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ORIGINAL RESEARCH PAPER

DEVELOPMENT OF A SYNBIOTIC BEVERAGE USING *Eucheuma cottonii* HYDROLYSATE: FERMENTATION CHARACTERISTICS AND ENHANCEMENT OF α-GLUCOSIDASE INHIBITORY ACTIVITY

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Abstract
The development of functional beverages containing probiotics and natural prebiotics has attracted increasing attention due to their potential role in preventing metabolic disorders. This study aimed to develop a synbiotic beverage using *Eucheuma cottonii* (EC) hydrolysate as a prebiotic source and to evaluate the effects of hydrolysate concentration and fermentation time on microbial growth, physicochemical properties, and α-glucosidase inhibitory activity. The hydrolysate was produced through thermal hydrolysis (100 °C, 3 h), and fermentation was carried out using *Lactobacillus plantarum* RJ83 with varying hydrolysate concentrations (0, 0.2, 0.4, and 0.6%) and fermentation times (0, 24, and 48 h). Fermentation enhanced lactic acid bacteria (LAB) growth, increased total titratable acidity, reduced pH, and decreased reducing sugar levels, indicating active microbial metabolism. The most intensive fermentation profile was observed in beverages supplemented with 0.6% hydrolysate, whereas the formulation containing 0.4% hydrolysate fermented for 24 h provided the most favorable balance between microbial viability, physicochemical characteristics, and functional properties. This treatment significantly improved α-glucosidase inhibitory activity compared with the control, increasing inhibition from 22.22 ± 0.76% to 92.17 ± 0.28% ($p < 0.001$). Dose–response analysis using a four-parameter logistic (4PL) model revealed a sigmoidal relationship, with an IC_{50} value of 1061 ppm, indicating moderate inhibitory activity. These findings suggest that *EC* hydrolysate contributed substantially to the observed α-glucosidase inhibitory activity and demonstrates strong potential as a functional

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ingredient for the development of synbiotic beverages with antidiabetic properties.

Keywords: synbiotic beverage, *Eucheuma cottonii*, seaweed hydrolysate, α-glucosidase inhibition, antidiabetic activity

Introduction

The global prevalence of metabolic disorders, particularly type 2 diabetes mellitus, continues to increase and remains a major public health challenge worldwide. Dietary strategies targeting gut microbiota modulation have gained increasing attention as potential approaches for the prevention and management of metabolic diseases (Fan and Pedersen, 2021). Functional foods containing probiotics, prebiotics, or their combination (synbiotics) have been extensively investigated due to their potential to modulate intestinal microbiota, improve gut health, and regulate host metabolic responses (Swanson *et al*., 2020).

Synbiotic products combine beneficial microorganisms with substrates that selectively stimulate their growth and activity. This synergistic interaction may enhance probiotic survival and promote the production of beneficial metabolites during fermentation. Previous studies have demonstrated that synbiotic supplementation can improve glucose homeostasis, modulate gut microbiota, and positively influence lipid metabolism in individuals with metabolic disorders, including type 2 diabetes mellitus (Moroti *et al*., 2012; Mahboobi *et al*., 2018; Mukesh *et al*., 2024). These beneficial effects are attributed to the synergistic interaction between probiotics and prebiotics, which promotes the growth of health-associated microorganisms and the production of bioactive metabolites that contribute to metabolic health.

Marine macroalgae have emerged as promising sources of functional ingredients for food applications. Among them, *Eucheuma cottonii* (EC) is widely cultivated in tropical regions and is known for its high content of sulfated polysaccharides such as carrageenan. After hydrolysis, these polysaccharides can produce low-molecular-weight oligosaccharides that may act as potential prebiotics (Lopez-Santamarina *et al.,* 2020; Shannon *et al.,* 2021). Prebiotics are food components that are not absorbed by the small intestine but can be metabolized by probiotic microorganisms in the large intestine, thus providing beneficial physiological effects, including glycemic control (Charoensiddhi *et al.,* 2020). Synbiotic products, which combine probiotics and prebiotics, have gained increasing attention due to their synergistic effects on gut health and metabolic regulation. Prebiotics such as polysaccharides derived from marine resources have been widely explored for their ability to selectively stimulate beneficial microbiota. Among marine macroalgae, *Eucheuma cottonii* is considered a promising source of bioactive compounds, including sulfated polysaccharides and oligosaccharides, which have been associated with prebiotic and potential antidiabetic activities. Recent studies have demonstrated that synbiotic interventions may improve metabolic parameters, including glycemic control, insulin sensitivity, and lipid metabolism, through modulation of gut microbiota and production of bioactive metabolites (Fan and Pedersen, 2021). Several studies have reported that seaweed-derived oligosaccharides can stimulate the growth of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* species (Lomartire *et al.*, 2022).

Thermal hydrolysis has been recognized as a simple and environmentally friendly approach for producing oligosaccharides from seaweed polysaccharides without requiring chemical reagents or enzymatic treatments. Thermal hydrolysis can depolymerize complex seaweed polysaccharides into low-molecular-weight oligosaccharides that are more readily utilized by probiotic bacteria during fermentation (de Moura *et al*., 2015; Chudasama *et al*., 2021). Despite the increasing interest in seaweed-derived prebiotics, their application in synbiotic fermented beverages remains relatively limited.

Fermentation parameters, including substrate concentration and fermentation time, strongly influence the microbial growth and physicochemical properties of fermented beverages. These factors determine the metabolic activity of lactic acid bacteria, which in turn affects acid production, sugar utilization, and bioactive compound formation. In addition to fermentation characteristics, the evaluation of α-glucosidase inhibitory activity is increasingly used to assess the antidiabetic potential of functional foods. Inhibition of α-glucosidase can delay carbohydrate digestion and reduce postprandial blood glucose levels (Lingxiao *et al.*, 2020; Carvalho *et al.*, 2023).

However, the application of seaweed-derived hydrolysates in synbiotic beverage systems remains underexplored, particularly in relation to their effects on fermentation performance and biofunctional properties associated with metabolic health. Despite the growing interest in seaweed-derived oligosaccharides as prebiotic sources, limited studies have explored their incorporation into synbiotic fermented beverages and their impact on both fermentation performance and antidiabetic-related bioactivity. Therefore, this study aims to develop a synbiotic beverage based on *EC* hydrolysate and to evaluate the effects of different hydrolysate concentrations and fermentation time on microbial growth, physicochemical characteristics, and α-glucosidase inhibitory activity. This study provides new insights into the utilization of seaweed hydrolysates as functional ingredients in synbiotic systems with potential antidiabetic properties.

Materials and methods

Materials

Fresh seaweed EC was obtained from seaweed cultivation areas in Takalar Regency, South Sulawesi, Indonesia. The probiotic strain *Lactobacillus plantarum* RJ83 was obtained from the culture collection of IPB University. De Man Rogosa Sharpe agar (MRSA) and broth (MRSB) were purchased from Merck (Germany). All other analytical reagents used were of analytical grade.

Preparation of seaweed hydrolysate

The seaweed samples were washed thoroughly with distilled water to remove impurities and salt residues. The cleaned seaweed was soaked for 24 h, and dried in a dehydrator at 50 °C for 3 h. The dried seaweed was ground into powder and sieved to obtain 60-mesh particles.

Seaweed powder was mixed with distilled water at a ratio of 1:50 (w/v) and allowed to hydrate for 2 h. Thermal hydrolysis was then carried out using a water bath at 100 °C for 3 h. After hydrolysis, the mixture was cooled and filtered to obtain the seaweed hydrolysate, which was stored at 4 °C until further use.

Preparation of synbiotic beverage and fermentation

Synbiotic beverages were prepared by adding seaweed hydrolysate to commercial pasteurized whole milk at concentrations of 0, 0.2, 0.4, and 0.6% (w/v). The mixture was heated at 40 °C in a water bath to facilitate homogenization.

The prepared medium was inoculated with 10% (v/v) of *Lactobacillus plantarum* RJ83 starter culture. Fermentation was carried out at 37 °C for 0, 24, and 48 h. After fermentation, the samples were stored at 4 °C before further analysis.

Preparation of starter culture

The probiotic culture was first grown on MRSA plates and incubated at 37 °C for 24 h. A single colony was then transferred into MRS broth and incubated anaerobically at 37 °C for 24 h to obtain an active culture. The culture was subsequently inoculated into sterile fresh milk and incubated at 30 °C for 24 h to produce the starter culture.

Microbiological analysis

Total lactic acid bacteria (LAB) counts were determined using the pour plate method. Samples were serially diluted using sterile Butterfield’s phosphate buffer. Aliquots from appropriate dilutions were plated on MRSA medium and incubated at 37 °C for 48 h. Colonies were counted and expressed as log CFU/mL.

Physicochemical analysis

Total titratable acidity

Total titratable acidity (TTA) was determined by titration using 0.1 N NaOH with phenolphthalein as an indicator and expressed as percentage of lactic acid.

pH measurement

The pH of the synbiotic beverages was measured using a calibrated pH meter at room temperature.

Determination of reducing sugar content

The reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method with slight modifications from Miller (1959). Briefly, 1 mL of sample was mixed with 1 mL of DNS reagent in a test tube. The mixture was then heated in a boiling water bath at 100°C for 5 min to allow color development. After cooling to room temperature, 8 mL of distilled water was added to dilute the solution. The absorbance was measured at 540 nm using a UV–Vis spectrophotometer.

A standard calibration curve was prepared using glucose solutions at concentrations ranging from 0 to 1 mg/mL. The reducing sugar content of the samples was calculated based on the standard curve and expressed as mg glucose equivalent per mL sample.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis *et al* (2007) with minor modifications. The assay was performed using α-glucosidase enzyme (from *Saccharomyces cerevisiae*) and p-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate.

Briefly, 50 µL of sample solution at various concentrations was mixed with 100 µL of 0.1 M phosphate buffer (pH 6.8) and 50 µL of α-glucosidase solution (1 U/mL). The mixture was pre-incubated at 37°C for 10 min. Subsequently, 50 µL of pNPG solution (5 mM) was added to initiate the reaction.

The reaction mixture was incubated at 37°C for 20 min and then terminated by adding 100 µL of 0.1 M Na_2CO_3. The absorbance was measured at 405 nm using a microplate reader.

The percentage of inhibition was calculated using equation 1.

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad (1)$$

where:
$A_{control}$ is the absorbance of the control (without sample)
A_{sample} is the absorbance in the presence of the sample

The IC_{50} value was determined using nonlinear regression based on a four-parameter logistic (4PL) model, by plotting inhibition percentage against sample concentration.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation. Statistical differences among treatments were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test at a significance level of $p < 0.05$.

Result and Discussion

Fermentation characteristics of synbiotic beverages

The fermentation characteristics of synbiotic beverages supplemented with *EC* hydrolysate were evaluated by monitoring microbial growth and physicochemical changes during fermentation. Key indicators including lactic acid bacteria (LAB) population, pH, titratable acidity (TTA), and reducing sugar content were measured to assess fermentation performance and the potential synbiotic effect of the seaweed-derived prebiotic substrate. All data presented in Figure 1 and Table 1 consistently represent fermentation up to 48 h.

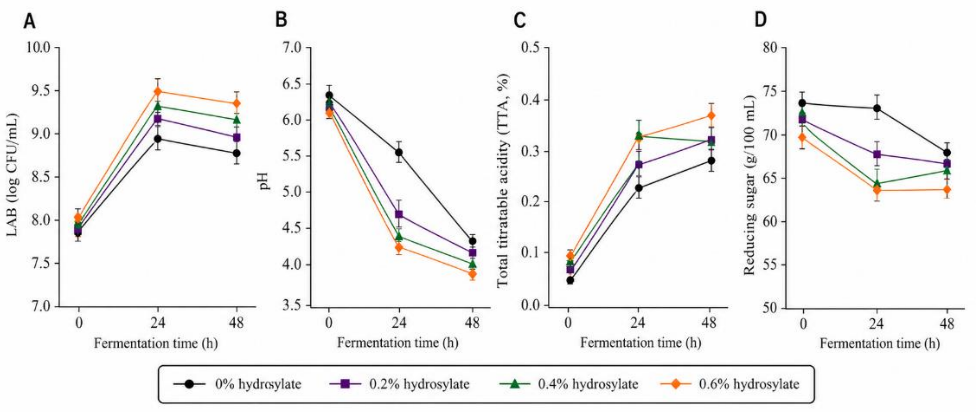

Figure 1. Changes in (A) lactic acid bacteria (LAB) counts, (B) pH, (C) total titratable acidity (TTA), and (D) reducing sugar content during fermentation of synbiotic beverages supplemented with different concentrations (0, 0.2, 0.4, and 0.6%) of EC hydrolysate over 48 h.

LAB counts increased significantly during the first 24 h of fermentation across all treatments, followed by a slight decline at 48 h. Supplementation with *EC* hydrolysate significantly influenced the growth of lactic acid bacteria during fermentation. The LAB population increased progressively in all treatments, indicating active microbial metabolism.

Table 1. Effects of *EC* hydrolysate concentration and fermentation time on microbial and physicochemical properties of synbiotic beverage.

Hydrolysate (%)	Fermentation Time (h)	LAB (log CFU/mL)	TTA (%)	pH	Reducing Sugar (g/100 mL)
	0	7.86 ± 0.09^a	0.037 ± 0.01^a	6.37 ± 0.13^a	74.21 ± 0.45^a
0	24	9.31 ± 0.03^b	0.273 ± 0.02^c	5.58 ± 0.02^b	73.81 ± 0.05^a
	48	9.03 ± 0.13^b	0.304 ± 0.02^d	4.22 ± 0.04^c	68.67 ± 0.21^b
	0	7.85 ± 0.21^a	0.075 ± 0.01^b	6.31 ± 0.03^a	71.91 ± 0.18^b
0.2	24	9.31 ± 0.03^b	0.287 ± 0.01^c	4.51 ± 0.04^c	68.07 ± 0.08^c
	48	9.03 ± 0.13^b	0.336 ± 0.05^d	4.08 ± 0.06^d	67.63 ± 0.25^c
	0	7.91 ± 0.07^a	0.085 ± 0.01^b	6.32 ± 0.04^a	73.18 ± 0.05^b
0.4	24	9.37 ± 0.05^c	0.345 ± 0.01^e	4.36 ± 0.04^c	64.66 ± 0.15^d
	48	9.26 ± 0.02^c	0.319 ± 0.01^e	4.02 ± 0.02^d	67.08 ± 0.05^c
	0	7.92 ± 0.02^a	0.091 ± 0.01^b	6.33 ± 0.01^a	70.23 ± 0.16^c
0.6	24	9.43 ± 0.04^c	0.325 ± 0.01^d	4.31 ± 0.04^c	64.18 ± 0.11^d
	48	9.35 ± 0.17^c	0.373 ± 0.02^f	3.91 ± 0.08^e	66.11 ± 0.74^c

Note: Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$).

Notably, hydrolysate-supplemented beverages exhibited higher LAB counts compared with the control. This finding suggests that oligosaccharides derived from *EC* may serve as fermentable substrates that promote probiotic growth. The enhanced bacterial proliferation further supports the potential prebiotic role of seaweed hydrolysates in synbiotic beverage formulations.

The fermentation process also resulted in a gradual decrease in pH accompanied by an increase in titratable acidity. The decline in pH is mainly attributed to the production of organic acids, particularly lactic acid, generated during carbohydrate metabolism by LAB. Treatments containing higher concentrations of *EC* hydrolysate showed a more pronounced pH reduction and higher acidity values, indicating more active fermentation. This observation suggests that the additional fermentable substrates from seaweed hydrolysates enhanced metabolic activity of the probiotic cultures.

In parallel, the reducing sugar content decreased during fermentation, reflecting microbial utilization of available carbohydrates. The faster reduction of sugar levels in synbiotic formulations indicates that the added seaweed hydrolysates contributed additional fermentable sugars, which were efficiently metabolized by LAB. These findings demonstrate that *EC* hydrolysate not only supports microbial growth but also improves fermentation efficiency.

Overall, the results presented in Table 1 demonstrate that the addition of *EC* hydrolysate enhances microbial growth and fermentation activity in synbiotic beverages. These findings indicate that seaweed-derived oligosaccharides can function as effective prebiotic components, supporting probiotic viability and contributing to improved functional beverage characteristics.

Changes in LAB growth, total titratable acidity, pH, and reducing sugar during fermentation

The addition of *EC* hydrolysate significantly affected LAB growth during fermentation (Figure 1). LAB populations increased during the first 24 h, indicating active microbial proliferation supported by fermentable oligosaccharides generated during thermal hydrolysis. These oligosaccharides may serve as readily available carbon sources for *Lactobacillus plantarum*, thereby enhancing bacterial growth and metabolic activity. Similar prebiotic effects of seaweed-derived oligosaccharides have been reported previously (Krangkratok *et al*., 2023). Likewise, Mutmainnah *et al*. (2023) demonstrated that hydrothermally degraded red seaweed polysaccharides promoted the growth of *L. plantarum* and improved fermentation characteristics, including LAB count, pH, titratable acidity, and reducing sugar utilization.

After 48 h of fermentation, LAB counts slightly decreased, likely due to organic acid accumulation and nutrient depletion during prolonged fermentation (Praiboon *et al*., 2023). Consistent with LAB growth, total titratable acidity increased significantly during fermentation, while pH decreased concurrently. This inverse relationship reflects the production of organic acids, primarily lactic acid, through carbohydrate metabolism by LAB.

Among the treatments, the beverage containing 0.4% hydrolysate exhibited the most balanced fermentation profile, characterized by high LAB viability and moderate acidification. This finding suggests that the selected concentration provided sufficient fermentable substrate without causing excessive viscosity or osmotic stress.

Reducing sugar levels gradually declined throughout fermentation, indicating active carbohydrate utilization by LAB (Figure 1). The reduction in sugars corresponded with increased acid production and microbial growth, confirming the conversion of fermentable carbohydrates into metabolic end-products such as lactic acid. Similar trends have been reported in fermented food systems, where sugar depletion is associated with enhanced microbial activity and acid formation (Zhang *et al*., 2022).

In addition, seaweed hydrolysate may provide low-molecular-weight carbohydrates that are more readily fermentable, thereby accelerating microbial metabolism and fermentation efficiency. Overall, these results demonstrate that *EC* hydrolysate supports LAB growth and metabolic activity, contributing to improved physicochemical characteristics of the synbiotic beverage.

Effect of seaweed hydrolysate on fermentation performance

The present study demonstrates that the incorporation of EC hydrolysate significantly enhanced the fermentation performance of synbiotic beverages, as indicated by increased LAB growth, decreased pH, and elevated titratable acidity during fermentation. The observed increase in LAB populations suggests that seaweed-derived oligosaccharides can serve as fermentable substrates that support probiotic growth. Fermentation has been widely recognized as a process that enhances the functional value of foods through the activity of beneficial microorganisms and their metabolites, thereby contributing to potential health-promoting effects (Leech *et al*., 2020). Marine macroalgae such as EC are known to contain polysaccharides including carrageenan-derived oligosaccharides that may act as prebiotic compounds, promoting the proliferation of beneficial bacteria in fermented food systems (Cherry *et al*., 2019).

The reduction in pH observed during fermentation is primarily attributed to the production of organic acids, particularly lactic acid, generated through carbohydrate metabolism by lactic acid bacteria. A similar decrease in pH during fermentation of synbiotic beverages has been reported in several studies, indicating active microbial metabolism and acidification of the fermentation medium (Ranadheera *et al*., 2017; Marco *et al*., 2017). Acidification not only indicates fermentation efficiency but also contributes to microbial stability and shelf-life of fermented beverages.

Relationship between fermentation parameters and probiotic growth

Correlation analysis was performed to evaluate the relationships among fermentation parameters and probiotic growth in synbiotic beverages supplemented with *EC* hydrolysate (Figure 2). The analysis revealed a strong positive correlation between lactic acid bacteria (LAB) counts and total titratable acidity (TTA), indicating that increased microbial growth was associated with enhanced organic acid production. In contrast, LAB counts showed a strong negative correlation with pH, confirming that acid accumulation during fermentation contributed to pH reduction.

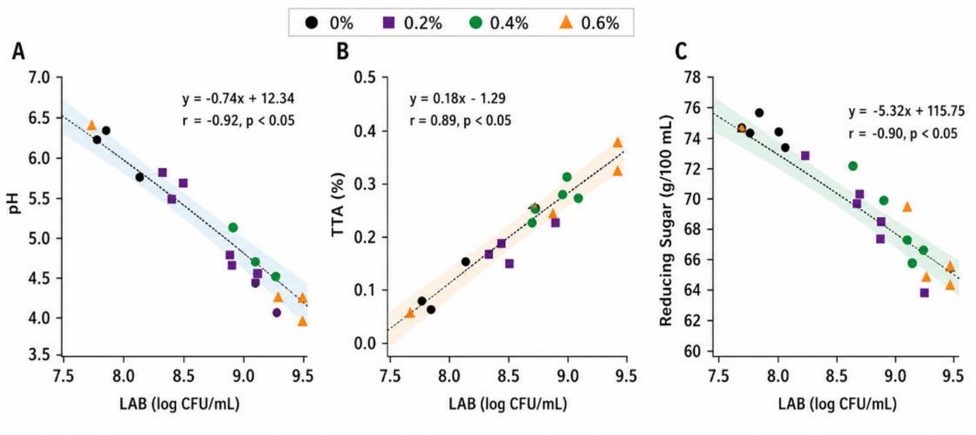

Figure 2. Correlation analysis among fermentation parameters of synbiotic beverages supplemented with *EC* hydrolysate.

Reducing sugar content was negatively correlated with LAB growth, suggesting active carbohydrate utilization by *Lactobacillus plantarum* during fermentation. This finding supports the role of fermentable substrates as primary energy sources for microbial proliferation and metabolite production. Similar relationships between sugar depletion, acid production, and LAB growth have been reported in seaweed-based fermentation systems (Allahgholi *et al*., 2023; Krangkratok *et al*., 2023).

The results further suggest that *EC* hydrolysate enhanced fermentation efficiency by providing low-molecular-weight oligosaccharides generated during thermal hydrolysis. These compounds are more readily utilized by LAB and may contribute to improved microbial activity and fermentation performance. However, although higher hydrolysate concentrations promoted initial microbial growth, prolonged fermentation likely induced acid stress due to excessive organic acid accumulation, which may inhibit further bacterial proliferation.

Overall, the correlation patterns demonstrate that fermentation dynamics in synbiotic beverages are strongly influenced by the interaction among substrate availability, microbial growth, and metabolic by-products. The addition of *EC* hydrolysate improved substrate utilization and supported probiotic activity, highlighting its potential application as a functional ingredient in synbiotic beverage formulations.

Role of seaweed oligosaccharides as prebiotic substrates

The enhanced LAB growth observed in hydrolysate-supplemented treatments suggests that *EC*-derived oligosaccharides may function as effective prebiotic substrates. Seaweed-derived oligosaccharides have been reported to selectively stimulate beneficial microorganisms such as *Lactobacillus* and *Bifidobacterium*, thereby supporting microbial growth and metabolic activity during fermentation (Carrasqueira *et al*., 2025).

In addition to their prebiotic potential, seaweed polysaccharides and their hydrolysis products have been associated with various biofunctional properties, including antioxidant and antidiabetic activities (Xie *et al*., 2024; Abo-Shady *et al*., 2023). One proposed mechanism involves inhibition of carbohydrate-hydrolyzing enzymes such as α-glucosidase, which may contribute to improved glycemic control (Moto *et al*., 2023). The α-glucosidase inhibitory activity observed in the present study supports the potential contribution of seaweed-derived bioactive compounds to the functional properties of the synbiotic beverage.

These findings suggest that incorporation of *EC* hydrolysate may provide multifunctional benefits by enhancing probiotic fermentation while also contributing bioactive compounds with potential metabolic health effects.

α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity of the synbiotic beverage was further evaluated to determine the contribution of *EC* hydrolysate. The dose–response relationship between sample concentration and inhibition percentage is presented in Figure 3.

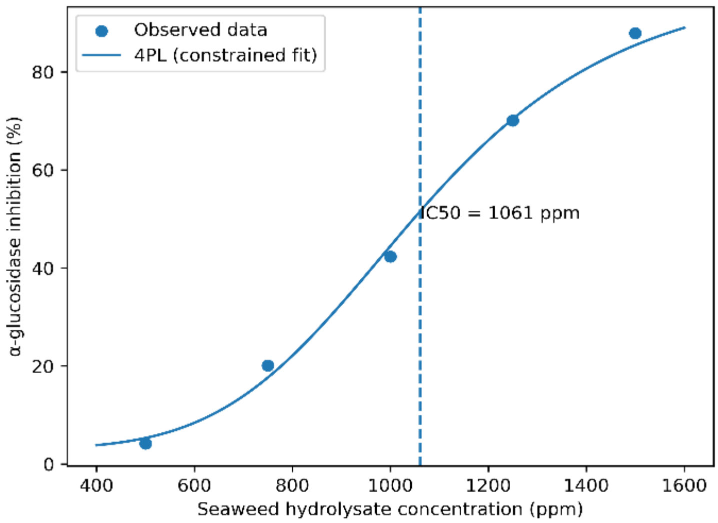

Figure 3. Dose–response curve of α-glucosidase inhibitory activity of *EC* hydrolysate fitted using a constrained four-parameter logistic (4PL) model. The estimated IC_{50} value was 1061 ppm.

The dose–response curve presented in Figure 3 demonstrates that the α-glucosidase inhibitory activity of the synbiotic beverage increased progressively with increasing concentration of *EC* hydrolysate. The sigmoidal pattern observed in the curve is characteristic of enzyme inhibition kinetics and reflects the gradual interaction between bioactive compounds and the active sites of α-glucosidase enzymes.

Based on the 4-parameter logistic (4PL) regression model, the estimated IC_{50} value was approximately 1061 ppm, indicating the concentration required to inhibit 50%

of enzyme activity. The relatively moderate IC_{50} value suggests that the hydrolysate possesses biologically relevant inhibitory activity and may serve as a potential natural antidiabetic ingredient. The increase in inhibition activity with increasing concentration may be associated with the higher availability of bioactive compounds derived from seaweed hydrolysis and fermentation. Thermal hydrolysis of EC polysaccharides likely generated low-molecular-weight oligosaccharides and sulfated polysaccharide fragments, which have been reported to exhibit α-glucosidase inhibitory properties (Zhu *et al*., 2020; Lee *et al*., 2022). Similar antidiabetic effects have been reported for seaweed-derived acidic polysaccharides, which showed significant α-glucosidase inhibitory activity and contributed to improved glycemic control in experimental models of type 2 diabetes (Moto *et al*., 2023). These compounds may interact with the catalytic region of the enzyme, thereby reducing carbohydrate hydrolysis and slowing glucose release.

In addition, fermentation by *Lactobacillus plantarum* may further enhance bioactivity through enzymatic biotransformation and metabolite production. LAB fermentation may enhance the release of bioactive metabolites and peptides with enzyme inhibitory potential through enzymatic biotransformation processes (Raveschot *et al*., 2020). The observed concentration-dependent inhibition is consistent with previous studies on fermented functional foods and marine polysaccharides (Lee *et al*., 2022; Canfora *et al*., 2019).

Overall, these findings demonstrate that the developed synbiotic beverage exhibits promising α-glucosidase inhibitory activity and supports the potential application of *EC*-based synbiotic beverages as functional foods for glycemic control.

Heatmap visualization of fermentation parameters

A heatmap analysis was conducted to visualize the overall patterns of fermentation parameters across different concentrations of *EC* hydrolysate and fermentation times. This heatmap visualization allows direct comparison of microbial and physicochemical parameters across hydrolysate concentrations and fermentation times. The resulting heatmap is shown in Figure 4.

Figure 4 presents a heatmap visualization of the fermentation parameters of synbiotic beverages supplemented with different concentrations of *EC* hydrolysate during fermentation. The heatmap provides an integrated overview of changes in lactic acid bacteria (LAB) counts, titratable acidity (TTA), pH, and reducing sugar content across hydrolysate concentrations and fermentation times.

A clear fermentation trend was observed throughout the study. LAB counts increased markedly after 24 h of fermentation in all treatments, reaching values above 9 log CFU/mL, indicating active microbial growth and successful adaptation of the starter culture. The highest LAB count was recorded in the beverage supplemented with 0.6% EC hydrolysate after 24 h of fermentation (9.43 log CFU/mL). This increase in LAB population was accompanied by a rise in TTA and a corresponding decline in pH, reflecting the production of organic acids as a result of carbohydrate metabolism by lactic acid bacteria. Similar trends have been widely reported in fermented dairy and non-dairy beverages, where microbial growth is associated with acid accumulation and pH reduction.

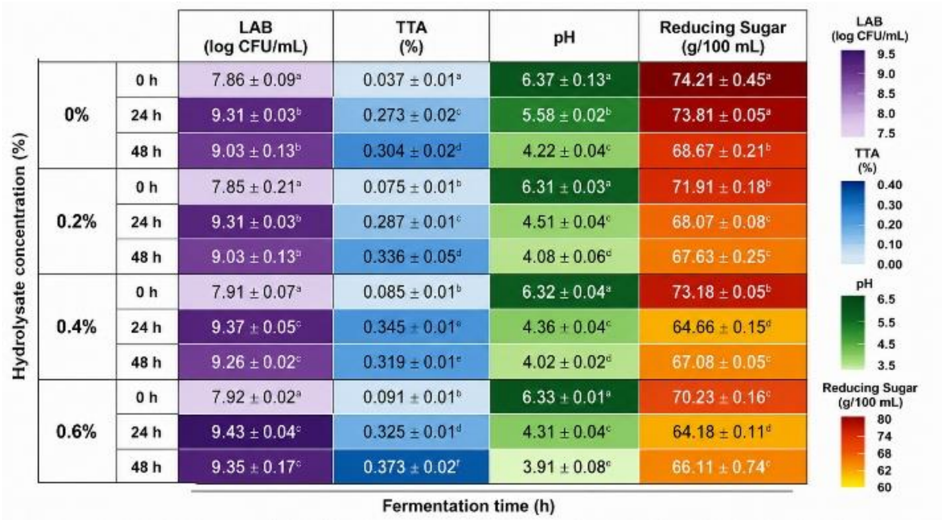

Figure 4. Heatmap of fermentation parameters of synbiotic beverages supplemented with *EC* hydrolysate.

Values are mean ± SD (n = 3). Different letters (a–f) within the same column indicate significant differences ($p < 0.05$) according to Tukey's HSD test. Colors indicate the magnitude of each parameter according to the corresponding scale.

The heatmap also illustrates the progressive utilization of reducing sugars during fermentation. Compared with unfermented samples, beverages fermented for 24 and 48 h generally exhibited lower reducing sugar contents, confirming that fermentable carbohydrates were utilized as substrates for bacterial growth and metabolic activity. The lowest reducing sugar content was observed in the 0.6% hydrolysate treatment after 24 h of fermentation (64.18 g/100 mL), indicating intensive carbohydrate consumption during the active fermentation phase.

Among the tested treatments, beverages supplemented with 0.4% and 0.6% EC hydrolysate exhibited the most pronounced fermentation responses. These treatments were characterized by higher LAB counts, increased TTA values, lower pH, and reduced sugar contents compared with the control treatment. The enhanced fermentation performance may be attributed to the availability of additional fermentable compounds derived from the hydrolysate, which supported microbial growth and metabolic activity. In particular, the 0.6% hydrolysate treatment showed the highest LAB count (9.43 log CFU/mL), the highest TTA (0.373%), and the lowest pH (3.91), indicating a more intensive fermentation process.

Furthermore, the heatmap clearly demonstrates the inverse relationships between LAB growth and reducing sugar content, as well as between pH and TTA. As LAB populations increased, reducing sugars decreased due to their utilization as energy sources, while the accumulation of organic acids resulted in increased TTA and decreased pH values. These relationships are consistent with the metabolic behavior of lactic acid bacteria during fermentation and confirm the effectiveness of the fermentation process in transforming available carbohydrates into organic acids.

Overall, the heatmap analysis confirms that both EC hydrolysate concentration and fermentation time significantly influenced the microbial and physicochemical characteristics of the synbiotic beverage. The 0.6% hydrolysate treatment exhibited the most intensive fermentation profile, characterized by the highest LAB count, highest TTA, and lowest pH values. However, when microbial viability, physicochemical characteristics, and α-glucosidase inhibitory activity were considered collectively, the beverage supplemented with 0.4% hydrolysate and fermented for 24 h was selected as the optimal formulation.

Functional mechanism and antidiabetic potential of the synbiotic beverage

To provide an integrated overview of the biological interactions underlying the functional properties of the developed synbiotic beverage, a conceptual mechanism is proposed in Figure 5. The proposed mechanism illustrates the synergistic interaction between seaweed-derived prebiotic compounds and probiotic metabolism, which may contribute to improved fermentation performance and potential antidiabetic activity.

Thermal hydrolysis of *EC* polysaccharides generates low-molecular-weight oligosaccharides that may serve as fermentable substrates for probiotic bacteria such as *Lactobacillus plantarum*. These oligosaccharides have been reported to exhibit prebiotic properties by selectively supporting the growth and metabolic activity of beneficial microorganisms (Zhu *et al*., 2020). The enhanced LAB growth observed in the present study further supports the role of seaweed hydrolysates as potential prebiotic ingredients in synbiotic beverage formulations.

During fermentation, probiotic metabolism converts available substrates into various bioactive metabolites, including organic acids and other low-molecular-weight compounds, which contribute to the physicochemical and functional characteristics of the beverage. The increase in total titratable acidity and reduction in pH observed during fermentation indicate active carbohydrate metabolism by LAB. In addition, fermentation may enhance the release and bioavailability of bioactive compounds derived from seaweed hydrolysates through enzymatic biotransformation processes.

Seaweed-derived compounds, particularly oligosaccharides and sulfated polysaccharide fragments generated during hydrolysis, have been associated with α-glucosidase inhibitory activity (Lee *et al*., 2022; Zhao *et al*., 2021). The concentration-dependent inhibition observed in the present study suggests that these compounds may interfere with carbohydrate hydrolysis, thereby reducing glucose release and potentially contributing to glycemic control. Furthermore, fermentation by *Lactobacillus plantarum* may enhance the biological activity of the beverage through the production of additional metabolites with enzyme inhibitory potential.

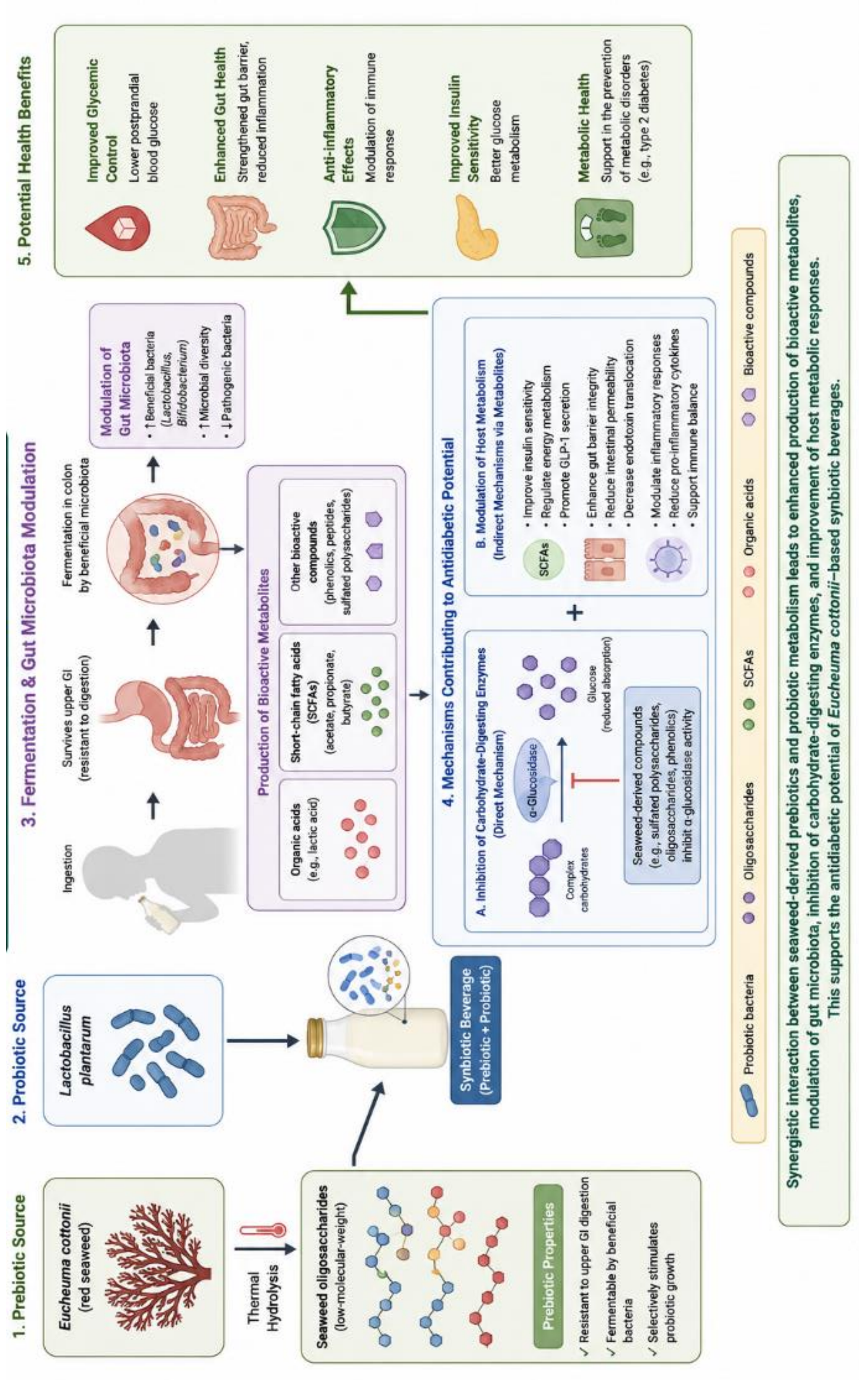

Figure 5. Proposed mechanism underlying the functional and antidiabetic potential of *EC*-based synbiotic beverages through probiotic fermentation, production of bioactive metabolites, modulation of gut microbiota, and inhibition of α-glucosidase activity.

Previous studies have also reported that synbiotic fermentation may influence gut microbial activity and metabolic responses through the production of fermentation metabolites, including short-chain fatty acids (SCFAs) and organic acids (Canfora *et al*., 2019; Liu *et al*., 2020). These metabolites have been associated with improved intestinal homeostasis and metabolic regulation. However, gut microbiota composition, SCFA production, and host metabolic responses were not directly evaluated in the present study. Therefore, further in vivo investigations are necessary to confirm the proposed mechanisms and physiological effects of the developed synbiotic beverage.

Overall, the findings suggest that the functional potential of the *EC*-based synbiotic beverage may arise from the combined effects of probiotic fermentation and seaweed-derived bioactive compounds. The synergistic interaction between prebiotic substrates and probiotic metabolism supports the potential application of this synbiotic beverage as a functional food with antidiabetic properties.

Limitations and future research

Although this study demonstrated the potential of *EC* hydrolysate as a prebiotic substrate, several limitations should be considered. First, the study focused primarily on in vitro fermentation and enzyme inhibition assays. Additional studies are needed to evaluate the stability, sensory properties, and consumer acceptance of the developed synbiotic beverage. Furthermore, in vivo studies are required to confirm the antidiabetic effects and gut microbiota modulation associated with the consumption of this beverage.

Future research should also investigate the structural characterization of seaweed-derived oligosaccharides and their specific interactions with probiotic bacteria. Understanding these mechanisms will provide valuable insights for optimizing synbiotic formulations and enhancing the functional properties of seaweed-based beverages.

Conclusion

This study demonstrated that thermally hydrolyzed *EC* has potential as a prebiotic substrate for the development of synbiotic beverages. The addition of seaweed hydrolysate enhanced lactic acid bacteria growth, improved fermentation characteristics, and increased α-glucosidase inhibitory activity. Although the highest fermentation intensity was observed at 0.6% hydrolysate, the formulation containing 0.4% hydrolysate fermented for 24 h provided the most favorable balance between microbial viability, physicochemical characteristics, and functional properties, making it the selected formulation for synbiotic beverage development. These findings highlight the potential application of seaweed-based synbiotic beverages as functional foods for glycemic control and metabolic health. However, further in vivo studies are required to validate the proposed antidiabetic mechanisms and physiological effects of the developed synbiotic beverage.

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