly promoting cholesterol catabolism **(Makki et al., 2018)**. Additionally, SCFAs from maltitol and sorbitol activate peroxisome proliferator-activated receptor (PPAR) pathways, enhancing cholesterol metabolism **(den Besten et al., 2013)**. *Taraxacum officinale* (dandelion) root extract, rich in inulin and bioactive polyphenols like chlorogenic acid, supports BSH activity by fostering probiotic growth and reducing oxidative stress. Chlorogenic acid inhibits cholesterol absorption by downregulating Niemann-Pick C1-like 1 (NPC1L1) protein expression in enterocytes and exerts anti-inflammatory effects via the TLR2/NF-κB pathway, further supporting cardiometabolic health . This study leverages a one-factor-at-a-time (OFAT) approach and response surface methodology (RSM) to optimize prebiotic blends for synbiotic formulations, aiming to enhance BSH activity and cholesterol reduction.

MATERIALS AND METHODS:

Plant materials and extraction procedure:

Dandelion roots (*Taraxacum officinale*) were obtained from Indian Jadibooti Pvt Ltd., a commercial supplier. A 25-gram sample of the roots was pulverized into a fine powder, stored in an airtight container to maintain dryness, and subjected to Soxhlet extraction. The extraction was conducted at 80°C using distilled water, ethanol, and hexane as solvents, with the process spanning six hours and encompassing seven cycles. The resulting extract volumes were measured, and the samples were stored at 4°C for subsequent analysis and characterization.

Characterization of plant extract

Determination of radical scavenging activity by DPPH assay

DPPH: The antioxidant capacity was evaluated using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay. Thirty microliters of extract were diluted with methanol to 3 mL, mixed with 0.004% (v/v) DPPH solution, and incubated in darkness for 30 minutes. After vortexing for 10 seconds, absorbance was measured at 517 nm using a UV-Vis spectrophotometer (N. Petkova et al., 2017).

$$Percentage\ inhibition = [Abs\ of\ control - Abs\ of\ sample/\ abs\ of\ control] * 100$$

Estimation of total carbohydrate content

Standard glucose solutions (200 µg/mL) were diluted with distilled water to 2 mL, mixed with 3 mL of anthrone reagent, and incubated in a boiling water bath for 8 minutes. After rapid cooling, absorbance was measured at 630 nm. TCC was calculated using a glucose calibration curve (Fayyazi et al., 2024).

Estimation of total phenolic content using the Fio-Ciocalteu reagent method

The Stintzing et al. method was adapted for TPC determination. Dandelion extract was added to the Folin-Ciocalteu reagent (1:1) and 0.8 mL of 7% Na_2CO_3 solution. Absorbance was recorded at 765 nm following 20-minute incubation in darkness (N. Tr. Petkova et al., 2015). The total phenolic content (TPC) of the samples was

quantified and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Estimating reducing sugars using the dinitrosalicylic acid (DNSA) method

A 0.4 mL aliquot of aqueous root extract was mixed with 1.2 mL of 3,5-dinitrosalicylic acid (DNSA) reagent in a centrifuge tube and incubated at 100°C for 10 minutes to trigger a colorimetric reaction. Absorbance was measured at 540 nm, and reducing sugar content was determined as 1 mg/mL using a glucose calibration curve (Jain et al., 2020).

Phytochemical profiling

HPLC and GC-MS

Chlorogenic acid was quantified using a C18 column (4.6 mm × 250 mm) in isocratic mode with a 60:40 (v/v) acetonitrile-water mobile phase at 1 mL/min. Samples and reference chlorogenic acid (0.4 mg/mL) were dissolved 1:1 in the mobile phase, and 20 µL was injected. Absorbance was measured at 331 nm. The quantification of the chlorogenic acid was done using the formula:

$$\frac{Sample\ area \times standard\ amount \times dilution\ of\ sample \times Mean\ weight}{Standard\ area \quad dilution\ of\ standard \quad sample\ amount}$$

Gas chromatography-mass spectroscopy (GC-MS)

A fused silica HP-5MS column (30 m × 0.25 mm ID × 250 µm, 5% biphenyl, 95% dimethylpolysiloxane) was used with helium as the carrier gas at 2 mL/min. One microliter of extract (1 mg/mL) was injected at 280°C. The oven temperature was held at 100°C for 2 minutes, ramped to 200°C at 10°C/min, then to 300°C at 25°C/min, and held for 10 minutes. Mass spectrometry settings included a 230°C ion source, 250°C inlet line, 70 eV ionization, and scans from 40 to 600 Da. Compounds were identified using the NIST (2014) library

Single factor analysis of inulin, maltitol, sorbitol, and fructooligosaccharides and *T. Officinale* root powder extract on bile salt hydrolase activity of *E. Faecalis* cgz 3

The effects of inulin, maltitol, sorbitol, fructooligosaccharides, and dandelion root extract (0.1–0.5 mg/mL) on BSH activity in *E. faecalis* CGz3 were tested. Substrates included 0.1% sodium glycocholate and 1% inoculum (OD600 = 1). Mixtures were incubated at 37°C and 120 rpm, sampled at 0 and 16 hours, and cholic acid levels were measured using a calibration curve.

Optimization of the prebiotic ratio by response surface methodology

Box-Behnken design: A Box-Behnken design evaluated sorbitol (A), maltitol (B), and dandelion root extract (C) at low (−1), medium (0), and high (1) levels across 17 runs. BSH activity was measured at 16 hours via cholic acid release, and data were analyzed using ANOVA (95% confidence level).

Table 1 3-factorial Box-Behnken design for prebiotic optimization.

Std	Run	A:Sorbitol	B:Maltitol	C:DRE
8	1	0.7 (1)	0.4 (0)	**0.5 (1)**
3	2	0.1 (-1)	0.6 (1)	**0.3 (0)**
10	3	0.4 (0)	0.6 (1)	**0.1 (-1)**
11	4	0.4 (0)	0.2 (-1)	**0.5 (1)**
9	5	0.4 (0)	0.2 (-1)	**0.1 (-1)**
1	6	0.1 (-1)	0.2 (-1)	**0.3 (0)**
17	7	0.4 (0)	0.4 (0)	**0.3 (0)**
12	8	0.4 (0)	0.6 (1)	**0.5 (1)**
13	9	0.4 (0)	0.4 (0)	**0.3 (0)**
4	10	0.7 (1)	0.6 (1)	**0.3 (0)**
16	11	0.4 (0)	0.4 (0)	**0.3 (0)**
15	12	0.4 (0)	0.4 (0)	**0.3 (0)**
5	13	0.1 (-1)	0.4 (0)	**0.1 (-1)**
14	14	0.4 (0)	0.4 (0)	**0.3 (0)**
7	15	0.1 (-1)	0.4 (0)	**0.5 (1)**
6	16	0.7 (1)	0.4 (0)	**0.1 (-1)**
2	17	0.7 (1)	0.2 (-1)	**0.3 (0)**

Bile salt hydrolase (BSH) assay

BSH activity was assessed using furfural. Cell-free supernatant (0.1 mL) from 0 and 16 hours was mixed with 0.1 mL of 1% (v/v) furfural and 16N H_2SO_4, incubated at 65°C for 15 minutes, and cooled in an ice bath for 85 seconds. A color shift from light brown to blue confirmed cholic acid production (Irvin et al., 1944).

Statistical analysis

Data were expressed as means ± standard deviations from three replicates. Significance ($p < 0.05$) was determined using one-way ANOVA with Duncan's Multiple Range Test in IBM SPSS (version 27.0).

RESULT

Characterization of root extracts: total carbohydrate content, total phenolic content, reducing sugars, and antioxidant activity (DPPH assay)

The aqueous extract showed the highest TPC (5.27 ± 5.74 mg GAE/g) compared to 95% ethanol (1.01 ± 0.64 mg GAE/g) and 60% ethanol (0.83 ± 1.00 mg GAE/g) extracts. TCC was highest in the aqueous extract (2.81 ± 0.07 g/L), followed by 95% ethanol (2.61 ± 0.04 g/L) and 60% ethanol (2.1 ± 0.75 g/L). The DPPH assay indicated superior antioxidant activity in the aqueous extract (82.5 ± 2.11% inhibition) compared to 95% ethanol (12.26 ± 1.06%) and 60% ethanol (18.39 ± 0.33%). Reducing sugar content in the aqueous extract was 9.03 ± 0.26 mg/mL.

Table 2 Characterization of *T. officinale* root extract. *DRE: Dandelion root extract.

Sample	Solvent	Total carbohydrate content (g/L)	TPC, mg GAE/g	Chlorogenic acid (mg/mL)	DPPH (% inhibition)
DRE	**Water**	2.81±0.07	5.27±5.74	0.14	82.5±2.11
	Ethanol (95%)	2.61±0.04	1.01±0.64	0.15	12.26±1.06
	Ethanol (60%)	2.1±0.75	0.83±1	0.14	18.39±0.33

Phytochemical profiling

HPLC

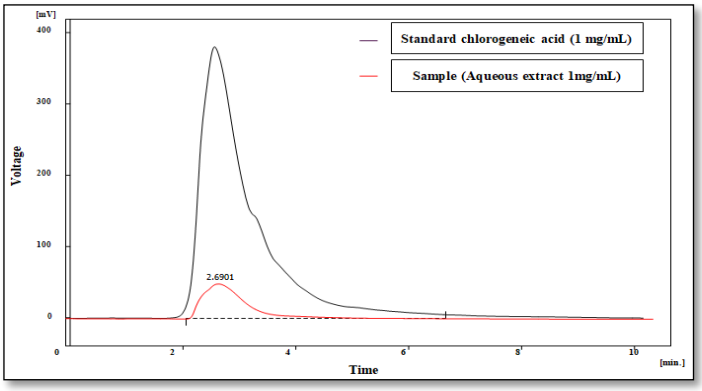

Figure 1 HPLC chromatograms of *T. officinale* root extract, chlorogenic acid peak standard (black); sample (red).

HPLC analysis quantified chlorogenic acid at 0.14 mg/mL (aqueous) and 0.15 mg/mL (ethanol) (**Table 2, Figure 4**).

Gas chromatography-mass spectroscopy

Phytochemical screening of the non-polar (hexane fraction) of *Taraxacum officinale* root extracts revealed the presence of 25 bioactive phytochemicals of significance **(Table 3).** The analysis identified 4-Methyl-2,6-dihydroxyquinoline as one of the major compounds, known for its anti-parasitic, anti-amoebic, and inotropic properties, with chronotropic activity (Majeed et al., 2023; Sadeghian et al., 2009a), p-tert-Butyldihydrocinnamaldehyde was identified as a significant compound, recognized for its antimicrobial, antioxidant, anti-diabetic, anti-cancer, and anti-inflammatory properties (Kumar & Parle, 2019; Wang et al., 2009a; Yang et al., 2015b; Zhu et al., 2017a) along with other major compounds with potential health benefits.

Table 3 Bioactive molecules hexane extract. *NA: No literature available on bioactivity; NF: No Bioactivity found.

RT	Compound name	Mol. formula	Probability %	Therapeutic benefits	References
5.3	Undecane	$C_{11}H_{24}$	9.76	Anti-allergic and anti-inflammatory on mast cells and keratinocytes	(Anti-inflammatory, 2020)
5.3	2,5,6-Trimethyldecane	$C_{13}H_{28}$	7.67	NF	NA
5.3	Nonane, 4,5-dimethyl	$C_{11}H_{24}$	5.57	NF	NA
5.3	Hexane octyl ether	$C_{14}H_{30}O$	3.48	NF	NA
5.3	Farnesane	$C_{15}H_{32}$	3.35	Antibiotic activity	(Masi et al., 2021)
5.3	5,6-Dimethyldecane	$C_{12}H_{26}$	2.96	NF	NA
5.3	n-Dodecane	$C_{12}H_{26}$	2.73	Antibacterial activity	(Padma et al., 2019)
8.3	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	58.2	NF	NA
8.3	2,2'-Ethylidenebis (5-methylfuran)	$C_{12}H_{14}O_2$	9.91	NF	NA
8.3	p-tert-Butyldihydrocinnamaldehyde /	$C_{13}H_{18}O$	5.11	Antimicrobial, antioxidant, anti-diabetic, anti-cancer, anti-inflammatory	(Nitish Kumar & Amrita Parle, 2019; Wang et al., 2009b; Yang et al., 2015a; Zhu et al., 2017b)
8.3	4-Methyl-2,6-dihydroxyquinoline	$C_{10}H_9NO_2$	1.54	Anti-parasitic/anti-amoebic, inotrophic and chronotropic activity	(Majeed et al., 2023; Mansouri et al., 2008; Sadeghian et al., 2009b)
11.4	Tetradecane	$C_{14}H_{30}$	10.6	Anti-microbial	(Sayed et al., 2022)
11.4	Hexadecane	$C_{16}H_{34}$	5.03	Anti-bacterial and anti-oxidant properties	(Yogeswari et al., 2012)
14.0	Heptadecane	$C_{17}H_{36}$	5.03	Anti-inflammatory, Anti-fungal, anti-oxidative	(Amudha et al., 2018; Kim et al., 2013)
15.9	Dibutyl phthalate (Genoplast B)	$C_{16}H_{22}O_4$	9.35	Anti-bacterial activity; anticancer, antitumor, fat burning, Hormone Balancing, Increase Vitamin K, D, Zinc and calcium Bioavailability	(Mini Shobi & Gowdu Viswanathan, 2018)
15.9	Phthalic acid	$C_{18}H_{26}O_4$	5.66	Antimicrobial activity	(Huang et al. 2021; M. Rowshanul Habib and M. Rezaul Karim 2009)
17.7	Eicosane	$C_{20}H_{42}$	6.70	Anti-inflammatory, analgesic, antipyretic, antiulcer, antifungal and wound healing	(Bhat et al., 2024; Chuah et al., 2018; OKECHUKWU, 2020; Wu et al., 2024)
17.7	Heneicosane	$C_{21}H_{44}$	5.66	Microbicidal	(Vanitha et al., 2020)
17.7	Heptacosane	$C_{27}H_{56}$	4.78	Overcoming multidrug resistance	(Labbozzetta et al., 2022)
17.7	Tetracosane	$C_{24}H_{50}$	3.56	Antioxidant and cytotoxic activities	(Paudel et al., 2019)
17.7	Pentadecane	$C_{18}H_{38}$	3.01	Anti-inflammatory, analgesic, antipyretic	(Bruno et al., 2015; Okechukwu, 2020)
21.1	(2E,6E,10E)-3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraenyl acetate	$C_{22}H_{36}O_2$	7.91	NF	NA
21.1	β-D-Mannofuranoside, farnesyl-	$C_{21}H_{36}O_6$	6.99	Antibacterial activity	(Altayb et al., 2022)
21.1	Farnesol formate	$C_{16}H_{26}O_2$	5.01	Antibiotic adjuvant; precursor to anticancer agents	(Akiyama et al., 2002; Chandran et al., 2011; Goldberg et al., 2009; Inoue et al., 2004; Jabra-Rizk et al., 2006)

Single factor analysis: Effect of prebiotics and plant root extract on the BSH activity of *E. Faecalis* cgz3

Dandelion root extract (0.3 mg/mL) yielded the highest BSH activity (458.06 ± 7.67 mg/mL), followed by maltitol (127.52 ± 3.44 mg/mL at 0.5 mg/mL), sorbitol (114.44 ± 1.47 mg/mL at 0.2 mg/mL), inulin (89.53 ± 3.35 mg/mL at 0.3 mg/mL), and fructooligosaccharides (89.15 ± 3.44 mg/mL at 0.1 mg/mL) (Figure 1). These findings highlight the potential of prebiotics and plant-based extracts in enhancing BSH activity, which may contribute to cholesterol metabolism and gut health.

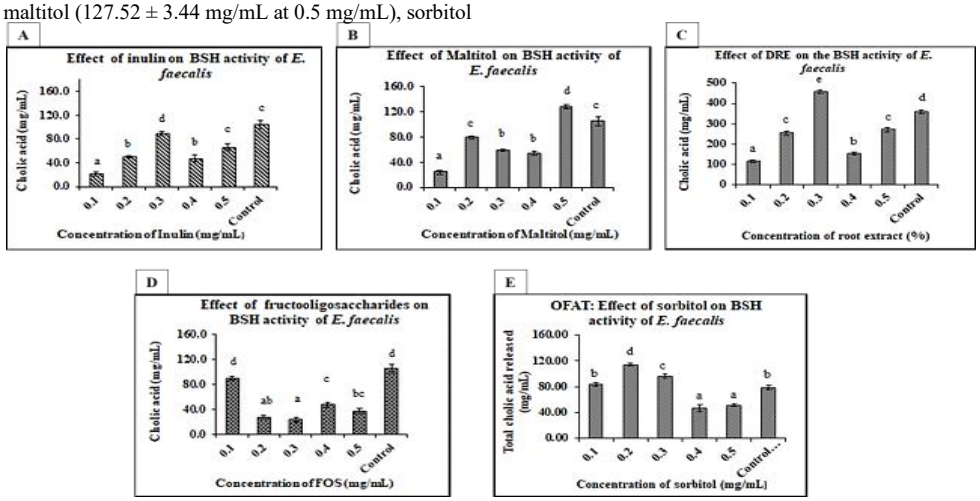

Figure 2 Analysis of individual factors: (A) the effect of inulin; (B) the effect of maltitol; (C) the effect of dandelion root extract; (D) the effect of fructooligosaccharides (FOS); and (E) the effect of sorbitol on the bile salt hydrolase (BSH) activity of *E. faecalis* CGz3. The data are presented as mean ± SD ($n = 3$). Using IBM SPSS software, ver. 27.0. One-way ANOVA revealed that values without a matching alphabet in the same column were substantially different ($p < 0.05$).

Determination of prebiotic ratio by response surface methodology: Central composite design and Box-Behnken design.

The Box-Behnken design identified an optimal formulation of sorbitol (0.4 mg/mL), maltitol (0.2 mg/mL), and dandelion root extract (0.5 mg/mL), achieving 182.5 mg/mL BSH activity. The model's R^2 (0.91) and p-value (0.0073) confirmed its robustness, with an insignificant lack of fit (p = 0.163). Response surface plots highlighted significant interactions between sorbitol and maltitol, and sorbitol and dandelion root extract. At 182.5 mg/mL, the most effective BSH activity was achieved by using an optimised prebiotic composition of 0.4 mg/mL (v/v) sorbitol, 0.2 mg/mL (v/v) maltitol, and 0.5 mg/mL (v/v) dandelion root extract (DRE). The model's p-value of 0.0073 further confirms its statistical significance. With the use of a response surface plot, we were able to see how each component interacted with the others to affect BSH activity; the colour red denoted the most active component, while blue denoted the least active **(Figure 3).**

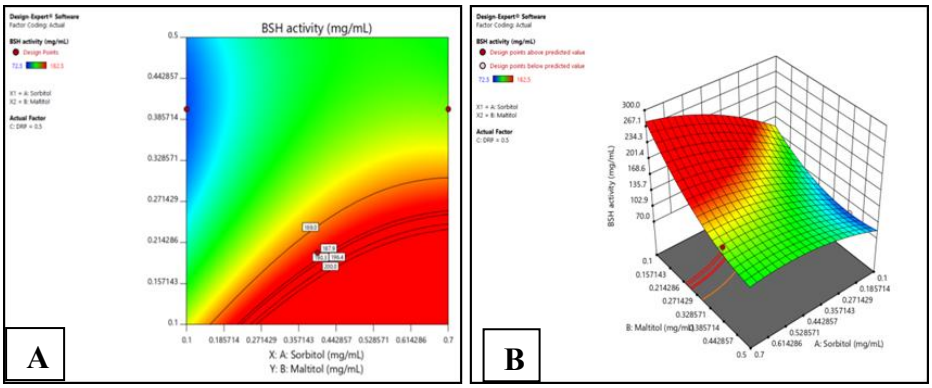

Figure 3. A) Contour plot showing the increase in BSH activity with the increase in concentration of maltitol; **B)** 3D-Interaction plot between factors A and B.
AB (Sorbitol and maltitol): The interaction is significant with a p-value of 0.0313 ($p<0.05$), which indicates that the effect of the sorbitol on the BSH activity of *E. faecalis* CGz3 depends on the level of maltitol and vice versa.
AC (Sorbitol and DRE): The interaction was found to be significant with a p-value of 0.0271 (p-value<0.05), in which the BSH activity of *E. faecalis* CGz3 is influenced by the combined levels of sorbitol and DRE suggesting a synergistic or antagonistic effect depending on their levels.
BC (Maltitol and DRP): The interaction was found to be non-significant with a p-value of 0.1194 ($p>0.05$), the combined effect of maltitol and DRE did not substantially influence the BSH activity of *E. faecalis* CGz3.
The model F-value of 7.49 signifies strong statistical significance, further supported by a p-value < 0.05. Notably, the terms **AB, AC, A^2, B^2**, and **C^2** made substantial contributions to the model. Additionally, the model demonstrated a good fit with experimental data, as indicated by the F-value of misfit (2.86), confirming that the lack of fit was insignificant compared to pure error.

DISCUSSION

To gain deeper insights into the complex interactions between dietary factors and health outcomes, researchers have increasingly adopted interdisciplinary approaches. Prebiotic, probiotic, and synbiotic research currently addresses nutrition, safety, quality, and functionality using multiple methods to fully comprehend their health advantages and applications (He et al., 2021). It's hard to evaluate a single product because probiotic strains have different effects and prebiotic efficacy varies. Kaewarsar et al. optimised a combined prebiotic formulation comprising maltitol, sorbitol, and *Taraxacum officinale* (dandelion) root powder extract to increase *E. faecalis CGz3's* BSH activity (He et al., 2021). This study *used E. faecalis CGz3* to test the effects of SCFA on *Lactobacillus rhamnosus strain HII117* and *Bifidobacterium animalis subsp. lactis* growth. Prebiotics' effect on BSH was examined. MRS media containing maltitol (0.3 mg/mL), sorbitol (0.2 mg/mL), and dandelion root extract (0.3 mg/mL v/v) had the highest BSH activity **(Figure 1).** In our quest to understand the phytochemical and bioactive characteristics of the dandelion root extract, we employed the Folin-Ciocalteu (F-C) technique to determine the total phenolic content (TPC) and the phenol-sulfuric acid (P-S) method to determine the total carbohydrate content (TCC). The water-based extract had the highest phenolic concentration (5.27 ± 5.74 mg GAE/g), in contrast to the ethanol-based extracts (95%; 1.01 ± 0.64 mg GAE/g) and 60% (0.83 ± 1 mg GAE/g). The overall carbohydrate content was not significantly different between the ethanol and water extracts, though **(Table 2).** The total carbohydrate content was recorded as 2.81 ± 0.07 g/L in the aqueous extract, while the 95% ethanol and 60% ethanol extracts contained 2.61 ± 0.04 g/L and 2.1 ± 0.75 g/L, respectively.
A polyphenol with strong antioxidant and free radical scavenging capabilities in vitro, chlorogenic acid (CGA) is included in several drinks. There is a lot of evidence that it helps protect cardiovascular health by making LDL cells more resistant to lipid peroxidation (Orhan et al., 2018). Through the TLR2/NF-κB pathway, Xu et al. showed that chlorogenic acid (CGA) has anti-inflammatory effects on bovine mammary epithelial cells activated with LTA (Xu et al., 2023).Chlorogenic acid concentrations in the water-based and alcohol-based extracts were determined to be 0.14 and 0.15 mg/mL, correspondingly, in the present investigation. **(Table 2, Figure 1).** The DPPH experiment revealed that the aqueous extract had the highest percentage inhibition of antioxidant activity (82.5 ± 2.11), whereas the 95% and 60% ethanol extracts showed the lowest inhibition.
We utilised response surface methodology (RSM) to find the ideal prebiotic ratio based on the outcomes of the one-factor-at-a-time strategy. For the first time, this research used a plant extract as a prebiotic to see how it affected the activity of bile salt hydrolase (BSH) in an organism that normally produces probiotics. The natural diuretic properties of dandelion roots are well-known for their ability to reduce water retention. Its prebiotic qualities are enhanced by the abundance of oligofructans and prebiotic fibres (N. Tr. Petkova et al., 2015). It is widely recognised that this fibre has a positive impact on fat metabolism and promotes the growth of good bacteria in the intestines. The inulin-rich dandelion roots also have other health advantages, such as limiting the growth of dangerous bacteria in the gastrointestinal tract (GIT), which in turn lowers the risk of cancer, obesity, and osteoporosis. The hepatoprotective effects of dandelion are highly regarded in traditional medicine. Believed to improve detoxification and maintain liver function, it is widely used as a liver tonic in traditional Russian, Chinese, and Indian medicine (Mudgil & Barak, 2013).
A standard optimisation method, the Box-Behnken design (BBD) finds the best possible combinations of variables with the fewest possible runs of experiments. With an R^2 value of 0.91, the BBD model proved to be more reliable than the central composite design (CCD), which investigates the interaction between maltitol and dandelion root extract. In comparison, the CCD model, while statistically significant, had a lower R^2 value of 0.79. In this study, the BSH activity of *E. faecalis* CGz3 was strongly impacted by Run number 04, which comprised 0.4 mg/mL (v/v) sorbitol, 0.2 mg/mL (v/v) maltitol, and 0.5 mg/mL (v/v) dandelion root extract (DRE) **(Table 1)**. The results of the ANOVA for the quadratic model ($p < 0.05$) corroborated this. Consequently, the formulation in Run 04 exemplifies the ideal prebiotic ratio for synbiotic compositions. **Figure 3** shows the three-dimensional interaction plots that demonstrated the beneficial effect of sorbitol-maltitol and sorbitol-dandelion root extract on BSH activity.
Furthermore, bioactive compounds with medicinal potential were identified using GC-MS analysis**. Table 3** shows that 25 bioactive chemicals with different health benefits were confirmed by comparing the mass spectra with the NIST (2014) library database. This study highlights the importance of utilizing plant extracts rich in bioactive compounds and prebiotic molecules like sorbitol, maltitol, and inulin for synbiotic formulations. The bioactive molecules in the plant extract, along with the BSH enzyme, known for its cholesterol-lowering effects, contribute to hepatic health. The BSH enzyme hydrolyzes bile salts into glycine and/or taurine, facilitating cholesterol reduction via gut microbiota metabolism, and ultimately promoting metabolic health. In the human colon, hydrolyzed bile salts are less absorbed, increasing free bile acid excretion in feces. The de novo synthesis of bile salts compensates for their loss, thereby effectively reducing serum cholesterol levels (Shehata et al., 2016). The excess fat removal facilitated by the synbiotic formulation offers a potential alternative for alleviating the symptoms of metabolic-associated fatty liver disease (MAFLD), contributing to hepatic health and metabolic regulation.

CONCLUSION

The study shows that prebiotics and *T. officinale* root extract optimise BSH activity in *E. faecalis CGz3*. This technique allows for cholesterol-lowering and metabolic health-promoting functional meals and therapies. Long-term in vivo impact and safety investigations are needed to confirm these findings.

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Declaration of interest: The authors declare that there is no potential conflict of interest.

REFERENCES

Akiyama, H., Oono, T., Huh, W. K., Yamasaki, O., Ogawa, S., Katsuyama, M., Ichikawa, H., & Iwatsuki, K. (2002). Actions of farnesol and xylitol against Staphylococcus aureus. *Chemotherapy*, *48*(3), 122–128. https://doi.org/10.1159/000064916

Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., & Hassan, F. A. (2013). Prebiotics as functional foods: A review. *Journal of Functional Foods*, *5*(4), 1542–1553. https://doi.org/https://doi.org/10.1016/j.jff.2013.08.009

Altayb, H. N., Yassin, N. F., Hosawi, S., & Kazmi, I. (2022). In-vitro and in-silico antibacterial activity of Azadirachta indica (Neem), methanolic extract, and identification of Beta.d-Mannofuranoside as a promising antibacterial agent. *BMC Plant Biology*, *22*(1), 1–14. https://doi.org/10.1186/s12870-022-03650-5

Amudha, P., Jayalakshmi, M., Pushpabharathi, N., & Vanitha, V. (2018). Identification of bioactive components in enhalus acoroides seagrass extract by gas chromatography–mass spectrometry. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(10), 313–317. https://doi.org/10.22159/ajpcr.2018.v11i10.25577

Anti-inflammatory, E. (2020). *molecules*.

Bhat, M. P., Kumar, R. S., Chakraborty, B., Nagaraja, S. K., Gireesh Babu, K., & Nayaka, S. (2024). Eicosane: An antifungal compound derived from Streptomyces sp. KF15 exhibits inhibitory potential against major phytopathogenic fungi of crops. *Environmental Research*, *251*, 118666. https://doi.org/https://doi.org/10.1016/j.envres.2024.118666

Bruno, F., Castelli, G., Migliazzo, A., Piazza, M., Galante, A., Verde, V. Lo, Calderone, S., Nucatolo, G., & Vitale, F. (2015). Cytotoxic screening and in vitro evaluation of pentadecane against leishmania infantum promastigotes and amastigotes. *Journal of Parasitology*, *101*(6), 701–705. https://doi.org/10.1645/15-736

Chandran, S. S., Kealey, J. T., & Reeves, C. D. (2011). Microbial production of isoprenoids. *Process Biochemistry*, *46*(9), 1703–1710. https://doi.org/10.1016/j.procbio.2011.05.012

Chuah, X., Okechukwu, P., Amini, F., & Teo, S. (2018). Eicosane, pentadecane and palmitic acid: The effects in in vitro wound healing studies. *Asian Pacific Journal of Tropical Biomedicine*, *8*(10), 490–499. https://doi.org/10.4103/2221-1691.244158

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D.-J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340. https://doi.org/10.1194/jlr.R036012

Fayyazi, M., Esmaeili, H., Moridi Farimani, M., & Mirjalili, M. H. (2024). Variations in phytochemical traits, total carbohydrate, and antioxidant activity of Iranian wild populations of greater burdock (Arctium lappa L.). *Genetic Resources and Crop Evolution*, *71*(2), 915–927. https://doi.org/10.1007/s10722-023-01672-y

Goldberg, L., Haklai, R., Bauer, V., Heiss, A., & Kloog, Y. (2009). New derivatives of farnesylthiosalicylic acid (Salirasib) for cancer treatment: Farnesylthiosalicylamide inhibits tumor growth in nude mice models. *Journal of Medicinal Chemistry*, *52*(1), 197–205. https://doi.org/10.1021/jm801165r

He, M., Tan, C. P., Liu, Y., & Xu, Y.-J. (2021). Foodomics: a new perspective on gut probiotics nutrition and health research. *Current Opinion in Food Science*, *41*, 146–151. https://doi.org/https://doi.org/10.1016/j.cofs.2021.04.004

Huang, L., Zhu, X., Zhou, S., Cheng, Z., Shi, K., Zhang, C., & Shao, H. (2021). Phthalic acid esters: Natural sources and biological activities. *Toxins*, *13*(7). https://doi.org/10.3390/toxins13070495

Inoue, Y., Shiraishi, A., Hada, T., Hirose, K., Hamashima, H., & Shimada, J. (2004). The antibacterial effects of terpene alcohols on Staphylococcus aureus and their mode of action. *FEMS Microbiology Letters*, *237*(2), 325–331. https://doi.org/10.1016/j.femsle.2004.06.049

Irvin, J. L., Johnston, C. G., & Kopala, J. (1944). a Photometric Method for the Determination of Cholates in Bile and Blood. *Journal of Biological Chemistry*, *153*(2), 439–457. https://doi.org/10.1016/s0021-9258(18)71986-8

Jabra-Rizk, M. A., Shirtliff, M., James, C., & Meiller, T. (2006). Effect of farnesol on Candida dubliniensis biofilm formation and fluconazole resistance. *FEMS Yeast Research*, *6*(7), 1063–1073. https://doi.org/10.1111/j.1567-1364.2006.00121.x

Jain, A., Jain, R., & Jain, S. (2020). *Quantitative Analysis of Reducing Sugars by 3, 5-Dinitrosalicylic Acid (DNSA Method)* (pp. 181–183). https://doi.org/10.1007/978-1-4939-9861-6_43

Jones, M. L., Martoni, C. J., & Prakash, S. (2012). Cholesterol lowering and inhibition of sterol absorption by Lactobacillus reuteri NCIMB 30242: a randomized controlled trial. *European Journal of Clinical Nutrition*, *66*(11), 1234–1241. https://doi.org/10.1038/ejcn.2012.126

Kim, D. H., Park, M. H., Choi, Y. J., Chung, K. W., Park, C. H., Jang, E. J., An, H. J., Yu, B. P., & Chung, H. Y. (2013). Molecular Study of Dietary Heptadecane for the Anti-Inflammatory Modulation of NF-kB in the Aged Kidney. *PLoS ONE*, *8*(3). https://doi.org/10.1371/journal.pone.0059316

Kumar, N., & Parle, A. (2019). Cinnamic acid derivatives: An ERA. *The Pharma Innovation Journal*, *8*, 580–595.

Labbozzetta, M., Poma, P., Tutone, M., McCubrey, J. A., Sajeva, M., & Notarbartolo, M. (2022). Phytol and Heptacosane Are Possible Tools to Overcome Multidrug Resistance in an In Vitro Model of Acute Myeloid Leukemia. *Pharmaceuticals*, *15*(3). https://doi.org/10.3390/ph15030356

M. Rowshanul Habib, & M. Rezaul Karim. (2009). Mb-37-31. *Mycobiology* , *37*(1), 31–36.

Majeed, J., Reang, J., Sharma, K., Acharya, P. C., & Chander Sharma, P. (2023). Chapter 12 - Antiamoebic drugs. In P. C. Acharya & M. Kurosu (Eds.), *Medicinal Chemistry of Chemotherapeutic Agents* (pp. 397–429). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-323-90575-6.00012-0

Makki, K., Deehan, E. C., Walter, J., & Bäckhed, F. (2018). The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host & Microbe*, *23*(6), 705–715. https://doi.org/10.1016/j.chom.2018.05.012

Mansouri, S. M.-T., Shafiee-Nick, R., Parsaee, H., Seyedi, S. M., Saberi, M.-R., & Sadeghian, H. (2008). Inotropic and chronotropic effects of 6-hydroxy-4-methylquinolin-2(1H)-one derivatives in isolated rat atria. *Iranian Biomedical Journal*, *12*(2), 77–84.

Markowiak, P., & Śliżewska, K. (2017). Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients*, *9*(9). https://doi.org/10.3390/nu9091021

Masi, M., Roscetto, E., Cimmino, A., Catania, M. R., Surico, G., & Evidente, A. (2021). Farnesane-type sesquiterpenoids with antibiotic activity from chiliadenus lopadusanus. *Antibiotics*, *10*(2), 1–12. https://doi.org/10.3390/antibiotics10020148

Mini Shobi, T., & Gowdu Viswanathan, M. B. (2018). Antibacterial activity of di-butyl phthalate isolated from Begonia malabarica. *Journal of Applied Biotechnology & Bioengineering*, *5*(2), 97–100. https://doi.org/10.15406/jabb.2018.05.00123

Mudgil, D., & Barak, S. (2013). Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: a review. *International Journal of Biological Macromolecules*, *61*, 1–6. https://doi.org/10.1016/j.ijbiomac.2013.06.044

Nitish Kumar, & Amrita Parle. (2019). Cinnamic acid derivatives: An ERA. *The Pharma Innovation Journal*, *8*(5), 580–595.

OKECHUKWU, P. N. (2020). Evaluation of Anti-Inflammatory, Analgesic, Antipyretic Effect of Eicosane, Pentadecane, Octacosane, and Heneicosane. *Asian Journal of Pharmaceutical and Clinical Research*, *13*(4), 29–35. https://doi.org/10.22159/ajpcr.2020.v13i4.36196

Okechukwu, P. N. (2020). EVALUATION OF ANTI-INFLAMMATORY, ANALGESIC, ANTIPYRETIC EFFECT OF EICOSANE, PENTADECANE, OCTACOSANE, AND HENEICOSANE. *Asian Journal of Pharmaceutical and Clinical Research*.

Orhan, I. E., Senol, F. S., Yılmaz, B. S., Altun, M. L., Özbilgin, S., Yazgan, A. N., Yüksel, E., & İşcan, G. S. (2018). Neuroprotective potential of Viburnum orientale Pallas through enzyme inhibition and antioxidant activity assays. *South African Journal of Botany*, *114*, 126–131. https://doi.org/https://doi.org/10.1016/j.sajb.2017.10.023

Padma, M., Ganesan, S., Jayaseelan, T., Azhagumadhavan, S., Sasikala, P., Senthilkumar, S., & Mani, P. (2019). Journal of drug delivery and therapeutics phytochemical screening and GC – MS analysis of bioactive compounds present in ethanolic leaves extract of Silybum marianum (L). phenol cardiac glycosides anthroquinones. *Journal of Drug Delivery and Therapeutics*, *9*(1), 85–89.

Paudel, M. R., Chand, M. B., Pant, B., & Pant, B. (2019). Assessment of antioxidant and cytotoxic activities of extracts of Dendrobium crepidatum. *Biomolecules*, *9*(9), 1–13. https://doi.org/10.3390/biom9090478

Petkova, N., Ivanova, L., Filova, G., Ivanov, I., & Denev, P. (2017). Antioxidants and carbohydrate content in infusions and microwave extracts from eight medicinal plants. *Journal of Applied Pharmaceutical Science*, *7*, 55–61. https://doi.org/10.7324/JAPS.2017.71008

Petkova, N. Tr., Ivanov, I. G., Topchieva, S. V, Denev, P. P., & Pavlov, A. (2015). *BIOLOGICALLY ACTIVE SUBSTANCES AND IN VITRO ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS FROM DANDELION (TARAXACUM OFFICINA L E) ROOTS.*

Sadeghian, H., Seyedi, S. M., Saberi, M. R., Nick, R. S., Hosseini, A., Bakavoli, M., Mansouri, S. M. T., & Parsaee, H. (2009a). Design, synthesis and pharmacological evaluation of 6-hydroxy-4-methylquinolin-2(1H)-one derivatives as inotropic agents. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*(4), 918–929. https://doi.org/10.1080/14756360802448063

Sadeghian, H., Seyedi, S. M., Saberi, M. R., Nick, R. S., Hosseini, A., Bakavoli, M., Mansouri, S. M. T., & Parsaee, H. (2009b). Design, synthesis and pharmacological evaluation of 6-hydroxy-4-methylquinolin-2(1H)-one derivatives as inotropic agents. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*(4), 918–929. https://doi.org/10.1080/14756360802448063

Sayed, M. A., Elbanna, S. A., Ragab, A. A., Hafez, E., Roushdi, M., & El Rhman, A. M. M. A. (2022). Effect of solid contents and the ratio of EVA/Octadecylacrylate blends on Paraffin Inhibition and pour point temperature of waxy crude oil. *Egyptian Journal of Chemistry*, *65*(3), 611–618. https://doi.org/10.21608/ejchem.2021.70234.3547

Shehata, M. G., El Sohaimy, S. A., El-Sahn, M. A., & Youssef, M. M. (2016). Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. *Annals of Agricultural Sciences*, *61*(1), 65–75. https://doi.org/https://doi.org/10.1016/j.aoas.2016.03.001

Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, *5*(4), 1417–1435. https://doi.org/10.3390/nu5041417

Vanitha, V., Vijayakumar, S., Nilavukkarasi, M., Punitha, V. N., Vidhya, E., & Praseetha, P. K. (2020). Heneicosane—A novel microbicidal bioactive alkane identified from Plumbago zeylanica L. *Industrial Crops and Products*, *154*(May), 112748. https://doi.org/10.1016/j.indcrop.2020.112748

Wang, R., Wang, R., & Yang, B. (2009a). Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. *Innovative Food Science & Emerging Technologies*, *10*(2), 289–292. https://doi.org/https://doi.org/10.1016/j.ifset.2008.12.002

Wang, R., Wang, R., & Yang, B. (2009b). Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. *Innovative Food Science and Emerging Technologies*, *10*(2), 289–292. https://doi.org/10.1016/j.ifset.2008.12.002

Wirngo, F. E., Lambert, M. N., & Jeppesen, P. B. (2016). The Physiological Effects of Dandelion (Taraxacum Officinale) in Type 2 Diabetes. *The Review of Diabetic Studies : RDS*, *13*(2–3), 113–131. https://doi.org/10.1900/RDS.2016.13.113

Wu, Z. K., Li, H. Y., Zhu, Y. L., Xiong, M. Q., & Zhong, J. X. (2024). Neuroprotective and anti-inflammatory effects of eicosane on glutamate and NMDA-induced retinal ganglion cell injury. *International Journal of Ophthalmology*, *17*(4), 638–645. https://doi.org/10.18240/ijo.2024.04.05

Xu, P., Xu, X., Fotina, H., & Fotina, T. (2023). Anti-inflammatory effects of chlorogenic acid from Taraxacum officinale on LTA-stimulated bovine mammary epithelial cells via the TLR2/NF-κB pathway. *PloS One*, *18*(3), e0282343. https://doi.org/10.1371/journal.pone.0282343

Yang, L., Wu, Q. Q., Liu, Y., Hu, Z. F., Bian, Z. Y., & Tang, Q. Z. (2015a). Cinnamaldehyde attenuates pressure overload-induced cardiac hypertrophy. *International Journal of Clinical and Experimental Pathology*, *8*(11), 14345–14354.

Yang, L., Wu, Q.-Q., Liu, Y., Hu, Z.-F., Bian, Z.-Y., & Tang, Q.-Z. (2015b). Cinnamaldehyde attenuates pressure overload-induced cardiac hypertrophy. *International Journal of Clinical and Experimental Pathology*, *8*(11), 14345–14354.

Yogeswari, S., Ramalakshmi, S., Neelavathy, R., & Muthumary, J. (2012). Identification and comparative studies of different volatile fractions from Monochaetia kansensis by GCMS. *Global Journal of Pharmacology*, *6*(2), 65–71.

Zhu, R., Liu, H., Liu, C., Wang, L., Ma, R., Chen, B., Li, L., Niu, J., Fu, M., Zhang, D., & Gao, S. (2017a). Cinnamaldehyde in diabetes: A review of pharmacology, pharmacokinetics and safety. *Pharmacological Research*, *122*, 78–89. https://doi.org/10.1016/j.phrs.2017.05.019

Zhu, R., Liu, H., Liu, C., Wang, L., Ma, R., Chen, B., Li, L., Niu, J., Fu, M., Zhang, D., & Gao, S. (2017b). Cinnamaldehyde in diabetes: A review of pharmacology, pharmacokinetics and safety. *Pharmacological Research*, *122*, 78–89. https://doi.org/10.1016/j.phrs.2017.05.019