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Strain-dependent lactic acid bacteria fermentation modulates nutritional quality and bioactive properties of phycocyanin-rich extract from *A. platensis*

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ABSTRACT

Phycocyanin-rich extracts from *Arthrospira platensis* (spirulina) are hailed for their bioactive properties, amino acid composition, and micronutrient content. However, their further valorisation through microbial fermentation remains largely underexplored. In this study, we applied strain-dependent lactic acid bacteria (LAB) fermentation as a process-engineering strategy to modulate the biochemical profile and functional potential of phycocyanin-rich extracts under controlled anaerobic conditions. *Lactiplantibacillus plantarum, Lactobacillus acidophilus*, and *Levilactobacillus brevis* all achieved high cell densities within 24 h, accompanied by strong acidification linked to glucose utilisation and lactic acid formation. Fermentation induced characteristic strain-specific changes, including reductions in phycobiliproteins, shifts in free amino acid profiles, biosynthesis of water-soluble vitamins (notably thiamine and pyridoxine), and accumulation of biogenic amines at levels well below safety thresholds. Fermented extracts also exhibited antimicrobial activity, particularly in *L. plantarum*-fermented samples. These findings suggest that LAB fermentation can significantly alter the nutritional quality and bio-functional potential of phycocyanin-rich extracts, providing mechanistic insights and knowledge for the development of a next-generation fermented algal-based functional ingredients for future food systems.

1. Introduction

In recent years, growing consumer awareness of the link between diet and chronic diseases has increased interest and demand for innovative functional ingredients with both nutritional and technological benefits (Fortuin et al., 2025). Among them, *Arthrospira platensis,* a photosynthetic cyanobacterium widely known as spirulina, has attracted considerable attention as a sustainable source of high-quality protein, essential amino acids, vitamins, minerals, and diverse bioactive compounds, which are incorporated as ingredients in the development of novel and functional food products (Lafarga et al., 2020). One of its most studied components, phycocyanin (PC), a water-soluble pigment-protein complex, is widely utilised in the food and pharmaceutical industries for its technological, functional and bioactive properties (Fernandes et al., 2023; Ziyaei et al., 2023).

Microbial fermentation is a well-established food processing technology that can enhance nutritional value, modify bioactive profiles, and contribute to product safety (Admassie, 2018; Xiang et al., 2019). Moreover, the health-beneficial properties of various fermented foods are attracting increasing attention from the scientific community and health-conscious consumers (Şanlier et al., 2019). Among fermenting microorganisms, lactic acid bacteria (LAB) play a central role in food systems due to their ability to produce organic acids, vitamins, and antimicrobial compounds, contributing to both functional and safety attributes of fermented products (Wang, Wu et al., 2021). LAB-mediated fermentations are increasingly applied in the development of functional foods, where their metabolic activities can modulate techno-functional properties and support nutritional enhancement (Mathur et al., 2020; Victoria Obayomi et al., 2024).

Building on this, algal fermentation has emerged as a strategy to further enhance the nutritional properties of algae, increase their activity, and expand their application in food, cosmetic, and functional products (Ahirwar et al., 2024; Babich et al., 2024; Sarıtaş et al., 2024). While recent studies have explored the fermentation of spirulina biomass by LAB, reporting enhanced probiotic growth and bioactivity (Kurt et al., 2023; Niccolai et al., 2019; Yay et al., 2024), systematic investigations on the fermentation's impact on the bio-functional role of spirulina protein isolates, especially phycocyanin-rich isolates, remain

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scarce.

This study applies LAB fermentation as a process-engineering strategy to enhance the nutritional and biofunctional properties of phycocyanin-rich extracts and to assess their potential as functional ingredients in food systems. To capture a wide range of possible biochemical transformations, three LAB strains with distinct metabolic traits were selected. *Lactiplantibacillus plantarum* was included due to its rapid acidification kinetics and bacteriocin production capacity; *Lactobacillus acidophilus* for its documented vitamin biosynthesis potential and proteolytic activity; and *Levilactobacillus brevis* for its heterofermentative metabolism and amino acid decarboxylation pathways. These metabolic profiles enable a comparative, strain-dependent evaluation of fermentation-driven modifications in phycocyanin-rich extracts. Overall, the objective of this work is to evaluate how strain-dependent LAB fermentation may influence PC stability, antioxidant capacity, amino acid profile, vitamin dynamics, and antimicrobial properties, thus providing critical insights for future development of functional foods and nutraceutical formulations.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid (3,4,5-trihydroxybenzoic acid, 99 %) was purchased from Sigma-Aldrich (Steinheim, Germany), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) from Sigma-Aldrich (St. Louis, USA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) purchased from Sigma-Aldrich Chemie (Taufkirchen, Germany), Folin-Ciocalteu's reagent from Sigma-Aldrich (Switzerland), sodium carbonate (Na_2CO_3) from Eurochemicals (Vilnius, Lithuania), sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), potassium chloride (KCl), potassium peroxodisulfate ($K_2S_2O_8$) purchased from Lach-Ner (Brno, Czech Republic), sodium hydrogen phosphate (Na_2HPO_4) from Merck KGaA (Darmstadt, Germany), methanol, copper(II) chloride ($CuCl_2$) from ThermoFisher (Kandel, Germany), neocuproine ($C_{14}H_{12}N_2$), from Sigma-Aldrich (St. Louis, USA), ammonium acetate ($NH_4CH_3CO_2$) from Reachem (Bratislava. Slovakia), acetonitrile (C_2H_3N), formic acid (HCOOH), ammonium sufate $(NH_4)_2SO_4$ from Eurochemicals (Vilnius, Lithuania), 17 amino acids standards including alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine from Sigma-Aldrich production (Buchs, Switzerland), water-soluble vitamin standards were purchased from Sigma-Aldrich. All solvents used were of analytical and HPLC grade.

2.2. Preparation of phycocyanin-rich extract

A. platensis biomass was purchased from UAB Mosus (Vilnius, Lithuania). The dry biomass was mixed with distilled water at a concentration of 100 g/L. The suspension underwent three successive freeze-thaw cycles to enhance cell disruption as previously described (Krakauskaitė et al., 2025). The mixture was then centrifuged at 5000 rpm for 15 min and filtered. The filtered extract was freeze-dried and stored in the freezer until further use.

2.3. Preparation of lactic acid bacteria cultures

L. plantarum DSM 24624, *L. acidophilus* DSM 20079, and *L. brevis* DSM 20556 cultures were provided by Biometrija (Kaunas, Lithuania). LAB was maintained on a slant De Man–Rogosa–Sharpe (MRS) agar EF 4017,282, Liofilchem, Italy) at +4 °C in a Liebherr ProfiLine refrigerator. For inoculum preparation, a single loopful of culture was transferred from the slant agar to 10 ml of MRS broth (REF 4017,292, Liofilchem, Italy). The inoculated tubes were incubated at 37 °C for 16–18 h. Overnight cultures were diluted by sterile saline solution (1:100) to obtain a working culture suspension containing lactic acid bacteria.

2.4. Fermentation of phycocyanin-rich extract

For the fermentations with phycocyanin-rich extract, 7 g of the freeze-dried powder was transferred to a 250 mL Duran flask containing 70 mL autoclaved, sterile, distilled water with 2 % glucose, and the mixture was mixed well under a laminar flow hood. Preliminary trials conducted without glucose supplementation resulted in negligible growth and acidification (data not shown). The mixture was then inoculated with *L. plantarum* DSM 24624 (7.9 log CFU/mL), *L. acidophilus* DSM 20079 (7.6 log CFU/mL), and *L.* brevis (7.8 log CFU/mL), individually, in three technical replicates under sterile conditions. The samples were placed in Thermo Scientific™ Oxoid™ AnaeroJar™ anaerostats (Thermo Scientific, UK) using Oxoid™ AnaeroGen™ Compact Sachet, and were maintained at 37 °C. Microbial counts, pH measurements, and pigment measurements for PC and APC were performed at 0, 24, and 48 h immediately after sampling, ensuring sterile conditions were maintained during sampling. The collected samples at 3 different time points were centrifuged (Orto alresa, Madrid, Spain) at 6000 rpm for 10 min, filtered through filter paper, and stored in the freezer at −45 °C for further analyses.

2.5. Microbiological analysis

Microbial enumeration was performed at 0, 24 and 48 h following established protocols using the pour-plating method. The number of viable bacterial cells grown in the sample was expressed as log colony-forming units per mL (log CFU/mL) and was determined at three time points (0, 24 and 48 h) by pour plating on de Man, Rogosa, and Sharpe media (MRS, Liofilchem, Italy) for the selective growth of lactobacillus strains. The MRS medium was prepared according to the manufacturer's recommendations by dissolving 14.04 g in 200 mL of distilled water and autoclaving at 121 °C for 15 min. The fermented sample was serially diluted 10-fold in physiological water (0.9 %, w/v NaCl) to obtain the required dilutions, and the media was poured in duplicate after cooling to 45–50 °C in a laminar flow hood to maintain aseptic conditions. The plates were then incubated at 37 °C for 48–72 h.

2.6. Spectrophotometric analysis of phycobiliproteins

Individual and total phycobiliprotein content was estimated spectrophotometrically using the equations of Bennet and Bogorad (Bennett & Bogorad, 1973) as previously reported (Syrpas et al., 2020). Briefly, absorbance measurements of optically clear supernatants were performed with a GENESYS 50 UV–Vis spectrophotometer (Thermo Fisher Scientific, Waltham, United States) immediately after sampling. Appropriate dilutions were prepared at each time point, and PC and allophycocyanin (APC) contents were estimated using Eqs. (1) and (2) below; analysis was performed in triplicate.

$$PC\left(\frac{mg}{mL}\right) = \frac{A_{620nm} - 0.474 \times A_{652nm}}{5.34} \quad (1)$$

$$APC\left(\frac{mg}{mL}\right) = \frac{A_{652nm} - 0.208 \times A_{620}}{5.09} \quad (2)$$

Where PC is phycocyanin, APC is allophycocyanin, A_{620nm} is the absorbance at 620 nm, and A_{652nm} is the absorbance at 652 nm.

2.7. Determination of in vitro antioxidant capacity

The in vitro antioxidant capacity of PC-rich samples was evaluated using the reducing power assay (Folin-Ciocalteu) and the cupric reducing antioxidant capacity (CUPRAC) assay, as previously reported (Bytautaitė et al., 2024; Nagybákay et al., 2023). Detailed descriptions of the methods are provided in the Supplementary Materials. All

measurements were performed on a GENESYS 50 UV–Vis spectrophotometer (Thermo Fisher Scientific, Waltham, United States). Analysis was performed in quadruplicate.

2.8. Chromatographic analyses

Determination of organic acids and glucose content was performed on a Shimadzu Prominence LC-20AD liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) coupled to a refractive index detector, as previously described in the literature (Dave et al., 2025). LC-MS/MS analyses were conducted in a Shimadzu LC-2050C3D UPLC system (Shimadzu Corporation, Kyoto, Japan) coupled to a Shimadzu LC-MS-8045 mass spectrometer. Free amino acid analysis, without prior derivatisation, was performed with slight modifications as previously reported (Wang et al., 2020). Water-soluble vitamins were analysed as described earlier (Aboobacker et al., 2025). Qualitative and quantitative determination of biogenic amines without prior derivatisation was performed following the methodology of Zhang et al., with slight modifications (X. Zhang et al., 2021). Detailed descriptions, along with MRM transitions and MS details, are provided in the supplementary material (Tables S1 and S2).

2.9. Determination of antimicrobial activity by agar diffusion method

The antibacterial properties of the samples against the reference strains *Escherichia coli* ATCC 8739, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25,923, and *Bacillus subtilis* ATCC 6633 were evaluated using the agar well diffusion method as previously reported (Grabauskaitė et al., 2024). To prepare the inoculum, the bacteria were cultured on a slant plate of count agar at 37 °C for 18–20 h. A loopful of the culture was then taken, resuspended in saline water, and adjusted to a 0.5 McFarland standard. The turbidity of the inoculum was adjusted to achieve a concentration equivalent to 10^8 CFU/mL. 1 mL of the test organisms was added to 100 mL of plate count media, poured onto plates, and allowed to solidify. Sterile pipette tips (9 mm in diameter) were used to create wells in the agar, into which 25 µL of each 24-hour fermented and unfermented control sample was dispensed. Subsequently, the plates were incubated at 37 °C for 48 h, after which the diameters of inhibition zones were measured in millimetres with an electronic digital calliper. Analysis was performed in triplicate.

2.10. Statistical analysis

Mean values and standard deviations of performed experiments were calculated using Microsoft Excel. Analysis of variance (ANOVA), followed by Tukey's post hoc test to compare the means that showed significant variation ($p < 0.05$), and Pearson's correlations were assessed using GraphPad Prism 10.4.2. software.

3. Results and discussion

3.1. Growth of LAB and acidification

In the first part of this study, the growth of the three inoculated lactic acid bacteria was monitored during fermentation. The growth dynamics of the three inoculated LAB strains during fermentation are presented in Fig. 1A. All strains were inoculated at an average initial load of 7.7 log CFU/mL and exhibited rapid proliferation within the first 24 h, after which growth stabilised. *L. plantarum* and *L. brevis* reached approximately 10.1 log CFU/mL (ΔLog ≈ 2.3), while *L. acidophilus* displayed comparatively lower growth, reaching 8.8 log CFU/mL (ΔLog ≈ 1.3).

Although growth characteristics depend on strain type, carbon source, and fermentation conditions, these observations align with previous reports showing that Spirulina biomass can be fermented with LAB. For instance, *L. plantarum* ATCC 8014 reached 10.6 log CFU/mL after 48 h when grown in spirulina-containing medium (Niccolai et al., 2019), while *L. plantarum* LMG 6907 increased from 7.0 to 8.5 log CFU/g within 24 h before stabilising (Jamnik et al., 2022). Similarly, mixed cultures of LAB (*L. plantarum, L. casei*, and *L. acidophilus*) achieved approximately 8.5 log CFU/mL after 24 h during spirulina fermentation (J. Yu et al., 2020). Additionally, strong proliferation (>9 log CFU/mL) of *L. plantarum* during mixed fermentation with *Bacillus subtilis* has also been reported (Bao et al., 2018).

As anticipated, growth trends strongly correlated with pH reduction, with results summarised in Fig. 1B The initial pH of the phycocyanin-rich extract was ~6.8 and decreased below 4.6 in all fermentations, a widely accepted cut-off value known to inhibit most foodborne pathogens (Peréz-Dıaz et al., 2013). Moreover, all strains exhibited rapid acidification, with samples fermented with *L. plantarum* reaching the lowest pH of 3.8 within 24 h, followed by *L. acidophilus* and *L. brevis* (Fig. 1B).

The observed acidification patterns are consistent with the metabolic characteristics of each strain. *L. plantarum* is frequently characterised by fast, homolactic carbohydrate fermentation and high lactic acid flux (Cui et al., 2021). *L. acidophilus* exhibited relatively slower initial acidification compared to *L. plantarum*, likely due to its more selective nutritional requirements, such as a preference for specific free amino acids or peptides, which may be less abundant in the phycocyanin-rich extract. On the other hand, *L. brevis*, an obligate heterofermentative strain, typically yields lower ATP per mole of glucose, which explains its weaker acidification compared to the other strains (Y. Zhang et al., 2022).

Although similar or even lower endpoints are frequently reported for fermented dairy or vegetable products (Medina et al., 2016), this is often not the case for algal fermentation. Notably, spirulina-based fermentations often exhibit limited acidification, typically remaining above pH 5 (Jamnik et al., 2022; Niccolai et al., 2019, 2020; Verni et al., 2021). The

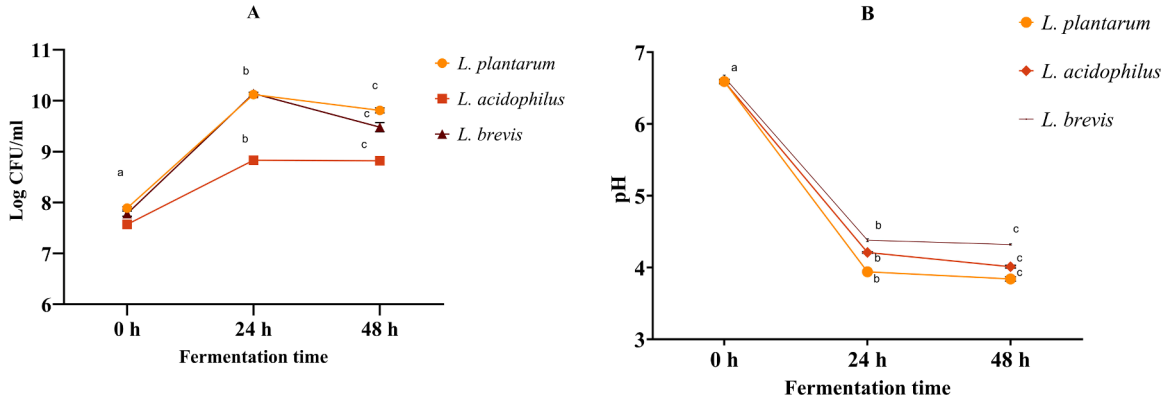

Fig. 1. Bacterial growth (A) and pH changes (B) during anaerobic fermentation of phycocyanin-rich *A. platensis* extract with *L. plantarum, L. acidophilus* or *L. brevis* at different time points. Values are expressed as mean ± standard deviation, $n = 3$.

more substantial acidification observed in this study can be attributed to glucose supplementation and the anaerobic conditions, as facultatively anaerobic LAB divert more carbon towards organic acid production. Nevertheless, comparable pH declines from 7 to ~4 after 24–36 h were also observed during spirulina fermentation with *L. plantarum* alone (de Marco Castro et al., 2019) or in mixed cultures with *B. subtilis* (J. Bao et al., 2018). The rapid pH drop observed has important implications for the subsequent biochemical changes. Acidification is a major driver of pigment destabilisation, amino acid liberation, vitamin utilisation, and antimicrobial metabolite formation, which will be further explored in the following sections.

3.2. Glucose consumption and organic acid production

Glucose uptake and subsequent organic acid production are summarised in Fig. 2A–C. In all treatments, glucose content decreased rapidly over the first 24 h, coinciding with the exponential growth phase of the LAB strains described in the previous section. *L. brevis and L. plantarum* showed the highest glucose utilisation, with glucose contents reducing from 23.4 g/L initially to 2.8 and 3.4 g/L after 48 h of fermentation, respectively (Fig. 2A). Samples fermented with *L. acidophilus* also showed high glucose utilisation, with their contents reducing to 12 and 9.6 g/L after 24 and 48 h of fermentation, respectively (Fig. 2A).

Swift depletion of monosaccharides can be highly anticipated as many LAB strongly prefer readily available carbohydrates, especially glucose, and is frequently reported in lactic fermentations of algal or plant-based substrates (Y. Wang, Wu et al., 2021). Nevertheless, LAB show either homofermentative or heterofermentative conversion of carbohydrates, resulting in organic acids and/or other end products (Augustiniene et al., 2022).

As expected, lactic acid was the dominant organic acid in all fermentations (Fig. 2B). *L. plantarum* produced the highest lactic acid concentration, reaching approximately 26.3 g/L after 48 h, followed by *L. acidophilus* (24.3 g/L) and *L. brevis* (20.6 g/L), with these values closely reflecting the observed acidification trends (Fig. 1B), thus confirming that carbohydrate catabolism was the primary driver of pH decline. The relationship between glucose utilisation, lactic acid formation, and pH reduction is well established in LAB fermentations (Leroy & De Vuyst, 2004).

Small quantities of acetic acid were detected in all treatments, with *L. brevis* showing the highest levels. This result is consistent with its obligate heterofermentative metabolism, where acetate is a key end product (Zheng et al., 2020). Meanwhile, *L. plantarum* generated only minor amounts of acetic acid, indicating predominantly homolactic metabolism under the anaerobic conditions used here. Nevertheless, the metabolic profiles obtained in this study are consistent with earlier reports on LAB fermentation of spirulina-based substrates. Furthermore, the strong inverse correlation between glucose concentration and pH (Supplementary Fig. S1) confirms that carbohydrate depletion directly contributed to the acidification kinetics described in Section 3.1. Organic acid levels in this study were markedly higher than previously reported. For example, Niccolai et al. observed lactic acid peaking at 24 h and acetic acid peaking at 72 h (Niccolai et al., 2020), while another study reported that *L. plantarum* produced 2.9 g/L lactic acid at 24 h and 3.7 g/L at 48 h (Niccolai et al., 2019). The greater acid production in this study likely reflects the glucose supplementation and strict anaerobic conditions used. These acids, in addition to driving acidification, may influence phycobiliprotein stability, a topic discussed in the next section.

3.3. Changes in phycobiliprotein content

PC, the major phycobiliprotein of *A. platensis*, has multiple applications but can be limited by its structural sensitivity to acidic conditions (Yuan et al., 2022). Although temperature, light, and pH are known to affect phycobiliprotein stability, the role of fermentation remains poorly studied (Z. Yu et al., 2024). Pretreatments such as pasteurisation or sterilisation, commonly used to reduce the microbial load in spirulina biomass, can also cause pigment loss or denaturation (Adjali et al., 2022). Extracts displayed distinct absorbance peaks at 620 nm (PC) and 652 nm (allophycocyanin, APC), which progressively declined during fermentation. After 24 h, PC dropped from 77 mg/g to 40 mg/g with *L. plantarum*, and to 67 and 62 mg/g with *L. acidophilus* and *L. brevis*, respectively (Fig. 3A). Similar trends were observed for APC (Fig. 3B) and total phycobiliproteins (Fig. 3C). As can be expected, prolonged fermentation (48 h) caused dramatic losses, reducing substantially individual and total phycobiliproteins (Fig. 3A–C).

From a mechanistic perspective, pigment loss in PC is driven by acidification below ~pH 6–5, where protonation can disrupt the α- and β-subunit folding and destabilise chromophore–protein linkages, ultimately leading to partial chromophore release (Parshina et al., 2024). This results in a decrease in λ max absorbance and spectral broadening, consistent with the observed colour shifts from blue to green-turquoise (Supplementary Fig. S3). Moreover, acid-induced conformational changes also impair chromophore coupling and energy transfer, resulting in shifts of the Q-band from its typical position near 620 nm to atypical wavelengths (Gwizdala et al., 2018; MacColl, 2004). Although large-scale aggregation was not observed, proximity to the isoelectric point and increased surface hydrophobicity under acidic conditions could promote partial aggregation (Li et al., 2021). Temperature (37 °C) further accelerates secondary and tertiary structure modifications compared to typical extraction conditions (25–30 °C), compounding instability (Pez Jaeschke et al., 2021). Correlation analysis revealed strong associations between pigment retention pH and lactic acid levels (Supplementary Material Fig S3). Lastly, residual glucose may partially mitigate degradation, as saccharides can stabilise phycocyanin by reducing colour loss over time (Faieta et al., 2020). However, this protective effect was insufficient under extended fermentation.

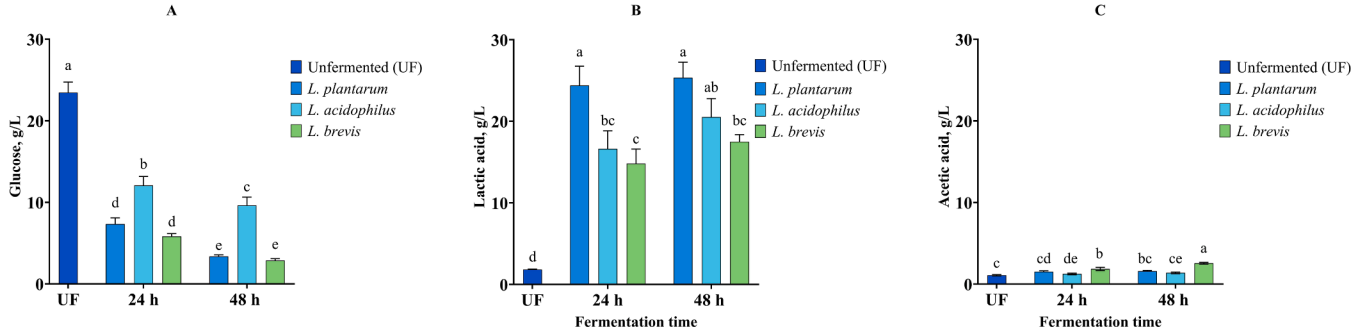

Fig. 2. Changes in glucose (A), lactic acid (B) and acetic acid (C) content during anaerobic fermentation of phycocyanin-rich *A. platensis* extract with *L.* plantarum, *L. acidophilus* or *L. brevis* at different time points. Values are expressed as mean ± standard deviation, $n = 3$. Different lowercase letters indicate significant differences (ANOVA, $p < 0.05$).

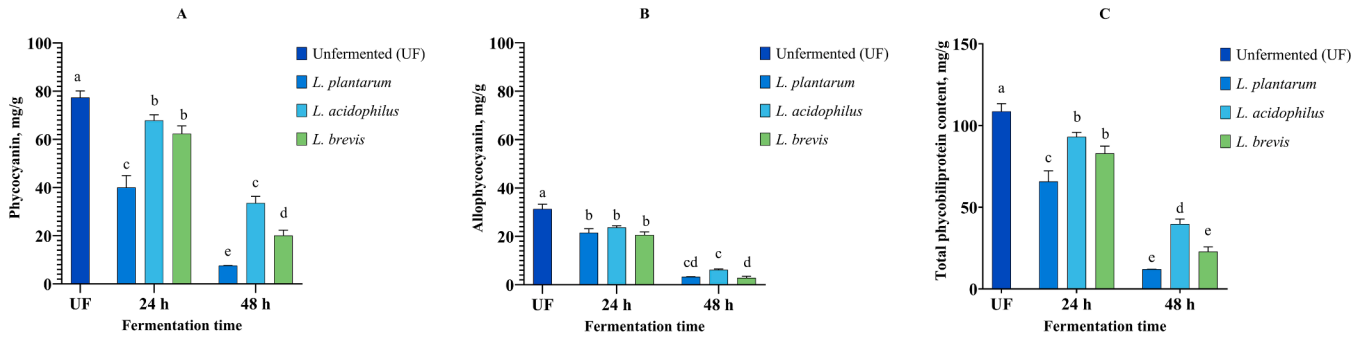

Fig. 3. Changes in phycocyanin (A), allophycocyanin (B) and total phycobiliprotein content (C) during anaerobic fermentation of phycocyanin-rich *A. platensis* extract with *L.* plantarum, *L. acidophilus* or *L. brevis* at different time points. Values are expressed as mean ± standard deviation, $n = 3$. Different lowercase letters indicate significant differences (ANOVA, $p < 0.05$).

Although little data are available in the literature, these findings align with previous reports: Niccolai et al. observed ~40 % PC loss during *L. plantarum* fermentation of *A. platensis* biomass (Niccolai et al., 2020), while Özyurt et al. reported a 95 % decrease after 72 h anaerobic fermentation (Özyurt et al., 2023). Liu et al. further demonstrated that PC supplementation alters microbial and biochemical profiles in silage systems (Liu et al., 2024). Overall, strain-specific acidification kinetics and structural sensitivity of phycobiliproteins explain the observed degradation patterns.

3.4. Utilisation and production of water-soluble vitamins

LAB are considered auxotrophic for several vitamins, with some strains also capable of producing or releasing vitamins during fermentation (Capozzi, Russo, Dueñas et al., 2012; Keyvan et al., 2025). This part of the study evaluated the consumption and production patterns of water-soluble vitamins by three LAB strains during anaerobic fermentation. Nicotinic acid (B_3) and thiamine (B_1) were the most abundant in the unfermented phycocyanin-rich extract, with riboflavin (B_2) also present at lower levels. Chromatographic data revealed significant shifts in these vitamin contents during fermentation, alongside pyridoxine (B_6) biosynthesis in specific strains (Fig. 4A–D). All strains depleted B_3 during fermentation (Fig. 4C), consistent with the absence of a complete *de novo* biosynthetic pathway for vitamin B_3. Instead, many LABs are auxotrophic for nicotinic acid, relying on uptake of this vitamin from their environment to support the synthesis of NAD and NADP via salvage

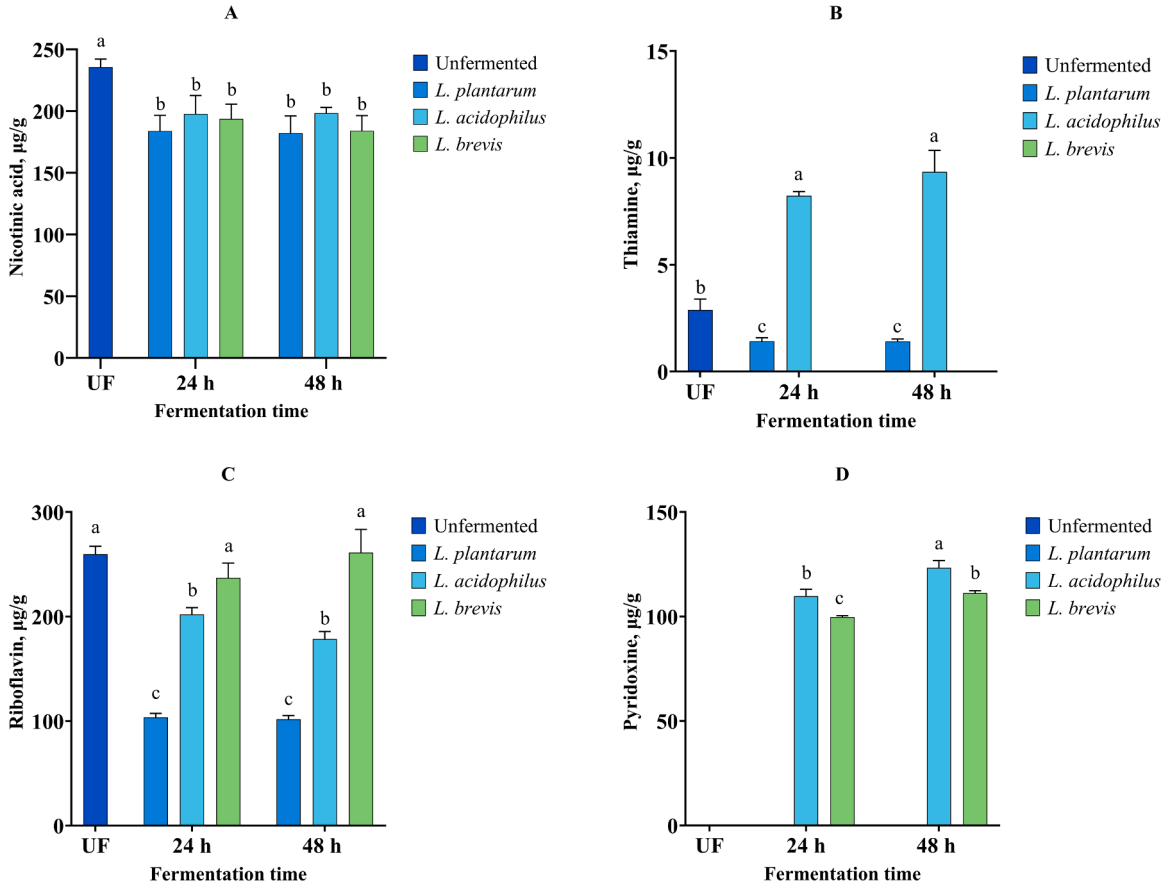

Fig. 4. Uptake and production of water-soluble vitamins during anaerobic fermentation of phycocyanin-rich *A. platensis* extract with *L.* plantarum, *L. acidophilus* or *L. brevis* at different time points: nicotinic acid (A), thiamine (B), riboflavin (C) and pyridoxine (D). Values are expressed as mean ± standard deviation, $n = 3$. Different lowercase letters indicate significant differences (ANOVA, $p < 0.05$).

pathways (LeBlanc et al., 2011). This observation can explain the uniform depletion across strains, as uptake supports cofactor regeneration under anaerobic conditions. Thiamine levels showed strain-specific trends with *L. plantarum* and *L. brevis* fully consuming B_1, indicating auxotrophy and dependence on external thiamine for pyruvate dehydrogenase and transketolase activity, while *L. acidophilus* showed a ~190 % increase, suggesting biosynthetic capability or active release mechanisms, which are rare and strain-dependent (Teran et al., 2021). Thiamine biosynthetic pathways are hypothesised to be end-product-regulated in a strain-specific manner, with reports indicating that further research is needed to clarify their control mechanisms and to optimise conditions to enhance thiamine production in LAB (Teran et al., 2021). Nevertheless, although some gut bacteria can synthesise B1, the amounts produced are insufficient to meet human needs, so daily intake must come from a diversified diet (Mrowicka et al., 2023).

Riboflavin levels remained stable in *L. brevis* (~250 μg/g) but decreased in *L. acidophilus* and *L. plantarum*, with the latter showing a ~60 % reduction. This general tendency for consumption is consistent with the metabolic profile of LAB. While a few select strains possess the capacity for de novo B_2 synthesis, the majority depend on uptake to regenerate flavin adenine dinucleotide and flavin mononucleotide, particularly under environmental or stress conditions (Diez-Ozaeta et al., 2025). B_6 production was observed in samples inoculated with *L. acidophilus* and *L. brevis,* with values exceeding 110 μg/g after 48 h. This trait, though uncommon among LAB, has been previously reported in fermented foods (Y. Wang, Wu et al., 2021). These results verify that vitamin metabolism in LAB is highly strain-specific and probably governed by incomplete biosynthetic pathways, salvage mechanisms, and regulatory adaptations to environmental stress. Regardless, selecting strains capable of producing vitamins could improve the nutritional value of algal fermentates. Future work should explore genomic determinants of vitamin biosynthesis genes and their regulation under fermentation conditions to optimise biofortification strategies.

3.5. Changes in the in vitro antioxidant capacity

Several studies confirm the antioxidant properties of spirulina and phycocyanin-rich extracts, with a recent meta-analysis showing spirulina intake enhances antioxidant defences (Naeini et al., 2021). The antioxidant capacity of the phycocyanin-rich extract was assessed using the Folin–Ciocalteu and CUPRAC assays, and the results are presented in Fig. 5A–B. Overall, fermentation decreased antioxidant activity across all treatments, with patterns closely reflecting the strain-dependent degradation of phycobiliproteins described in Section 3.3. Fermented samples showed lower antioxidant values than untreated ones, with the greatest decline after 48 h. *L. plantarum* caused the most significant reduction, with total reducing power dropping 15 % and 23 % at 24 and 48 h, and CUPRAC values decreasing by 21 % and 30 % (Fig. 5B). *L. acidophilus* and *L. brevis* showed similar trends, though less pronounced (Fig. 5A and B), consistent with their slower acidification rates and less extensive phycobiliprotein degradation. These results contrast with reports suggesting fermentation enhances antioxidant capacity (Pérez-Alva et al., 2022). This discrepancy may be associated with thermal pretreatment, which often inactivates PC, and observed increases in other studies could stem from proteolysis or release of bound antioxidants. Nevertheless, a few recent reports have also shown a reduction in the overall antioxidant capacity over time. For example, de Marco Castro et al. reported that after 36 h of fermentation, the total antioxidant capacity peaked but diminished thereafter (de Marco Castro et al., 2019)

Correlation analysis confirmed strong positive associations ($r > 0.8$, $p < 0.05$) between phycobiliprotein content and antioxidant capacity (Supplementary Fig. S2). Overall, LAB fermentation reduced the antioxidant capacity in a strain-dependent manner, probably due to acid-mediated pigment degradation. At the same time, this observation may represent a trade-off in antioxidant functionality and the retention of other micronutrients and bioactive compounds. Fermentation might convert native antioxidant compounds, such as PC, into novel derivatives with potentially beneficial biological effects not fully captured by these in vitro assays. Avenues for process optimisation, such as controlled pH fermentation or protective encapsulation, to improve antioxidant preservation could be explored in future applications.

3.6. Changes in the amino acid content and profile

Amino acids have a critical role in anaerobic fermentations, serving as essential nutrients and intermediates (Zhou et al., 2024). *A.platensis* biomass is recognised as a rich source of amino acids at FAO's recommended levels (Wang, Tibbetts et al., 2021). In this part of the study, we investigated the differences in total free essential amino acids (TFEAA), total free non-essential amino acids (TFNEAA), and total free amino acids (TFAA) between unfermented and LAB-fermented phycocyanin-rich extracts (Fig. 6A–C). Moreover, heatmaps of individual amino acids can be found in the supplementary material (Supplementary Materials, Figs. S4–6). Unfermented samples contained 57.3 mg/g TFAA, dominated by arginine, glutamic acid, leucine, phenylalanine, and alanine (63.2 %). TEFAA and TNEFAA contributed 21.8 mg/g (38 %) and 35.5 mg/g (62 %), respectively, consistent with previous reports (J. Bao et al., 2018; Özyurt et al., 2023).

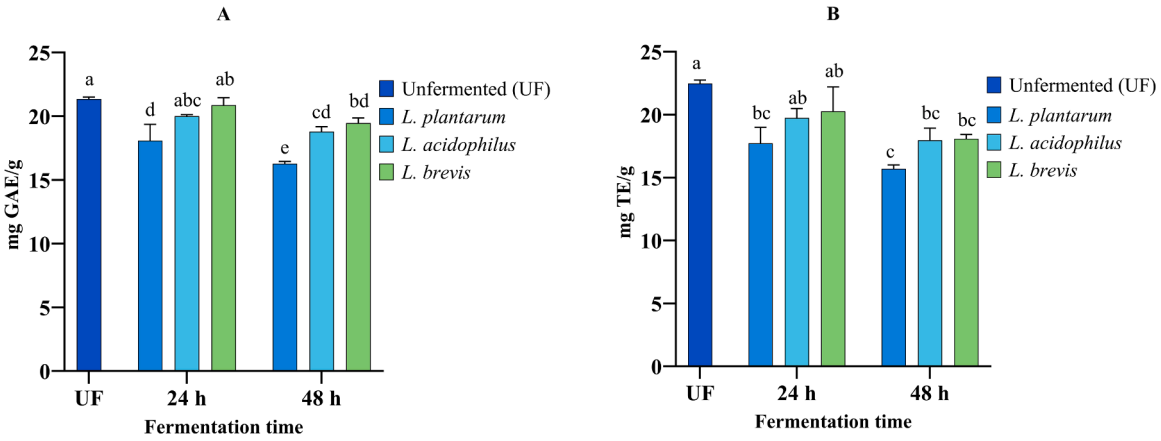

Fig. 5. Changes in the reducing power by Folin Ciocalteu's assay (A) and in vitro antioxidant capacity by CUPRAC assay (B) during anaerobic fermentation of phycocyanin-rich *A. platensis* extract with *L.* plantarum, *L. acidophilus* or *L. brevis* at different time points. Values are expressed as mean ± standard deviation, $n = 4$. Different lowercase letters indicate significant differences (ANOVA, $p < 0.05$).

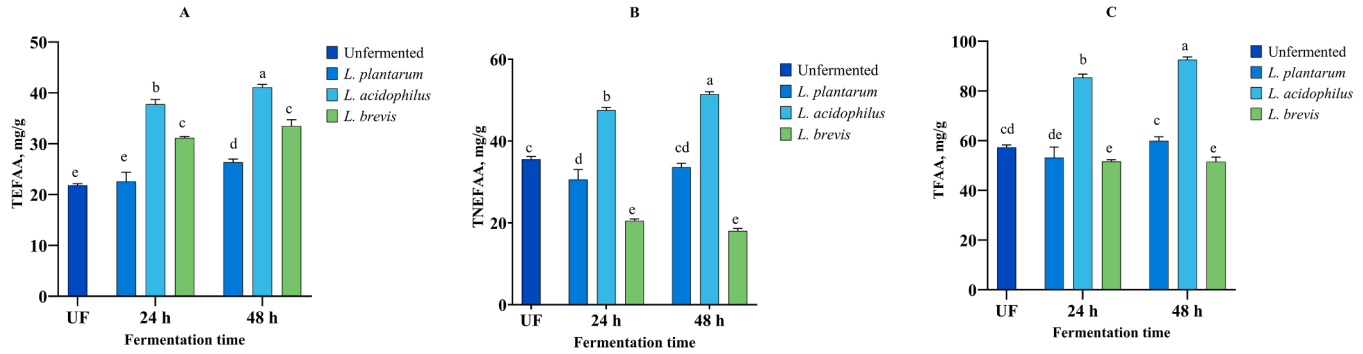

Fig. 6. Changes in the total essential free amino acid content (A), total non-essential free amino acid content (B) and total free amino acid content (C) during anaerobic fermentation of phycocyanin-rich *A. platensis* extract with *L. plantarum, L. acidophilus* or *L. brevis* at different time points. Values are expressed as mean ± standard deviation, $n = 3$. Different lowercase letters indicate significant differences (ANOVA, $p < 0.05$).

The TFAA content of fermented samples varied among strains, with different strains exhibiting distinct metabolisms and amino acid uptake. Fermentation altered amino acid profiles in a strain-dependent manner. Specifically, *L. plantarum* maintained TFAA, *L. brevis* showed a slight decrease, while *L. acidophilus* increased TFAA, indicating proteolysis (Fig. 6C).

To the best of our knowledge, no prior studies have examined the effects of fermentation on free amino acids in phycocyanin-rich extracts, and reports on spirulina biomass remain inconsistent. For example, Bao et al. reported an 18–35 % decrease in TFAA but an increase in TEFAA after mixed fermentation with *L. plantarum* and *B. subtilis* (J. Bao et al., 2018). Yu et al. showed a reduction in TFAA and TFEAA contents in samples fermented with *Bacillus* strains (J. Yu et al., 2020), while Ozturk et al. also noted *decreases using L. helveticus and K. marxianus* (Ozturk et al., 2025). Conversely, another study showed that TFAA increased under controlled pH and aeration with the same strains (Yay et al., 2024), and Verni et al. reported increases after fermentation with *L. plantarum* (Verni et al., 2021). Similarly, Kurt et al. observed that TFAA increased with *L.* rhamnosus and *L.* casei but decreased with *Bacillus* strains (Kurt et al., 2023).

Despite variability, TEFAA generally increased post-fermentation, thereby shifting the TEFAA: TNEFAA ratio. In unfermented samples, TEFAA accounted for 37 % of TFAA. After fermentation, *L. plantarum* and *L. acidophilus* increased TEFAA to ~44 %, while *in L. brevis* reached nearly a 2:1 TEFAA: TNEFAA ratio. As could be expected, the amino acid profiles also varied by strain. *L. plantarum* showed a decrease in arginine and glutamic acid but retained leucine, arginine, and glutamic acid (~15 % each) as major components, along with phenylalanine and alanine (~10 %) (Supplementary Fig. S4). These reductions suggest bacterial assimilation, consistent with reports that *L. plantarum* requires the specific amino acids for growth (A. Zhang et al., 2021). In samples fermented with *L. brevis,* the leucine content (~22 %) nearly doubled, but sharply reduced glutamic acid (from 14.3 mg/g to 1.3 mg/g) and arginine (>90 %), explaining its TEFAA shift (Supplementary Fig. S6). This observation aligns with known glutamate and tyrosine decarboxylase activity in this strain (Moreno-Arribas & Lonvaud-Funel, 2001; Park & Oh, 2007; Zhang & Ni, 2014) In contrast, fermentation with *L. acidophilus* increased the content of practically all analysed amino acids with a notable exception of tyrosine, whose levels decreased by >90 % within 24 h of fermentation (Supplementary material, Fig. S5). Many LAB are known to possess proteolytic systems and amino acid biosynthesis genes, enabling amino acid production (Akpoghelie et al., 2025). However, proteolytic activity is known to be strain- and species-dependent (Raveschot et al., 2018). For example, *L. plantarum* RG14 showed high proteolytic activity at pH 5, which substantially increased at pH 8, whereas *L. plantarum* RS5 exhibited the highest activity at pH 6.5 (Lim et al., 2019). These observations, in parallel with the decrease in pH observed for the strains detected in this study, could potentially explain the variation in proteolytic activity among the tested strains. Nevertheless, these changes have important implications for nutritional quality, as increases in EAAs and bioactive amino acids may enhance the functional properties of the fermented extracts.

3.7. Formation of biogenic amines

Although LAB is generally considered non-toxic and non-pathogenic, certain strains can produce biogenic amines, low-molecular-weight nitrogenous bases, formed through the decarboxylation of specific amino acids (Spano et al., 2010). Their accumulation in fermented foods can pose a health risk, with severity influenced by an individual's susceptibility, overall health, and the specific characteristics of the food (del Rio et al., 2024; Spano et al., 2010).

Despite these concerns, regulation is limited to histamine in fish products (Saha Turna et al., 2024). Results of biogenic amines analysis in this study are summarised in Fig. 7A–D. Histamine increased from 31 to 133 μg/g after 48 h in *L. acidophilus* samples, and showed a slight increase in *L. plantarum* (Fig. 7A). A notable exception was in *L. brevis* samples, where it dropped to zero (Fig. 7A), consistent with reports of *L. brevis* reducing histamine in sufu (W. Bao et al., 2020). Tyramine appeared only in *L. acidophilus* and *L. brevis* samples after 24 h, increasing at 48 h (Fig. 7B). As could be expected, histamine correlated positively with histidine, while tyramine correlated negatively with tyrosine (Supplementary Fig. S7). Histamine and tyramine are major dietary amines linked to acute effects (Latorre-Moratalla et al., 2017).

Putrescine and 2-phenylethylamine were also detected (Fig. 7C and D). Putrescine rose significantly only in *L. brevis* samples (1.11 mg/g at 24 h) and correlated negatively with arginine, but is considered low-risk due to its low toxicity (del Rio et al., 2024). 2-Phenylethylamine appeared in *L. acidophilus* and *L. brevis* samples (Fig. 7D), reaching 88 μg/g at 48 h, values well below the suggested 30 mg/kg limit (Saha Turna et al., 2024).

Several LAB groups are histamine and tyramine producers (Deepika Priyadarshani & Rakshit, 2011; Lee et al., 2023), with the ability of these decarboxylation products to accumulate being strain-dependent (Ma et al., 2024). Both tyramine and histamine are considered primary dietary bioactive amines associated with acute adverse health effects. The observations in this study align with reported characteristics of the strains. For example, *L. brevis* is known for its decarboxylase activity, especially its ability to convert tyrosine to tyramine and ornithine to putrescine (Moreira et al., 2024). Also, the moderate acidification exhibited by *L. brevis*, discussed in Section 3.1, may have further supported decarboxylase expression, as biogenic amine production is often enhanced under mildly acidic conditions (Dankar et al., 2025; Spano et al., 2010). Moreover, fermentations with *L. plantarum* resulted in no biogenic amine formation or significantly lower accumulation than with other strains. These findings are consistent with the observation that

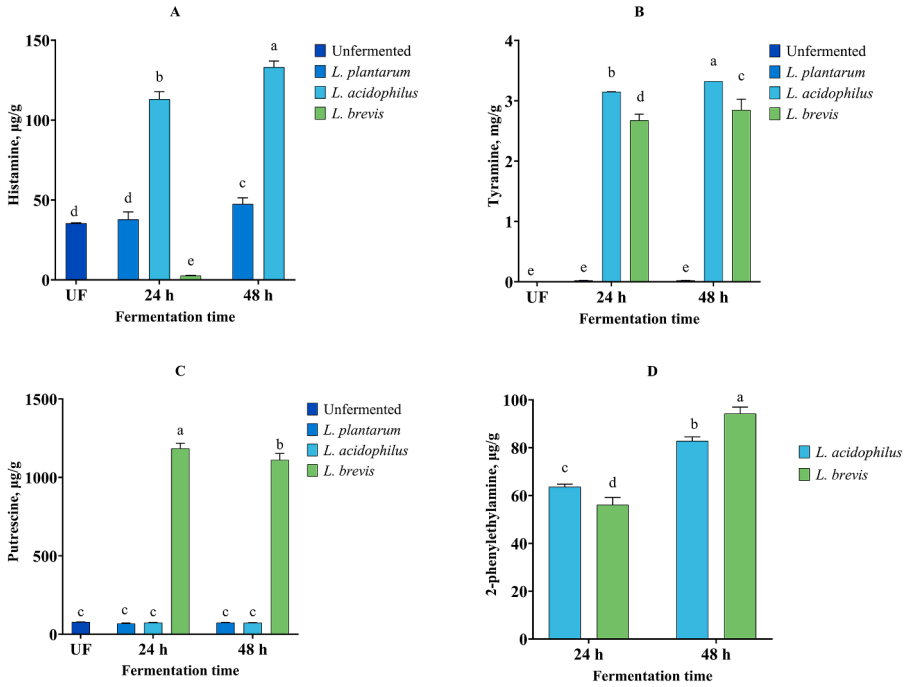

Fig. 7. Changes in biogenic amine content during anaerobic fermentation of phycocyanin-rich *A. platensis* extract with *L.* plantarum, *L. acidophilus* or *L. brevis* at different time points: histamine (A), tyramine (B), putrescine (C) and 2-phenylethylamine (D). Values are expressed as mean ± standard deviation, $n = 3$. Different lowercase letters indicate significant differences (ANOVA, $p < 0.05$).

many *L. plantarum* strains lack amino acid decarboxylase activity, or even show amine-degrading capacity (Capozzi, Russo, Ladero et al., 2012). Generally, the concentrations measured in this study are substantially below regulatory or guideline thresholds. The European Food Safety Authority notes that tyramine levels below ~100–200 mg/L are generally considered safe for most consumers ('Scientific Opinion on Risk Based Control of Biogenic Amine Formation in Fermented Foods', 2011). While fermentation of the phycocyanin-rich extract did not result in significant biogenic amine formation, the clear strain-dependent differences reveal the unique metabolic traits of each LAB strain and underscore the importance of selecting the right strain when developing fermented spirulina-based functional products.

3.8. Antimicrobial properties of fermented and unfermented phycocyanin-rich extracts

Microbial pathogens pose major risks to food safety and public health. LAB fermentation often produces antimicrobial compounds that inhibit pathogens or affect spoilage organisms (Gao et al., 2019). In this study, antimicrobial activity of 24-hour fermented and unfermented samples was tested against four pathogens using agar diffusion assay (Table 1).

Unfermented samples showed no activity. In contrast, fermented samples have shown different activities against the tested pathogens (Table 1). All fermented samples inhibited *E. coli.* Extracts fermented with *L. plantarum* showed the strongest antibacterial effects. This outcome corresponds to the extensive production of lactic acid and the rapid pH reduction described earlier. For the other strains, their efficacy against *E. coli* also corresponded to their lactic acid production. LAB antimicrobial effects are linked to organic acids, bacteriocins, and other metabolites (Ibrahim et al., 2021). Lactic acid lowers pH and can permeabilise Gram-negative membranes (Alakomi et al., 2000), though it is more effective against Gram-positive bacteria. Nevertheless, the antimicrobial activity typically cannot be attributed to a single molecule (Figueroa et al., 2024). The activity of *L. plantarum* against all the tested strains could probably be attributed to plantaricins, which have received special attention due to their potential applications in various areas, such as food bio-preservation agents, reduction of irritable bowel syndrome symptoms, and protective role against urinary infections (Abdulhussain Kareem & Razavi, 2020). Overall, the results indicate that LAB fermentation could significantly enhance the antimicrobial properties of phycocyanin-rich spirulina extracts, with *L. plantarum* generating the most potent inhibitory effects. These observations support the potential application of fermented spirulina-derived ingredients as natural antimicrobial agents in food systems, particularly in formulations where acidic pH and microbial stability are desirable.

Table 1
Inhibition zone (mm) of 24 h fermented samples and control samples against four pathogens (values are expressed as mean ± standard deviation, $n = 3$).

Sample	Inhibition zone, mm			
	E. coli	*M. luteus*	*B. subtilis*	*S. aureus*
Unfermented	-^{NA}	-^{NA}	-^{NA}	-^{NA}
L. plantarum	11.46 ± 0.5	14.01 ± 0.38	8.23 ± 0.49	9.22 ± 0.23
L. acidophilus	9.4 ± 0.12	-^{NA}	-^{NA}	-^{NA}
L. brevis	7.72 ± 0.09	-^{NA}	-^{NA}	-^{NA}

-^{NA}: not active.

4. Conclusions

In conclusion, this study demonstrates the anaerobic fermentation of phycocyanin-rich extracts derived from *A. platensis* using selected LAB, providing new insight into how fermentation influences the functional and nutritional properties of this substrate. All strains achieved substantial growth and acidification, driving notable biochemical changes. Among them, *L. plantarum* showed the fastest acidification rate and lactic acid production, while the resulting decrease in pH was closely associated with reductions in individual and total phycobiliproteins. Differences in amino acid dynamics were also strain-dependent. Specifically, while total free amino acids decreased in samples fermented with *L. plantarum* and *L. brevis, L. acidophilus* showed proteolytic activity that increased TFAA content. Nevertheless, all strains improved the

essential-to-non-essential amino acid ratio, indicating an overall enhancement of protein quality post-fermentation. Strain-specific increases in water-soluble vitamins were also observed, including thiamine biosynthesis by *L. acidophilus* and pyridoxine accumulation in both *L. acidophilus* and *L. brevis*. Moreover, the fermented extracts displayed antimicrobial properties, with *L. plantarum* exhibiting the broadest inhibition, thereby expanding the potential applications of these extracts. Although antioxidant capacity declined across treatments due to pigment degradation, the strong relationship between phycobiliprotein levels and antioxidant activity highlights the need to optimise fermentation conditions to balance nutrient enhancement with functional preservation. Overall, these findings show that fermentation may enhance the nutritional profile and antimicrobial potential of phycocyanin-rich spirulina extracts. However, careful strain selection and process optimisation remain essential, and future work should explore strategies to stabilise phycobiliproteins and evaluate functional performance in food-relevant systems to expand the application potential of algal ingredients.

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Ethical statement

This article contains no interventional studies performed by the authors with human participants or animals. The funding source did not play any role in study design, in the collection, analysis, and interpretation of data, in the report's writing, and in the decision to submit the article for publication.

CRediT authorship contribution statement

Shahana Aboobacker: Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Hakki Bilgin:** Writing – review & editing, Investigation. **Vaida Kitrytė-Syrpa:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Aušra Šipailienė:** Writing – review & editing, Methodology, Formal analysis. **Michail Syrpas:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.101704.

Data availability

Data will be made available on request.

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