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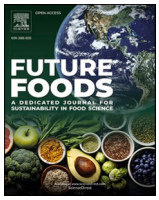

Sustainable co-fermentation of seagrape (*Caulerpa lentillifera*) and coffee cherry pulp: Enhancing bioactivity and stability for functional beverages

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ABSTRACTS

Fermentation of seagrape seaweed (*Caulerpa lentillifera*) with *Lactiplantibacillus plantarum* and supplemented with coffee cherry pulp enhances microbial viability, bioactive compound content, and antioxidant activity while reducing cytotoxicity and improving sensory acceptance. The co-fermented formulation containing 50 g/L seagrape, 25 g/L coffee cherry pulp, *L. plantarum* TISTR 2070, and 30 g/L sucrose exhibited the highest total phenolic content and antioxidant capacity, with DPPH, FRAP, and ABTS values significantly exceeding those of the unfermented control ($p \leq 0.05$). Cold storage at 4 °C demonstrated that the fermented beverage retained its antioxidant properties and maintained microbial viability with minor changes in sugar content and pH. Additionally, cytotoxicity testing on human colon cells demonstrated that fermentation markedly reduced cytotoxicity, with the CC_{50} increasing from 16,020 ± 445.6 μg/mL (unfermented SG) to 25,860 ± 336.6 μg/mL in the co-fermented sample ($p \leq 0.05$), indicating improved safety for human consumption. Sensory evaluation confirmed that the fermented beverage was more accepted than the unfermented counterpart, with improvements in taste, mouthfeel, and overall preference. Multivariate analysis by PCA further revealed strong correlations between microbial activity, antioxidant capacity, and sensory attributes. LC-QTOF-MS analysis confirmed the release and biotransformation of diverse bioactive metabolites during fermentation, contributing to the enhanced functional properties of the final product. With its enhanced bioactivity, stability, reduced cytotoxicity, and superior sensory attributes, co-fermentation of seagrape and coffee cherry pulp presents a promising strategy for the development of health-promoting functional beverages.

1. Introduction

Seagrape seaweed (*Caulerpa lentillifera*, SG) is a nutritionally significant green algae with a rich profile of bioactive compounds, including vitamins, minerals (iodine, sodium, magnesium), omega-3 fatty acids, fiber, and antioxidants (Zhang et al., 2020). It has been traditionally consumed in fresh and dried forms, incorporated into dietary supplements, and processed into seaweed-derived beverages (Peerakietkhajorn et al., 2024). Recent studies have suggested that fermentation may enhance the bioavailability of bioactive compounds in seagrape,

Abbreviations: SG, seagrape seaweed; CC, coffee cherry pulp; LP2070, *Lactiplantibacillus plantarum* TISTR 2070; LC2286, *Lactiplantibacillus paracasei* TISTR 2286; AP102, *Acetobacter pasteurianus* TISTR; LAB, lactic acid bacteria; AAB, acetic acid bacteria; MRS, de Man, Rogosa, and Sharpe media; GYC, glucose, yeast extract, and calcium carbonate media; TPC, total phenolic content; DPPH, 2,2-diphenyl-1-picrylhydrazy radical scavenging assay; ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid radical scavenging ability; FRAP, ferric reducing antioxidant power assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ND., Indicates not detectable; Suc, sucrose.

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potentially increasing its functional properties and health benefits (Melini and Melini, 2021; Sawant et al., 2025).

Coffee cherry pulp (CC), a byproduct of coffee bean processing, has received increasing recognition for its high content of phenolic compounds and potent antioxidant properties (Rosas-Sanchez et al., 2021). It contains bioactive molecules such as chlorogenic acids and flavonoids, which exhibit strong radical-scavenging activity and may contribute to reduced risks of chronic diseases such as cardiovascular disease and cancer (Chomphoosee et al., 2025; Geremu et al., 2016). However, despite its high nutritional potential, coffee cherry pulp is often discarded as waste, leading to environmental concerns. Fermenting CC with functional ingredients such as SG not only enhances its nutritional value but also supports sustainability by upcycling this nutrient-rich byproduct (Pongsiriyakul et al., 2024).

Fermentation is a widely recognized biotechnological process that enhances the nutritional and functional properties of food by modulating its chemical composition through microbial metabolism (Sawant et al., 2025). This process leads to the production of bioactive metabolites, including phenolic acids, exopolysaccharides (EPS), and organic acids, which may contribute to improved antioxidant activity, probiotic viability, and sensory properties (Wu et al., 2023). Additionally, fermentation is known to reduce potentially harmful compounds, making food products safer for human consumption (Melini and Melini, 2021). Several studies have demonstrated that fermenting plant-based ingredients with *Lactiplantibacillus plantarum* can enhance phenolic content and increase antioxidant capacity (Li et al., 2021; Myo et al., 2021). However, limited research has explored the synergistic effects of fermenting seagrape with coffee cherry pulp and probiotic strains, despite their potential for enhancing both bioactive properties and consumer acceptability.

One particularly underexplored aspect is the effect of fermentation on cytotoxicity. While seagrape and coffee cherry pulp contain beneficial phenolic compounds, some phenolic derivatives may exhibit cytotoxic effects at high concentrations. Fermentation may help mitigate cytotoxicity by modifying these bioactive compounds, thus improving the safety of the final product for human consumption (Magoni et al., 2018; Sawant et al., 2025). Additionally, there is limited information on how fermentation alters the sensory characteristics of seagrape and coffee cherry beverages. Given that fermentation is known to influence taste, texture, and aroma, understanding its impact on consumer perception is critical for the development of functional food products.

This study aimed to investigate the impact of fermentation on the bioactive properties, cytotoxicity, and sensory attributes of seagrape when co-fermented with coffee cherry pulp and *L. plantarum*. Specifically, the study evaluated microbial viability, sugar metabolism, lactic acid production, total phenolic content, antioxidant activity, and consumer acceptance of the fermented beverage. By examining the interplay between microbial metabolism and functional properties, this research seeks to provide insights into the potential application of fermented seagrape and coffee cherry as a novel functional beverage.

2. Materials and methods

2.1. Raw material preparation

Seagrape (SG) was sourced from a commercial farm in Trang Province, Thailand. Coffee cherry pulp (CC) was obtained from Thep Sadet District, Chiang Mai Province, Thailand. Both raw materials were harvested from a single batch and used consistently throughout all experimental replicates to ensure reproducibility.

Seagrape (SG) and coffee cherry pulp (CC) were dried at 90 °C for 6 h in a hot air oven, ground using a multifunctional blender (HR2061, Philips, Indonesia), sieved through an 18-mesh sieve, and subsequently stored in airtight containers at room temperature until further use.

2.2. Proximate composition analysis

The proximate composition of seagrape and coffee cherry pulp was analyzed, including moisture, protein, fat, ash (AOAC, 2000), and carbohydrate content, which was calculated based on the method of Sullivan and Carpenter (1993).

2.3. Selection of fermenting bacteria for bioactive compound enhancement

2.3.1. Bacterial strain preparation

The fermentation utilized *Lactiplantibacillus plantarum* TISTR 2070 (LP2070), *Lactiplantibacillus paracasei* TISTR 2286 (LC2286), and *Acetobacter pasteurianus* TISTR 102 (AP102), obtained from the Thailand Institute of Scientific and Technological Research (TISTR). The initial bacterial inoculum was standardized to 7–8 log CFU/mL. A calibration curve correlating OD_{600} with viable cell count was previously established for each strain under the same culture conditions. Inoculum OD_{600} was then adjusted accordingly to achieve the target concentration.

For *L. plantarum* TISTR 2070 and *L. paracasei* TISTR 2286, a single colony was picked from MRS agar, transferred to 10 mL of MRS broth, and incubated at 30 °C for 18–24 h. The optical density (OD) was adjusted to 1.0 at 600 nm before being scaled up in 100 mL of MRS broth at 30 °C for 8–12 h, reaching an OD of approximately 0.8. The bacterial suspension was centrifuged at 6000 × *g*, 4 °C for 15 min, washed twice with 0.2 g/L sodium chloride solution, and vortexed to ensure homogeneity before use.

For *A. pasteurianus* TISTR 102, a single colony from GYC agar was transferred to 10 mL of GYC broth and incubated at 30 °C for 48–72 h under shaking conditions. The culture was then scaled up in 100 mL of GYC broth and incubated under the same conditions until an OD of 0.8 was reached. The bacterial suspension was processed as described above.

2.3.2. Fermentation process

The fermentation medium contained 20 g/L of seagrape powder dissolved in distilled water and sterilized at 121 °C for 20 min. Each bacterial strain (LP2070, LC2286, and AP102) was inoculated individually at 50 mL/L (7–8 log CFU/mL), with and without supplementation of 30 g/L sucrose. All fermentations were carried out in sterile flasks covered with cotton plugs, under static, semi-anaerobic conditions at 30 °C without shaking. All experiments were conducted in triplicate (n = 3), with independent biological replicates.

2.3.3. Microbiological determination

Microbial enumeration was conducted using the plate method to determine the viable counts of lactic acid bacteria (LAB) and acetic acid bacteria (AAB) during fermentation. LAB were cultured on de Man, Rogosa, and Sharpe (MRS) agar supplemented with bromocresol purple and incubated at 30 °C for 24–48 h until distinct colonies were observed for enumeration, whereas AAB were cultured on glucose, yeast extract, and calcium carbonate (GYC) agar (Himedia, India) at 30 °C for 48 h. The results were expressed as log colony-forming units per milliliter (log CFU/mL).

2.3.4. Analysis of pH, sugars and organic acids

The pH of the fermentation samples was measured using a pH meter (OAKTON). Total sugar and lactic acid concentrations were analyzed by high-performance liquid chromatography (HPLC) equipped with a refractive index detector (RID) and a diode array detector (DAD). The separation was performed using an Aminex HPX-87H column (300 × 7.8 mm) at 40 °C with 5 mM H_2SO_4 as the mobile phase at an isocratic flow rate of 0.6 mL/min. Filtered samples (0.22 μm) were injected at a volume of 10 μL, and the detection of sugars (sucrose, glucose, fructose) and organic acids (lactic acid and acetic acid) was carried out based on calibration curves. Method validation demonstrated excellent linearity

($R^2 > 0.99$), specificity, and recovery (>95 %) for each quantified compound, ensuring the accuracy and reliability of the analytical results. The final results were expressed as g/L (Chomphoosee et al., 2025).

2.3.5. Analysis of total phenolic content (TPC)

The total phenolic content (TPC) was quantified using the Folin-Ciocalteu method (Geremu et al., 2016). A sample of 80 μL was added to a reaction mixture containing 400 μL of 2 M Folin reagent, followed by the addition of 320 μL of 0.7 M Na_2CO_3. The mixture was incubated in the dark at room temperature for 45 min, and the absorbance was measured at 765 nm. TPC values were expressed as μg gallic acid equivalents per mL (μg GAE/mL) based on a standard curve of gallic acid.

2.3.6. Antioxidant capacity

Antioxidant activity was assessed using three different assays. The DPPH radical scavenging activity was measured by mixing 250 μL of the sample with 500 μL of DPPH solution, vortexed briefly, and incubated at room temperature in the dark for 30 min before measuring the absorbance at 517 nm (Xia et al., 2014). The ABTS radical scavenging activity was determined by combining 200 μL of ABTS working solution with 10 μL of the sample, thoroughly mixed, and incubated in the dark for 7 min before measuring the absorbance at 734 nm (Xiao et al., 2020). The ferric-reducing antioxidant power (FRAP) assay was performed by mixing 180 μL of FRAP reagent with 5 μL of the sample, followed by incubation at 37 °C for 15 min, and absorbance was recorded at 593 nm (Xiao et al., 2020). The antioxidant activities of all assays were expressed as μg Trolox equivalents per mL (μg TE/mL).

2.4. Effects of coffee cherry supplementation on the fermentation of seagrape by L. plantarum TISTR 2070

To investigate the impact of coffee cherry supplementation on the fermentation of seagrape, 50 g/L of seagrape powder and 25 g/L of coffee cherry powder were dissolved in distilled water, sterilized (121 °C for 20 min), and fermented with *L. plantarum* TISTR 2070 (LP2070) at 30 °C for 72 h. Samples were collected every 24 h for microbial viability, pH, sugar and organic acid content, total phenolic content (TPC), and antioxidant activity analyses.

2.5. Liquid chromatography–electrospray ionization quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) analysis and identification of bioactive compounds

Bioactive compounds in the fermented and unfermented samples were analyzed using liquid chromatography–electrospray ionization quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). An Agilent 1290 Infinity II LC system coupled with an Agilent 6545B QTOF-MS and equipped with an Agilent Poroshell 120 EC—C18 column (2.1 × 150 mm, 2.7 μm) was used. The mobile phase consisted of 0.1 g/L formic acid in water (eluent A) and 0.1 g/L formic acid in acetonitrile (eluent B), delivered at a flow rate of 0.2 mL/min. The gradient elution was programmed as follows: 0 min, 5 % B; held at 5 % B until 1 min; increased to 17 % B at 10 min and held until 13 min; ramped to 95 % B at 20 min and held until 25 min; returned to 5 % B by 27 min; and re-equilibrated at 5 % B until 35 min. The column temperature was maintained at 35 °C, and the injection volume was 1 μL.

Mass spectrometric analysis was performed using an electrospray ionization (ESI) source under the following conditions: gas temperature 300 °C, gas flow rate 10 L/min, nebulizer pressure 35 psig, sheath gas temperature 350 °C, sheath gas flow rate 11 L/min, capillary voltage (Vcap) 3500 V, nozzle voltage 1000 V, fragmentor voltage 175 V, Skimmer 1 voltage 65 V, and Octopole RF voltage 750 V. The mass range was set from m/z 100 to 1100, with a scan rate of 2 spectra per second. Data acquisition and processing were performed using Agilent MassHunter Data Acquisition and MassHunter Qualitative Analysis software (version B.08.00).

2.6. Cytotoxicity assay (MTT assay)

The cytotoxicity of fermented extracts was assessed using human colon cells cultured in RPMI-1640 medium supplemented with 100 mL/L FBS at 37 °C under 5 % CO_2. Cells were seeded at 1×10^4 cells/well in a 96-well plate, treated with 0–16,000 μg/mL extract, and incubated for 72 h. MTT solution (0.5 mg/mL) was added for 3 h, followed by DMSO extraction, and absorbance was measured at 570 nm to determine the CC_{50} value.

2.7. Sensory analysis

The sensory evaluation was conducted following approval by the Chiang Mai University Research Ethics Committee (CMUREC No 66/331) on November 21, 2023. All participants provided informed consent before participation and received financial compensation. The study adhered to ethical guidelines and regulations for human research protection, including the Declaration of Helsinki, the International Conference on Harmonization in Good Clinical Practice (ICH-GCP), and The Belmont Report. The evaluation method was adapted from Amaral et al. (2018) with modifications.

The fermented beverage produced was a functional fermented drink characterized by a slightly turbid appearance and mild acidity, without filtration to retain its natural texture and bioactive components. For sensory evaluation, three formulations were tested: (1) unfermented SG, (2) SG fermented with *L. plantarum* TISTR 2070 and sucrose (SG+LP2070+Suc), and (3) SG co-fermented with coffee cherry pulp, *L. plantarum* TISTR 2070, and sucrose (SG+CC+LP2070+Suc). All samples were stored at 4 °C and served chilled (4–6 °C) to the panelists.

A panel of 50 trained participants (aged between 21 and 50 years old, 24 % male and 76 % female) with prior experience in food sensory evaluation was recruited. Samples were freshly prepared and randomly served to minimize bias. The participants evaluated appearance, color, smell, texture, mouthfeel, acidity, sweetness, saltiness, overall taste, and overall acceptability using a 5-point hedonic scale, where 1 = least preferred and 5 = most preferred.

2.8. Statistical analysis

All experimental data were expressed as mean ± standard error (SE). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test at a significance level of $p \leq 0.05$. The analysis was conducted using IBM SPSS Statistics 17.0. Data are presented as mean ± standard error (SE) from three independent experiments ($n = 3$).

3. Results and discussion

3.1. Proximate composition analysis of seagrapes and coffee cherry pulp

The proximate composition analysis of seagrape (*C. lentillifera*) on a dry weight basis in this study revealed 63.02±0.21 % carbohydrates, 11.36±0.01 % protein, 0.29±0.03 % fat, 19.55±0.09 % ash, and 5.78 ±0.14 % moisture. These values differ from a previous study conducted in Indonesia, which reported significantly lower protein (14.40 %), higher fat (0.85 %), and similar moisture content (5.40 %) (Sinurat and Fadjriah, 2019). In contrast, an analysis of seagrape from Taiwan found higher carbohydrate content (66.97 %) but lower protein levels (9.26 %), with an ash content of 22.20 % (Nguyen et al., 2011). These variations suggest that the proximate composition of *C.* lentillifera is influenced by geographical origin and environmental factors.

For coffee cherry pulp, the composition was determined as 73.54

±0.20 % carbohydrates, 7.21±0.01 % protein, 1.70±0.08 % fat, 8.72 ±0.15 % ash, and 8.82±0.07 % moisture. These values are consistent with previously reported data, which indicated carbohydrate content ranging from 78.47 % to 78.70 %, protein levels between 9.70 % and 11.13 %, and fat content between 1.50 % and 4.10 %. Variations in ash and moisture content were also observed, with reported values ranging from 0.32 % to 4.20 % for ash and 5.98 % to 8.82 % for moisture (Rosas-Sanchez et al., 2021; Sangta et al., 2021). These differences may be attributed to variations in geographic origin, environmental conditions, and processing methods.

The findings of this study highlight the importance of selecting appropriate raw materials for the development of fermented beverages. The high ash content in seagrape suggests a rich mineral profile, which may offer potential health benefits. Meanwhile, the high carbohydrate content in coffee cherry pulp could serve as an essential energy source in fermented beverages.

3.2. Microbial viability during fermentation of seagrape juice

The fermentation process of seagrape juice revealed distinct survival patterns among lactic and acetic acid bacteria, particularly with sucrose supplementation (Table 1). Among the tested strains, LP2070 with 30 g/L sucrose exhibited the highest viability (6.54±0.01 Log CFU/ml at 72 h), showing a minimal reduction from its initial count of 7.04±0.02 Log CFU/ml. LC2286 also exhibited greater stability with sucrose supplementation, maintaining a cell count of 6.36±0.07 Log CFU/ml at 72 h, compared to a more pronounced decline to 5.43±0.03 Log CFU/ml in the non-supplemented condition. AP102 exhibited the lowest survival, with final counts of 5.42±0.04 Log CFU/ml without sucrose and 5.68 ±0.02 Log CFU/ml with sucrose.

The survival capability of LP2070 and LC2286 highlights their potential for application in probiotic-rich fermented beverages. This finding is in agreement with previous studies demonstrating that sucrose serves as both an energy source and a protective agent against environmental stress (Yin et al., 2018). *L. plantarum* strains are recognized for their adaptability to acidic and alkaline conditions (Tuerhong et al., 2024), which likely enhances their survival throughout fermentation. This pH tolerance is crucial for maintaining viability in functional fermented beverages, where microbial survival directly impacts probiotic efficacy and product stability (Tripathi and Giri, 2014). Selecting highly adaptable strains, such as LP2070 and LC2286, may enhance both microbial stability and functional benefits in the final product (Bentahar et al., 2024).

3.3. Changes in sugar content, pH, and organic acids during fermentation

During fermentation, microbial activity and biochemical transformations were closely interconnected (Sawant et al., 2025). The metabolic activities of lactic acid bacteria (LAB) and acetic acid bacteria (AAB) led to the depletion of carbon sources and the production of organic acids, primarily lactic and acetic acids. As shown in Table 2, sucrose-supplemented fermentations resulted in lactic acid accumulation (0.45–0.48 g/L), whereas acetic acid production was only detected in the AP102+Suc group (0.28 g/L). These metabolic processes corresponded with a significant pH reduction from an initial near-neutral range (6.11–6.18) to notably lower values after 72 h ($p \leq 0.05$). The most pronounced acidification was observed in the LP2070+Suc and LC2286+Suc groups, with final pH values of 3.10±0.02 and 3.12±0.01, respectively, suggesting efficient sucrose metabolism into lactic acid. In contrast, the AP102+Suc group exhibited a higher final pH (3.61±0.01), likely due to the predominant production of acetic acid rather than lactic acid.

The results further highlight a strong correlation between bacterial metabolism and sugar depletion. In non-supplemented groups, bacterial viability significantly declined ($p \leq 0.05$), likely due to limited carbon availability. In contrast, sucrose supplementation supported LAB survival and led to greater sugar reduction (Sionek et al., 2024). Among all tested conditions, LP2070+Suc exhibited the highest sugar depletion (~13.84 %), indicating its superior metabolic efficiency in carbohydrate utilization.

Interestingly, residual sugars remained in sucrose-supplemented samples, suggesting that some strains did not fully metabolize all available carbohydrates within the given fermentation period. This could influence the final product's sweetness and sensory attributes, emphasizing the need for further optimization of fermentation parameters (Tlais et al., 2024).

Table 1
Total viable cell counts of fermented seagrape-based beverages.

Experiment	Total viable cell count (Log CFU/ml)			
Time (h)	0	24	48	72
LP2070	7.02 ± 0.01^{Ab}	7.06 ± 0.01^{Ca}	6.86 ± 0.01^{Bc}	5.67 ± 0.02^{Cd}
LP2070+Suc	7.04 ± 0.02^{Ab}	7.48 ± 0.02^{Aa}	7.03 ± 0.02^{Ab}	6.54 ± 0.01^{Ac}
LC2286	7.07 ± 0.01^{Aa}	7.11 ± 0.01^{Ba}	6.65 ± 0.04^{Cb}	5.43 ± 0.03^{Dc}
LC2286+Suc	7.10 ± 0.01^{Ab}	7.42 ± 0.06^{Aa}	6.99 ± 0.01^{Ab}	6.36 ± 0.07^{Bc}
AP102	7.04 ± 0.02^{Aa}	6.96 ± 0.01^{Ca}	6.39 ± 0.07^{Db}	5.42 ± 0.04^{Dc}
AP102+Suc	7.08 ± 0.00^{Ab}	7.20 ± 0.01^{Ba}	6.97 ± 0.01^{Ac}	5.68 ± 0.02^{Cd}

Different lowercase letters indicate a significant difference within a row, and different uppercase letters indicate a significant difference within a column ($p \leq 0.05$), according to the Tukey test.
SG (20 g/L) and sucrose (30 g/L) were used in the experiment.

3.4. Total phenolic content and antioxidant activity of fermented seagrape juice

The fermentation process significantly influenced the antioxidant activity and total phenolic content (TPC) of seagrape juice (Fig. 1). Fermentation for 48–72 h led to a significant increase in antioxidant activity ($p \leq 0.05$), particularly in the groups supplemented with 30 g/L sucrose (Fig. 1B, 1C and 1D). Among the tested strains, LP2070+Suc exhibited the highest antioxidant capacity, as measured by DPPH radical scavenging activity (22.87±0.12 μg TE/ml), FRAP (10.60±0.45 μg TE/ml), and ABTS (6.69±0.19 μg TE/ml). Additionally, this group displayed the highest total phenolic content (TPC) at 30.68±0.24 μg GAE/ml (Fig. 1A), which was significantly higher than other experimental groups ($p \leq 0.05$).

Comparing the results with the control and other experimental groups, it is evident that the release of phenolic compounds may result from the breakdown of phenolic-bound sugar complexes during fermentation, potentially through cell wall degradation and enzymatic hydrolysis mediated by microbial activity (Elhalik et al., 2024). This is supported by previous studies demonstrating that lactic acid bacteria, particularly *L. plantarum*, enhance antioxidant activity by producing bioactive metabolites and promoting the release of bound phenolics (Shi et al., 2019). Moreover, *L. plantarum* has been reported to exhibit strong free radical scavenging activity, which is associated with potential anti-inflammatory effects, apoptosis induction in cancer cells, and improved gut survival under harsh conditions (Wu et al., 2023).

These findings highlight the potential of *L. plantarum* TISTR2070 in functional fermented beverages. Its high metabolic efficiency in phenolic compound biotransformation suggests that it could be a promising strain for developing health-promoting probiotic products with enhanced antioxidant properties. Further optimization of fermentation conditions is necessary to maximize the release of bioactive compounds while maintaining sensory acceptability and product stability.

3.5. Enhanced microbial viability, sugar metabolism, and antioxidant activity in fermented seagrape juice supplemented with coffee cherry pulp

To develop a functional beverage with enhanced bioactive

Table 2
Changes in pH, sugar content, lactic acid and acetic acid of fermented seagrape-based beverages.

Experiment	pH		Sugar concentration (g/L)		Lactic acid (g/L)		Acetic acid (g/L)	
Time (h)	0	72	0	72	0	72	0	72
Unfermented	6.18±0.00 ^{Aa}	6.20±0.00 ^{Aa}	ND	ND	ND	ND	ND	ND
LP2070	6.11±0.00 ^{Ba}	5.70±0.01 ^{Cb}	ND	ND	ND	ND	ND	ND
LP2070+ Suc	6.14±0.01 ^{Ba}	3.10±0.02 ^{Fb}	32.06±0.08 ^{Ba}	27.62±0.04 ^{Bb}	ND	0.45±0.01 ^{A}	ND	ND
LC2286	6.16±0.01 ^{Aa}	5.54±0.02 ^{Db}	ND	ND	ND	ND	ND	ND
LC2286+ Suc	6.15±0.00 ^{Aa}	3.12±0.01 ^{Fb}	33.34±0.12 ^{Aa}	29.06±0.05 ^{Ab}	ND	0.48±0.00 ^{A}	ND	ND
AP102	6.18±0.01 ^{Aa}	5.96±0.02 ^{Bb}	ND	ND	ND	ND	ND	ND
AP102+ Suc	6.17±0.02 ^{Aa}	3.61±0.01 ^{Eb}	31.89±0.06 ^{Ca}	27.10±0.11 ^{Bb}	ND	ND	ND	0.28±0.02 ^{A}

Different lowercase letters indicate a significant difference within a row, and different uppercase letters indicate a significant difference within a column ($p \leq 0.05$), according to the Tukey test.
SG (20 g/L) and sucrose (30 g/L) were used in the experiment.
ND indicates 'Not Detectable'.
Sugar content represents the sum of fructose, glucose, and sucrose concentrations.

properties, coffee cherry pulp (CC), which has been reported to be rich in bioactive compounds that increase after fermentation (Chomphoosee et al., 2025), was incorporated into the fermentation of seagrape (*C. lentillifera*, SG). The co-fermentation of SG and CC with *L. plantarum* TISTR 2070 (LP2070) demonstrated a significant impact on microbial viability, sugar consumption, acid production, total phenolic content, and antioxidant activity. At 72 h, the viable cell count in the CC-supplemented group (5.77±0.05 Log CFU/ml) was significantly higher than in the control group without CC (5.02±0.04 Log CFU/ml) ($p \leq 0.05$) (Fig. 2A).

Sugar metabolism analysis also revealed that CC supplementation resulted in a greater reduction in total sugar concentration (Fig. 2B). The SG+CC+LP2070+Suc group exhibited a final sugar concentration of 22.64±0.13 g/L, significantly lower than the SG+LP2070+Suc group (24.79±0.85 g/L) ($p \leq 0.05$). This indicates that coffee cherry pulp contributes additional fermentable sugars, such as glucose and fructose, facilitating enhanced microbial activity (Sangta et al., 2024). Correspondingly, lactic acid production was significantly higher in the CC-supplemented group (Fig. 2D), reaching 1.21±0.02 g/L, more than double that of the non-supplemented group (0.60±0.01 g/L). This increased lactic acid accumulation correlated with a greater pH reduction, where the final pH (3.03±0.06) in the CC group was significantly lower than in the non-supplemented group (pH 3.38±0.00) ($p \leq 0.05$) (Fig. 2C). The higher acidification rate suggests that CC not only serves as an additional energy source but also enhances microbial metabolic efficiency (Picon et al., 2024).

Phenolic compounds have been shown to enhance microbial resilience against oxidative stress and fermentation-associated environmental fluctuations (Kwaw et al., 2018). In this study, fermentation significantly increased the total phenolic content (TPC) and antioxidant activity of seagrape juice supplemented with coffee cherry (Table 2E). The TPC in the SG+CC+LP2070+Suc group increased from 2250.00 ±19.09 μg GAE/ml to 2620.75±20.97 μg GAE/ml after fermentation ($p \leq 0.05$), a significant rise compared to the unfermented control groups. In contrast, the unfermented SG and CC controls exhibited either no increase or a slight decline in TPC.

Similarly, Chomphoosee et al. (2025) reported that fermentation alters phenolic profiles in coffee cherry pulp extract, mainly yielding hydroxycinnamic and hydroxybenzoic acids. Among these, hydroxycinnamic derivatives—such as 5-caffeoylquinic acid, feruloylquinic acid, and isoferulic acid—were predominant. Fermentation also enhanced the production of organic acids and bioactive compounds, including 3-aminobenzoic acid, chlorogenic acid, quinic acid, trigonelline, feruloylquinic acid, isoferulic acid, and propionic acid, in coffee cherry water kefir with sucrose, indicating an improvement in bioactive compound bioavailability. These findings suggest that co-fermentation promotes the release of bound phenolic compounds, likely due to enzymatic degradation of plant cell matrices by microbial activity.

In addition to TPC, antioxidant activity significantly increased in the SG+CC+LP2070+Suc group. The DPPH radical scavenging activity increased from 1136.88±4.88 μg TE/ml to 1236.02±5.54 μg TE/ml ($p \leq 0.05$), FRAP activity rose from 446.43±7.29 μg TE/ml to 619.64±3.86 μg TE/ml, and ABTS activity increased from 436.57±20.71 μg TE/ml to 568.89±5.85 μg TE/ml (Table 2F-H). These results suggest that coffee cherry enhances the antioxidant potential of the fermented product (Chomphoosee et al., 2025).

The increased antioxidant activity in the coffee cherry-supplemented group may be linked to the production of bioactive metabolites, such as phenolic acids and exopolysaccharides (EPS), which are known for their radical-scavenging properties (Elhalik et al., 2024). These bioactive components may stabilize bacterial cell membranes, enhance stress tolerance, and influence secondary metabolite production, leading to prolonged bacterial survival and enhanced metabolic efficiency. These findings highlight the potential of coffee cherry as a functional ingredient in fermented beverages, enhancing both the metabolic activity of *L. plantarum* and the overall biochemical characteristics of the final product.

3.6. Effects of cold storage (4 °C) on co-fermented seagrape and coffee cherry pulp beverage

Cold storage (4 °C) played a crucial role in preserving the stability of co-fermented SG and CC beverage, particularly in microbial viability, sugar consumption, pH, and organic acid production. *L. plantarum* TISTR 2070 showed a gradual decline in viability over four weeks (Fig. 3A), a typical trend in probiotic beverages due to dormancy, lysis, or oxidative stress (Davis, 2014).

Sugar concentration decreased (Fig. 3B), likely due to residual microbial activity. The greater reduction in SG+CC+LP2070+Suc suggests additional fermentable substrates from coffee cherry pulp (Chomphoosee et al., 2025). pH remained stable (~3.0) in both fermented samples (Fig. 3C), an advantage in inhibiting spoilage microorganisms (Barcenilla et al., 2023). Lactic acid content increased slightly in early storage before stabilizing (Fig. 3D), indicating continued metabolic activity. This aligns with previous findings in probiotic beverages where post-fermentation acidification occurs due to residual sugar metabolism (Nag and Das, 2013).

Antioxidant activity (TPC, DPPH, FRAP, ABTS) remained stable throughout storage (Fig. 3E-H), suggesting fermentation enhances phenolic bioavailability while cold storage preserves functionality. The higher initial antioxidant levels in fermented samples highlight their potential as functional beverages (Tang et al., 2023). Overall, cold storage effectively maintained viability and biochemical stability, reinforcing the potential of this fermented beverage as a functional drink.

3.7. LC-QTOF-MS analysis of bioactive compounds

LC-QTOF-MS analysis was performed to further elucidate the

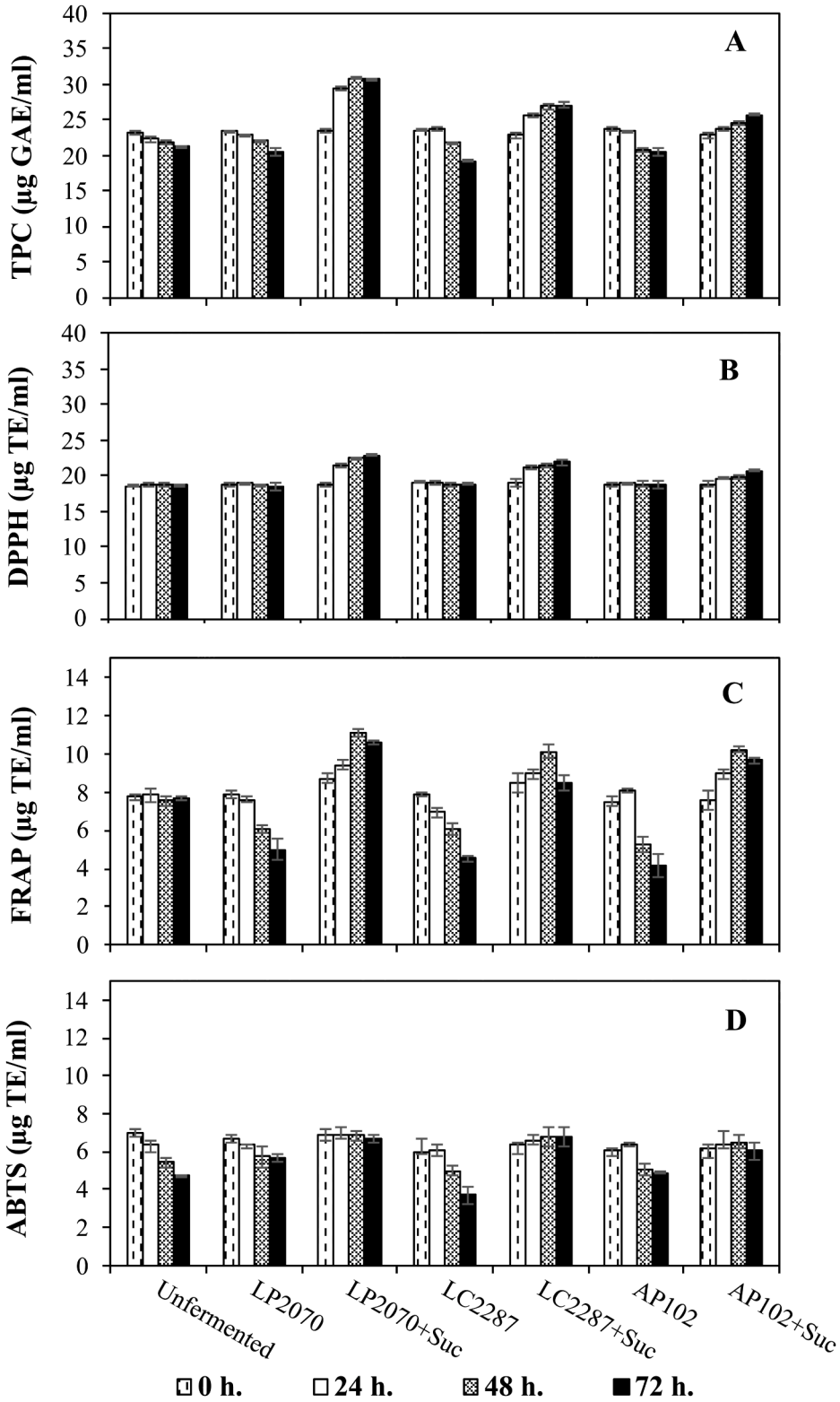

Fig. 1. Changes in TPC (A), DPPH (B), FRAP (C), and ABTS (D) during fermentation with different bacterial strains over 72 h.

bioactive compounds contributing to the antioxidant activity of the fermented beverages (Table 3). The metabolite profiles revealed substantial differences between unfermented and fermented samples, particularly in the SG+CC+LP2070+Suc formulation. Fermentation led to the enhancement of various classes of bioactive compounds, including phenolic acids, organic acids, amino peptides, and alkaloids.

Notably, chlorogenic acid, 5Z-caffeoylquinic acid, and methyl chlorogenate were detected exclusively in the co-fermented samples, correlating strongly with the increased antioxidant capacity measured by DPPH, FRAP, and ABTS assays. These phenolic compounds are well known for their potent radical scavenging properties and ability to modulate oxidative stress (Luo et al., 2025; Park et al., 2023). The presence of organic acids such as d-glucaric acid and l-gulonate further supports their contribution to the antioxidant defense system through

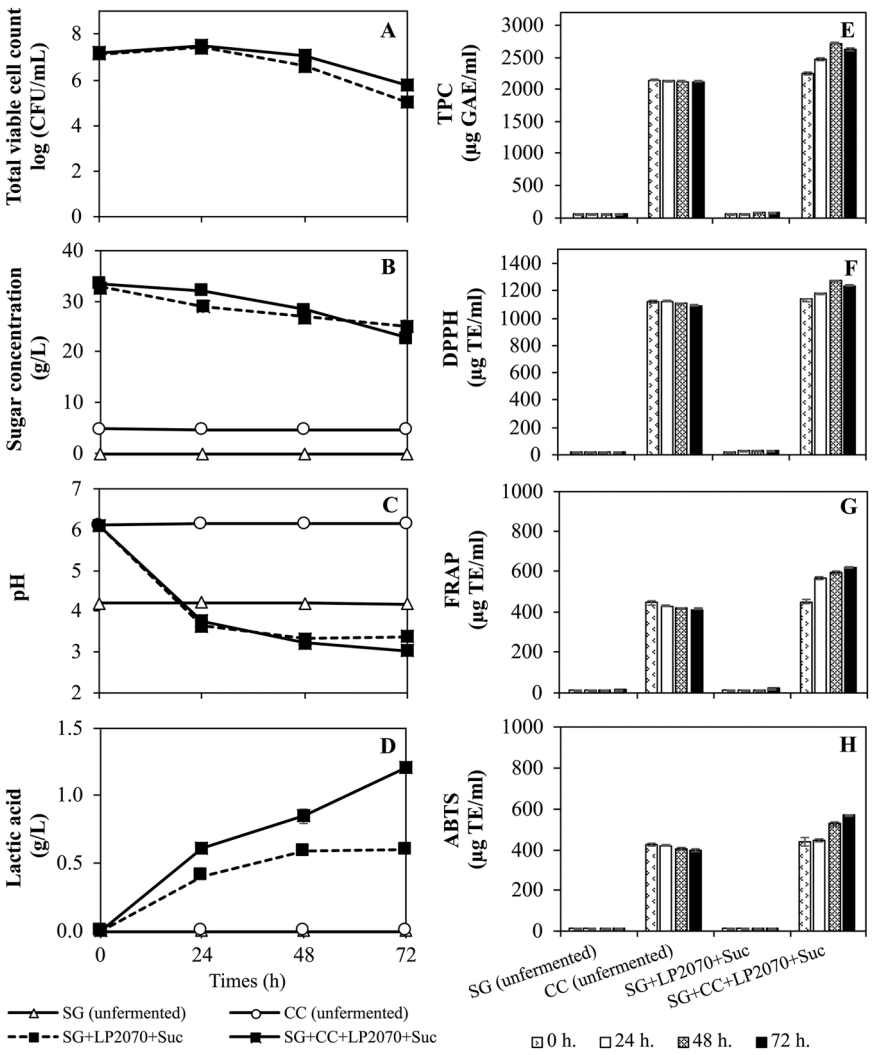

Fig. 2. Changes in total viable cell count (A), sugar concentration (B), pH (C), and lactic acid (D), TPC (E), DPPH (F), FRAP (G), and ABTS (H), during the fermentation of SG and CC with *L. plantarum* TISTR 2070 (LP2070) and sucrose over 72 h.

detoxification and vitamin C biosynthesis pathways (Karachaliou and Livaniou, 2024).

In addition, amino peptides such as l-isoleucyl-l-proline and l-valine, along with the alkaloid trigonelline, were identified in the fermented beverage. These metabolites are known to contribute to antioxidant mechanisms, cellular repair, and additional health benefits, including neuroprotective and antidiabetic effects (Nguyen et al., 2024). The detection of dethiobiotin, a precursor of vitamin B7, further suggests a potential role in metabolic health (Karachaliou and Livaniou, 2024).

Overall, the LC-QTOF-MS results confirm that co-fermentation of SG and CC with L. *plantarum* TISTR 2070 enhances the release and biotransformation of diverse bioactive metabolites. These findings align with the observed increases in antioxidant activity and suggest a synergistic contribution of multiple metabolite classes to the functional quality of the final product (Darko et al., 2025).

3.8. Cytotoxicity test on human colon cells

The cytotoxicity of fermented SG and CC beverage was assessed using human colon cells, with CC_{50} values representing the concentration required to induce 50 % cell death. The unfermented SG exhibited a CC_{50} of 16,020 ± 445.6 µg/ml, indicating moderate cytotoxicity. When 30 g/L sucrose was added to the SG, the CC_{50} value increased to 18,350 ± 728.8 µg/ml ($p \leq 0.05$), suggesting a reduction in cytotoxicity. This may be due to the role of sucrose in modulating the bioactivity of phenolic compounds or diluting potential cytotoxic constituents.

A significant improvement was observed when CC was co-fermented with SG and sucrose, leading to the highest CC_{50} value of 25,860 ± 336.6 µg/ml, which was significantly different from both the SG and SG+Suc groups ($p \leq 0.05$). This substantial reduction in cytotoxicity suggests that bioactive metabolites produced during fermentation, particularly phenolic compounds and their derivatives, may contribute to enhanced cellular protection (Magoni et al., 2018). The presence of coffee cherry appears to modulate the extract's bioactivity, possibly by altering the composition of phenolic compounds and secondary metabolites during fermentation. These findings highlight the potential application of SG+CC+Suc fermentation as a strategy to reduce cytotoxicity while maintaining beneficial bioactive properties. The results support the feasibility of using this fermentation system for the development of functional beverages with improved safety profiles for human consumption (Melini and Melini, 2021; Sawant et al., 2025).

3.9. Sensory analysis

Sensory evaluation assessed consumer acceptance of fermented and unfermented seagrape-based beverages using a 5-point hedonic scale. Three formulations were compared: (1) SG (unfermented), (2) SG+LP2070+Suc (fermented with *L. plantarum* TISTR 2070 and

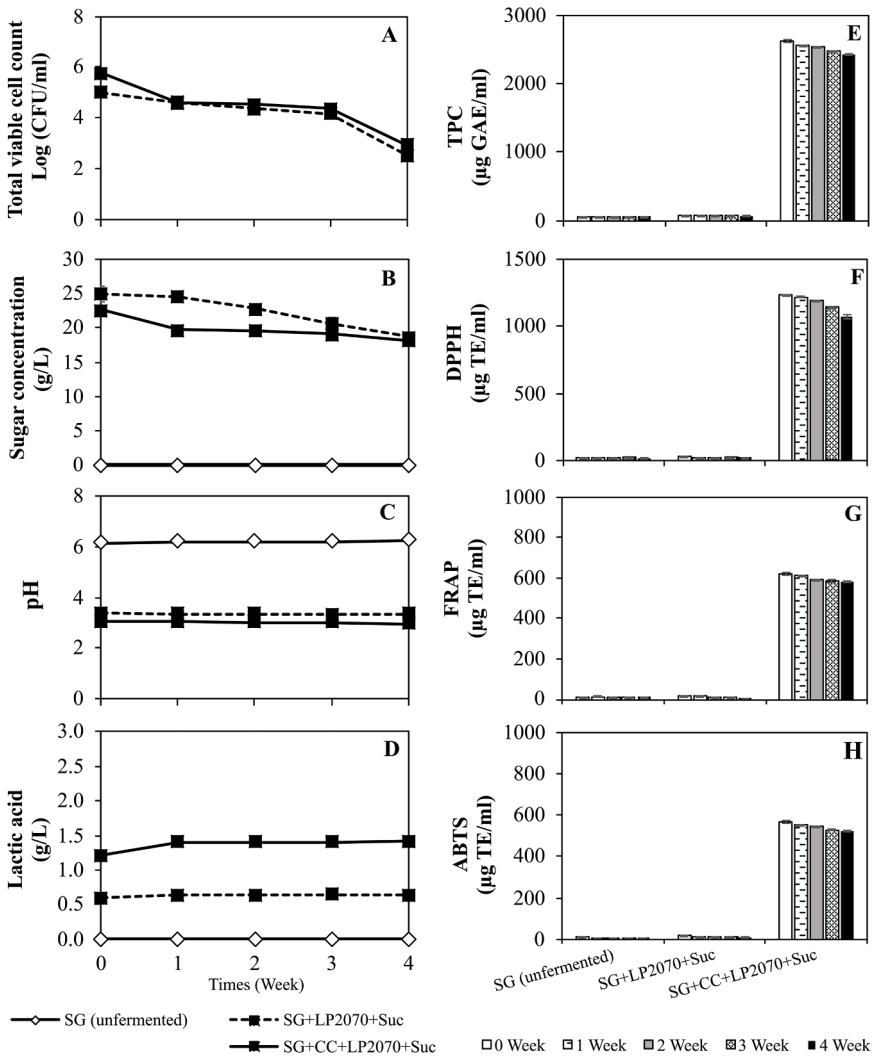

Fig. 3. Changes in total viable cell count (A), sugar concentration (B), pH (C), lactic acid (D), TPC (E) and antioxidant properties (F–H) during cold storage of fermented SG and CC with *L. plantarum* TISTR 2070 and sucrose.

sucrose), and (3) SG+CC+LP2070+Suc (co-fermented with coffee cherry pulp, *L. plantarum* TISTR 2070, and sucrose).

As shown in Fig. 4, SG (unfermented) received the highest appearance and color scores, likely due to fermentation-induced pigment degradation. In contrast, fermented formulations were rated significantly higher in smell, texture, mouthfeel, acidity, sweetness, overall taste, and acceptability ($p \leq 0.05$). SG+LP2070+ Suc and SG+CC+LP2070+Suc exhibited comparable high scores in taste complexity, mouthfeel, and overall acceptability, indicating that both fermentation strategies improved sensory perception relative to the unfermented control. This suggests that the fermentation process led to noticeable changes in visual characteristics, potentially due to pigment degradation or precipitation of bioactive compounds from microbial activity. These observations align with previous studies indicating that fermentation can modify the color stability of plant-based substrates due to enzymatic reactions and microbial metabolism (Wu et al., 2023).

The acidity of SG+CC+LP2070+Suc was significantly higher than the other formulations, attributed to increased lactic acid production. Additionally, the sweetness balance was optimized in SG+CC+LP2070+Suc, with 83.33 % of panelists rating it as Just About Right (JAR). Saltiness perception was also improved in the co-fermented formulation, mitigating the excessive saltiness observed in SG (unfermented). Texture and mouthfeel improvements in the fermented beverage may be associated with exopolysaccharide (EPS) production by *L. plantarum*, which has been reported to enhance the viscosity and body of fermented beverages (Elhalik et al., 2024).

These results suggest that coffee cherry pulp enhances not only bioactivity but also sensory appeal, improving taste balance and acceptability. Further optimization could refine visual attributes while maintaining functional and sensory benefits. The findings align with previous reports on the impact of fermentation on probiotic beverage characteristics, demonstrating the potential of co-fermentation in enhancing consumer preference and functional properties (Picon et al., 2024; Tlais et al., 2024).

3.10. Principal component analysis (PCA) of physicochemical properties, bioactive compounds and antioxidant capacity

PCA was performed to explore the interrelationships among physicochemical properties, bioactive compounds, and antioxidant capacity of the seagrape-based fermented beverages. The first two principal components accounted for 78.81 % (PC1) and 27.19 % (PC2) of the total variance, indicating that the model effectively represented the data structure.

The PCA plot (Fig. 5) revealed clear associations among the measured variables. PC1 was primarily driven by lactic acid, lactic acid bacteria viability, FRAP, and ABTS, highlighting the central role of microbial fermentation in enhancing both bioactivity and antioxidant

Table 3
Identification of bioactive compounds in unfermented and fermented seagrape-based beverages using LC-QTOF-MS analysis.

No.	Proposed Compounds	Molecular Formula	SG (unfermented)		CC (unfermented)		SG+LP2070+Suc		SG+CC+LP2070+Suc	
			Mass	Matching score (%)	Mass	Matching score (%)	Mass	Matching score (%)	Mass	Matching score (%)
	Organic acids									
1	Orthothymotinic acid	$C_{11}H_{14}O_3$	193.0870	99.62	193.0869	99.56	193.0869	99.85	193.0870	99.74
2	Phloionolic acid	$C_{18}H_{36}O_5$	331.2492	98.15	331.2488	98.97	331.2492	83.50	331.2489	99.46
3	L-Gulonate	$C_6H_{12}O_7$	ND	–	ND	–	ND	–	195.0515	98.61
4	D-Glucaric acid	$C_6H_8O_7$	ND	–	191.0195	86.43	ND	–	191.0195	98.51
5	D-Galacturonic acid	$C_7H_{12}O_6$	ND	–	ND	–	ND	–	215.0532	85.04
	Amino and peptides									
6	5-Epi-valiolone	$C_7H_{12}O_6$	ND	–	ND	–	ND	–	191.0562	99.71
7	L-Valine	$C_5H_{11}NO_2$	118.0864	99.78	118.0864	87.57	118.0866	87.15	118.0863	99.57
8	L-isoleucyl-L-proline	$C_{11}H_{20}N_2O_3$	ND	–	ND	–	ND	–	229.1552	84.17
	Polyphenols and Phenolic acids									
9	Methyl chlorogenate	$C_{17}H_{20}O_9$	ND	–	367.1037	94.88	ND	–	367.1037	99.17
10	6-Hydroxycoumarin	$C_9H_6O_3$	ND	–	163.0391	97.62	ND	–	163.0394	98.86
11	5Z-Caffeoylquinic acid	$C_{16}H_{18}O_9$	ND	–	353.0875	83.96	ND	–	353.0874	98.00
12	Salicin	$C_{13}H_{16}O_8$	ND	–	299.0771	97.29	ND	–	299.0771	97.76
13	11,12,13-trihydroxy-9-octadecenoic acid	$C_{18}H_{34}O_5$	329.2331	84.77	329.2328	82.58	329.233	98.97	329.233	94.57
14	Chlorogenic acid	$C_{16}H_{18}O_9$	ND	–	353.0882	87.93	ND	–	353.0882	89.25
15	Quinic acid	$C_7H_{12}O_6$	ND	–	ND	–	ND	–	191.0556	75.71
	Alkaloids									
16	Trigonelline	$C_7H_8NO_2$	ND	–	138.0553	98.56	ND	–	138.0555	97.85
17	Xestoaminol C	$C_{14}H_{31}NO$	230.2488	96.56	230.2487	96.67	230.2488	96.04	230.2488	96.39
	Coumarin									
18	6-OH—Coumarin	$C_9H_6O_3$	ND	–	163.0391	97.62	ND	–	163.0394	98.86
	Vitamins and Coenzymes									
19	Dethiobiotin	$C_{10}H_{18}N_2O_3$	215.1395	85.60	ND	–	ND	–	215.1393	82.71
20	25 - Hydroxyvitamin D2 - 25 - (beta-glucuronide)	$C_{34}H_{52}O_8$	ND	–	ND	–	ND	–	611.3559	83.31

Matching score (%) indicates the quality of correlation between precursor ions and their corresponding fragment ion peaks for each compound; higher values reflect greater confidence in compound identification.
ND indicates not detected.

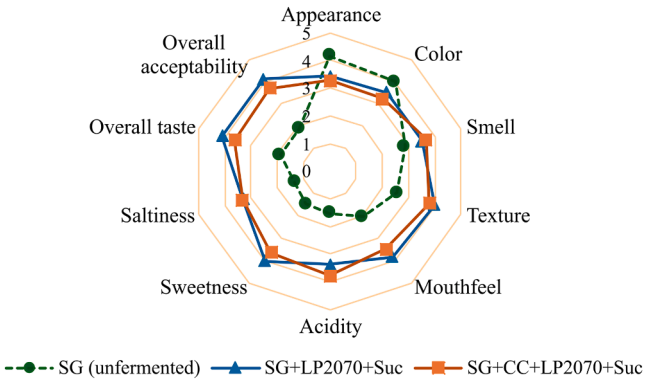

Fig. 4. Sensory evaluation of unfermented and fermented SG and CC with *L. plantarum* TISTR 2070, assessing appearance, taste, texture, and overall acceptability.

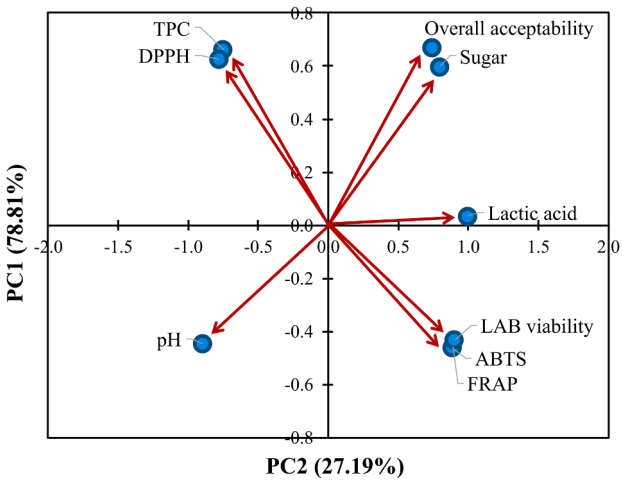

Fig. 5. Principal Component Analysis (PCA) of physicochemical properties, bioactive compounds and antioxidant capacity.

capacity (Sui et al., 2024). In contrast, pH exhibited a strong negative loading on PC1, reflecting the expected acidification during fermentation. The positioning of TPC and DPPH on PC1 also suggested a substantial contribution of phenolic compounds to the observed antioxidant effects. However, the negative loadings of TPC and DPPH may reflect the relatively high initial levels of these compounds in the unfermented samples. Consequently, the magnitude of change post-fermentation might have been less pronounced. Additionally, the distinct loading patterns observed among different antioxidant assays imply that the fermentation process could have selectively modified specific antioxidant compound classes, thereby influencing their relative contributions to the overall antioxidant profile (Tlais et al., 2024).

PC2 captured variation related to sensory attributes, with Overall acceptability and Sugar displaying high positive loadings. This indicates that fermentation not only modulated the biochemical properties but also influenced sensory quality. The alignment of sensory variables with antioxidant-related parameters suggests that the improved bioactive profile contributed positively to consumer-perceived acceptability (Filannino et al., 2013).

Overall, these findings confirm that the co-fermentation strategy effectively enhanced the functional and sensory properties of the seagrape-based beverages. The observed associations between bioactivity, microbial activity, and sensory outcomes provide valuable insights for optimizing fermentation processes aimed at developing

functional fermented beverages with improved health benefits and consumer appeal.

4. Conclusion

This study highlights the potential of seagrape (*C. lentillifera*) as a novel ingredient for functional beverage development. Traditionally consumed fresh or dried, seagrape possesses a rich profile of bioactive compounds, and fermentation further amplifies its health benefits. When co-fermented with coffee cherry pulp and *L. plantarum* TISTR 2070, the resulting beverage exhibited enhanced antioxidant activity, increased bioactive compound content, and improved microbial viability, while also reducing cytotoxicity. The synergistic interaction between seagrape and coffee cherry pulp not only boosted phenolic compound release but also enriched the functional properties of the final product.

Cold storage at 4 °C preserved the stability of the fermented beverage, maintaining its bioactivity and microbial viability with minimal alterations in sugar content and pH. This ensures that the beverage remains functionally beneficial over time, supporting its feasibility as a shelf-stable product. Sensory evaluation confirmed that fermentation improved taste complexity, mouthfeel, and overall acceptance, with the co-fermented formulation achieving an optimal balance of flavors. The integration of coffee cherry pulp, an underutilized but nutrient-rich byproduct, further enhanced the beverage's functionality while promoting sustainability through food waste reduction.

Multivariate analysis further confirmed strong correlations between microbial activity, antioxidant capacity, and sensory attributes, while LC-QTOF-MS analysis revealed the release and biotransformation of diverse bioactive metabolites contributing to the product's enhanced functional profile.

By combining the unique properties of seagrape and coffee cherry pulp through fermentation, this study presents a compelling approach to functional beverage innovation. The co-fermented product not only delivers enhanced bioactivity and improved safety but also offers a well-balanced sensory experience, positioning it as a promising candidate for health-oriented consumers.

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CRediT authorship contribution statement

Churairat Moukamnerd: Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Supanut Pothimoi:** Writing – review & editing, Visualization, Data curation. **Saranya Peerakietkhajorn:** Writing – review & editing, Resources. **Chittipong Tipbunjong:** Writing – review & editing, Investigation.

Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100688.

Data availability

Data will be made available on request.

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