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Synergistic interactions of nisin and *Spirulina platensis* extracts for foodborne pathogen control

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ABSTRACT. The search for naturally derived antimicrobials has emerged due to an increasing concern about the use of synthetic ones. In this investigation, we examined the antibacterial mechanism and potential synergistic effect of *Spirulina platensis* extracts and Nisin in inhibiting the growth of 23 strains of Gram-positive and Gram-negative foodborne pathogens. We conducted four different extraction protocols to obtain *S. platensis* compounds, ultimately selecting the alcoholic acid-formic acid-sonication extraction due to its antimicrobial activity against 95.6% of the tested strains. *Escherichia coli, Listeria monocytogenes*, and *Staphylococcus aureus* were selected for further analysis. Tests for Minimum Inhibitory Concentration (MIC) and the Checkerboard test revealed MIC values ranging from 0.5 to 0.12 µg mL^{-1} for Nisin and from 0.5 to 0.06 µg mL^{-1} for the selected *S. platensis* extract. When combined, the MIC range was 0.12 to 0.078 µg mL^{-1}. The combination of *S. platensis* extracts and Nisin showed synergistic effects (Fractional Inhibitory Concentration Index: 0.302 to 0.18). The growth of foodborne pathogens tested was inhibited after 2, 6, 12, and 24 h with the combination of the compounds. Flow cytometry analysis showed that bacterial membrane permeability of the *L. monocytogenes* strain increased, while Scanning Electron Microscopy (SEM) illustrated the leakage of intracellular material, indicating cell membrane disruption. These observations suggest that combining *S. platensis* and nisin could be a promising natural alternative to synthetic preservatives in the food industry.

Keywords: Antimicrobial synergism; bacteriocin; natural antimicrobial; *Arthrospira platensis*.

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Introduction

The increasing consumer concern regarding the consumption of synthetic chemical preservatives has prompted the food industry to prioritize the "clean label" concept. The use of these preservatives has been associated with adverse effects, including a heightened risk of carcinogenicity and the potential to trigger allergic reactions (Marrez et al., 2025; Maruyama et al., 2021).

Microalgae have received increasing attention for their potential industrial applications, particularly due to their bioactive compounds with anticancer, anti-inflammatory, and antioxidant properties, as well as their antimicrobial potential against foodborne pathogenic bacteria (Scaglioni & Badiale-Furlong, 2023). Many species of cyanobacteria, i.e., *Spirulina* and *Chlorella*, have been indicated as important producers of metabolites such as polyphenols, phenolic acids, tocopherols, and linolenic acid (Maddiboyina et al., 2023; Özogul et al., 2021). Several *in vitro* and *in vivo* studies have shown that *S. platensis* extracts (ethanolic and methanolic extracts) possess significant antifungal, antiviral, and antibacterial properties (Alshuniaber et al., 2021). However, the specific bioactive compounds responsible for these antimicrobial effects have not yet been fully elucidated. These constituents may contribute to antimicrobial activity by disrupting bacterial cell walls, inhibiting bacterial proliferation, or interfering with microbial metabolic processes (Marrez et al., 2025; Scaglioni & Badiale-Furlong, 2023).

However, while these compounds exhibit potential against foodborne pathogens, further investigation is required to confirm their effectiveness in food preservation. Additionally, the characteristic odor and flavor of *S. platensis* extracts may limit their application as food preservatives (Abdel-Moneim et al., 2022). The synergistic combination of natural compounds presents a promising strategy for controlling foodborne pathogens, as it may enhance antimicrobial efficacy while potentially reducing the concentrations required, thereby mitigating sensory limitations and preserving the sensory qualities of food products (Shi et al., 2017).

Nisin (NIS), a bacteriocin from *Lactococcus lactis,* is a peptide with a strong affinity for lipid II (the peptidoglycan precursor), forming a complex. This difficult peptidoglycan synthesis leads to pore formation (Bag & Chattopadhyay, 2017; Suganthi et al., 2012) that dissipates the proton motive force and affects some energy-dependent reactions in the cell, releasing the constituents of cytoplasm and leading to cell inactivation (Ramalho et al., 2023; Wang et al., 2023). Reports on the NIS effect on G-bacteria can yield varying results, ranging from ineffective to significant impacts. This variation in action has limited the use of this antimicrobial peptide in controlling foodborne pathogens (Bag & Chattopadhyay, 2017; Grenier et al., 2020; Suganthi et al., 2012). However, its use in the food industry has limitations, such as its narrow spectrum of action, the development of resistance, and the cost of production and commercialization (Márquez et al., 2020; Soltani et al., 2022). The combination of NIS and some antibiotics and/or chemical preservatives has already been reported, with the additive or synergistic antibacterial effect as the most satisfactory combination (Soltani et al., 2022; Wang et al., 2023). Marrez et al. (2025) reported the combination of NIS and *S. platensis* against *E. coli* and *Pseudomonas*; however, they did not identify the potential bactericidal action through membrane disruption. Furthermore, expanding the spectrum of target microorganisms and reducing applied concentrations is possible (Mathur et al., 2017; Sani et al., 2022). So, this work aimed to study the synergistic antimicrobial activity of *S. platensis* extracts with NIS against foodborne pathogens to contribute to new strategies for replacing synthetic chemical additives with natural antimicrobials in foods.

Material and methods

Bacterial strains, NIS and *Spirulina platensis*

The study was conducted with Gram-positive (G+) and Gram-negative (G-) microorganisms described in Table 1. The bacterial strains belong to the Basic and Applied Microbiology Laboratory (LAMBA) of the *Universidade Tecnológica Federal do Paraná* and are stored at -80°C in Brain Heart Infusion broth (BHI Neogen Culture Media, USA) containing 20% glycerol. Before use, the isolates will be reactivated in 10 mL of BHI broth and kept at 37ºC for 24 hours.

Commercial NIS from Zhejiang Silver-Engineering (⩾ 900 IU mg-1) Co-Ltda was prepared at a final concentration stock of 1028 mg mL-1 in ultrapure water and sterilized by filtration through 0.22 µm polystyrene membranes (Millipore®).

The dehydrated biomass of *S. platensis* was obtained from a natural product store in Foz do Iguaçu, state of Paraná, Brazil, and had the following composition: 58.9% protein, 4.10% total lipids, 2.90% moisture, 7.4% ash, and 26.6% total carbohydrates (AOAC International, 1997).

Extraction of bioactive compounds from *Spirulina platensis*

Bioactive compounds from *S. platensis* were extracted via four extraction protocols: (Extraction A) Alcoholic extraction of antimicrobial compounds from *S. platensis* biomass, followed by a protocol described by Parisi et al. (2009), with modifications: 2.0 g of the dehydrated microalgae biomass was dissolved in 20 mL of methanol. The solution was then stirred for 1 hour at 25°C. After stirring, the solution was allowed to stand for 30 minutes at room temperature, and an additional 20 mL of methanol was added. The extraction with agitation was repeated. Subsequently, the solution was filtered through Whatman No. 40 paper, and the filtrate was sent to a rotary evaporator to remove the solvent (methanol) completely, starting from its boiling point at 78°C. Finally, the dry extract was eluted in ultrapure water at 500 µg mL^{-1} final concentration; (Extraction B) The protocol used an ultrasound bath for cell disruption of the microalgae and extraction of antimicrobial compounds, as described by Bermejo et al. (2006). A suspension of 10 g of *S. platensis* was prepared in 100 mL of acetate buffer (pH 5.5), followed by homogenization. The solution was frozen for 24 hours and then thawed, followed by 10 minutes of ultrasound (ultrasonication) with a 2-minute pause. The post-sonication solution was centrifuged at 3.075 × g at 10°C for 10 min., and the supernatant was collected, filtered, and sent to a rotary evaporator to remove the solvents completely. The dry extract was diluted at a final concentration of 500 µg mL^{-1} with ultrapure water; (Extraction C) The extraction with alcohol-acetone was carried out using the methodology described by Machu et al. (2015). In a solution of 30 mL of methanol, 1 mL of acetic acid, and 69 mL of sterile distilled water, 10 g of *S. platensis* were added. The mixture was subjected to a water bath for four hours at 70°C. Following, 70 mL of methanol was added and subjected to 70°C for 1 hour. Subsequently, 50 mL of acetone was added, homogenized, and maintained at 30°C for 1 hour;

after this period, 70 mL of methanol was added, followed by incubation at 20°C for 4 hours. After this period, the solution was centrifuged at 3.075 × g for 10 min. and protected from light. The supernatant was filtered and sent to a rotary evaporator to remove the solvents completely. The dry extract was diluted at a final concentration of 500 µg mL^{-1} with ultrapure water; (Extraction D) Alcoholic-formic acid-sonication extraction followed a protocol of Martelli et al. (2020), with modifications. *S. platensis* (10 g) was homogenized in methanol-formic acid (99:1). The solution was mixed at 25±2°C for 15 min., followed by a 15 min. sonication. This step was repeated twice. The solution was centrifuged at 4.427 × g for 15 min. After centrifugation, the supernatant was filtered through Whatman No. 40 paper, and the material retained on the paper was subjected to the extraction described above again. After centrifugation, both supernatants were mixed and taken to a rotary evaporator to completely remove the solvents, starting from their boiling points at 78°C and 100.5°C, respectively. The dry extract was diluted at a final concentration of 500 µg mL^{-1} in ultrapure water.

Antimicrobial activity - disc diffusion method

The antimicrobial tests to evaluate the extracts obtained by methods A, B, C, and D were conducted following the CLSI (Clinical and Laboratory Standards Institute, 2018). For that, 100 µL of bacterial suspension adjusted to 1×10^8 CFU mL^{-1} was spread on BHI agar. Subsequently, paper discs (± 6 mm) were impregnated with 10 µL of the sample extracts A, B, C and D (final concentration of the extracts: 500 µg mL^{-1} final) and NIS solution (concentration of 1028 µg mL^{-1}), deposited on the agar surface, incubated at 37°C for 24 hours, and the inhibition zones were measured using a caliper. The extract that provided the largest zone of inhibition was considered to have the potential to inhibit the target bacteria. By observing the results of this stage, three bacterial isolates were selected to continue the tests.

Minimum Inhibitory Concentration (MIC)

The MIC values for selected bacteria were determined using the microdilution methodology in 96-well microplates (Clinical and Laboratory Standards Institute, 2018). The NIS solution (1028 µg mL^{-1}) and *S. platensis* extract (500 µg mL^{-1}) were diluted 1:1 in the wells. Bacterial suspensions (0.5 McFarland scale) were prepared, followed by inoculation in wells of microplates at a concentration of approximately 10^6 CFU mL^{-1}. These microplates were then placed in triplicate in an incubator set at 37°C for 24 hours. Post incubation, 10 µL of 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-carboxanilide (XTT /Sigma®) 0.125% solution was added, and incubated for an additional two hours at 37°C. The optical density (OD) was measured using a spectrophotometer (BIOCHOROM) at an absorbance of 595 nm. The MIC values were identified as the lowest concentrations of compounds that effectively inhibited bacterial growth.

Synergy test of *Spirulina* extract + NIS against foodborne pathogens

To assess the synergistic effect of *S. platensis* extracts and the NIS solution against bacterial isolates, the Checkerboard microdilution technique was performed according to the European Committee on Antimicrobial Susceptibility Testing (2000), in a microplate to obtain the fractional inhibitory concentration index (FICI).

The bacterial suspension was standardized at 5×10^5 CFU mL^{-1} per well and incubated at 37 °C for 24 hours. The concentrations evaluated for NIS were 1028 µg mL^{-1}, and for *S. platensis* extract were 500 µg mL^{-1}. The microplate assay was arranged as follows: NIS was diluted two-fold along the x-axis, while *S. platensis* extract was diluted two-fold along the y-axis. The Fractional Inhibitory Concentration Index (FICI) was calculated as $FICI_A + FICI_B$, where $FICI_A$ and $FICI_B$ were the minimum concentrations that inhibited the bacterial growth for NIS and *S. platensis* extract, respectively. Thus, FICIs were calculated as $FICI_A$ (MIC_A combination /MIC_A alone) and $FICI_B$ (MIC_B combination /MIC_B alone). The results were interpreted as synergic action ($FICI \leqslant 0.5$), partial synergism ($0.5 < FICI \leqslant 0.75$), no effect ($0.75 < FICI \leqslant 2$) or antagonism ($FICI > 2$) (Fadli et al., 2012). All assays were performed in duplicate.

Time-kill assay

To determine bactericidal activity, the time-kill curve was monitored to evaluate the impact of *S. platensis* extract and NIS on the growth of the selected isolates, according to Souza et al. (2024), with modifications. Bacteria at 5×10^5 CFU mL^{-1} were exposed to three treatments along with a control. At intervals of 0, 2, 6, 12, and 24 hours, samples from the cultures were extracted and plated on BHI agar and incubated at 37°C for 24 hours. The colonies were enumerated and converted into Log CFU mL^{-1}.

Flow cytometry

To determine the effects on bacterial membrane integrity caused by *S. platensis* extract and NIS, FACS (Fluorescence-Activated Cell Sorting) analyses were carried out as described by Souza et al. (2024), with modifications. Then, 30 µg mL^{-1} propidium iodide (PI/ Santa Cruz Biotechnology) was added to each solution and traced at a rate of < 15.000 events per second by flow cytometry of BD Accuri™ 6 Plus Personal Flow Cytometer. The control consisted of untreated cells, and the analyses were conducted in triplicate.

Scanning electron microscopy (SEM) analysis

Ultrastructural changes in untreated and treated (NIS and/or *S. platensis* extract) cells were assessed using FEI Quanta 200 Scanning Electron Microscope, as described by Santos et al. (2025). The control and treated cells were fixed by immersing the coverslips in 2.5% glutaraldehyde solution prepared in PBS at 4°C for 4 hours. After fixation, the samples were immersed sequentially in ethanol solutions at concentrations of 25, 35, 50, 60, 70, 80, 90, 95, and 100%, each step lasting 7 min. at room temperature. Following dehydration, the samples were treated with hexamethyldisilazane (HMDS). This step involved immersion in increasing concentrations of HMDS in methanol (33% and 66%), followed by 100% HMDS. Coverslips were subsequently left to air-dry to ensure complete evaporation of the solvent. Once dried, the specimens were mounted on aluminum stubs using carbon tape and sputter-coated with gold using a cathodic sprayer, and examined under a scanning electron microscope.

Statistical analysis

To determine bacterial growth inhibition using nisin and *S. platensis* extract, the growth on the positive control (no inhibition) was used as a reference (growth = 100%). All experiments were performed in duplicate or triplicate on different days. No formal confirmatory statistical analysis was carried out. Graphical displays of the results were prepared using Excel Office LTSC 2024.

Results and discussion

Inhibitory effect of NIS and *S. platensis* extracts

The values for the halo inhibition zones (mm) formed by the *S. platensis* extracts and NIS for the test bacteria are presented in Table 1. At 500 µg mL^{-1}, the alcoholic-formic acid-sonication extract from *S. platensis* (extract D) was the only extract with antimicrobial activity, with inhibition zones ranging from 10 to 30 mm for G+ and G- bacteria. On the other hand, NIS presented values from 13 to 19, and 11 to 19 for G+ and G- bacteria, respectively (Table 1). The results collected for the *S. platensis* extract are similar to those obtained by Martelli et al. (2020), whose methodology was used to obtain extract D; these authors observed halos of approximately 12 to 15 mm for *Salmonella* spp., *L. monocytogenes*, *E. coli,* and *S. aureus*.

Table 1. Halo inhibition (mm) values for Gram-positive and Gram-negative bacteria with *S. platensis* extracts (A, B, C, and D) and NIS.

	A	B	C	D	NIS
Gram-positive bacteria					
Enterococcus faecium ATCC – 6569	-	-	-	-	-
Listeria innocua CLIST 2050	-	-	-	18	-
Listeria innocua CLIST 2052	-	-	-	18	13
Listeria ivanovii CLIST 2056	-	-	-	15	18
Listeria monocytogenes CLIST 2032	-	-	-	15	17
Listeria monocytogenes CLIST 2048	-	-	-	18	17
Listeria monocytogenes CLIST 2049	-	-	-	16	18
Listeria monocytogenes CLIST 2042 3b	-	-	-	30	19
Listeria monocytogenes CLIST 2044	-	-	-	14	14
Listeria monocytogenes CLIST 2044 1/2[a]	-	-	-	10	15
Listeria seeligeri CLIST 2067	-	-	-	14	17
Staphylococcus aureus ATCC-25923	-	-	-	15	18
Staphylococcus aureus P2306	-	-	-	15	16
Gram-negative bacteria					
Escherichia coli ATCC 25922	-	-	-	19	16
Escherichia coli ATCC 43888	-	-	-	20	11
Escherichia coli ETEC Bac 17ST	-	-	-	15	16
Klebsiella pneumoniae ATCC 2378	-	-	-	15	18

Klebsiella pneumoniae ATCC 10031	-	-	-	15	17
Pseudomonas aeruginosa P2381	-	-	-	18	17
Pseudomonas aeruginosa ATCC 9027	-	-	-	16	18
Salmonella choleraesuis ATCC 292013	-	-	-	30	19
Salmonella enteritidis P3116	-	-	-	14	14
Salmonella Typhimurium ATCC 24028	-	-	-	10	15

(-) absence of halo

In this study, commercial NIS showed inhibition against all G- bacteria tested. The extract D from *S. platensis* biomass also effectively controlled the G- bacteria tested. The extraction protocol greatly influences antimicrobial activity (Abedin & Taha, 2008). Protocol D used alcohol and formic acid followed by sonication, and the total volume of solvent was evaporated from their boiling point until complete drying after the extraction, so we can consider that this protocol was more effective in extracting bioactive compounds of *S. platensis* with antimicrobial activity. *S. platensis* is an excellent source of bioactive secondary metabolites in addition to carbohydrates, vitamins, proteins, minerals, essential fatty acids, and dietary fiber (Machu et al., 2015). Bioactive secondary metabolites include polyphenols, phenolic acids, tocopherols, and linolenic acid, which exhibit several activities such as anticancer, anti-inflammatory, antioxidant, neuroprotective, hepatoprotective, and antimicrobial properties (El-Sheekh et al., 2014; Mazur-Marzec et al., 2015).

Similar results are highlighted by El-Sheekh et al. (2014). According to these authors, the solvents used in the extraction were evaporated from their boiling point until complete drying, supporting the results obtained in this study. The antimicrobial activity of *S. platensis* is due to the presence of phenolic compounds, which comprise large aromatic chain molecules with a hydroxyl group. Various factors can influence these compounds, such as the alga's geographical origin and environmental, physiological, and nutritional variations (Marinho-Soriano et al., 2006). In addition to these compounds, gamma-linolenic, lauric, and palmitoleic acids may also be related to the antimicrobial activity of this microalga (Mendiola et al., 2006). All these associated factors may indicate the potential to use these compounds synergistically with other antimicrobial substances.

For the MIC, synergy tests, and flow cytometry, the strains *E. coli* ATCC 25922, *L. monocytogenes* ATCC 2049, and *S. aureus* ATCC 25923 were selected randomly because they represent important foodborne bacteria. SEM was conducted with *L. monocytogenes*, collecting images of untreated cells, cells treated with NIS and *S. platensis* extract, and cells treated with both compounds.

Checkerboard test and inhibitory effect

The MIC values of the components in combination outperformed for the three tested bacteria, and the values presented demonstrated a synergistic antibacterial interaction between the compounds NIS/Spirulina against *S. aureus* ATCC 25923 (FICI 0.30), *L. monocytogenes* ATCC 2049 (FICI 0.25), and *E. coli* ATCC 25922 (FICI 0.18) (Table 2). The FICI ranged from 0.18 to 0.30, indicating a synergistic interaction for selected isolates (Fadli et al., 2012).

Table 2. Fractional Inhibitory Concentration Index (FICI) of NIS with *Spirulina* extract combined against cells of food-origin bacteria.

Microorganism	Test Substance	MIC (μg mL^{-1})		FICI	Efect
		Isolated	Combined		
S. aureus ATCC 25923	NIS	0.12	0.0078	0.30	Synergism
	S. platensis	0.50	0.120		
L. monocytogenes ATCC 2049	NIS	0.50	0.007	0.25	Synergism
	S. platensis	0.06	0.015		
E. coli ATCC 25922	NIS	0.50	0.030	0.18	Synergism
	S. platensis	0.50	0.062		

The combination of NIS with *S. platensis* extract and their potential synergistic interactions makes it easier to use them as natural food preservatives, expanding their spectrum of action. In this study, the synergistic action reduced MIC values for NIS acting against *E. coli, L. monocytogenes*, and *S. aureus* (Table 2). A minimal amount of NIS (0.12 and 0.5 μg mL^{-1}) combined with *S. platensis* extract demonstrates antimicrobial efficiency against the mentioned pathogens. The same can be observed by analyzing MIC values for *S. platensis* extract isolated and combined (MIC values decrease for *S. platensis* and NIS combined against *L. monocytogenes* and *E. coli*). Reports of synergistic action between NIS and *S. platensis* extract are still scarce. Synergistic combinations of NIS have been established with antimicrobials (Kitazaki et al., 2017), essential oils (Li et al., 2022), plant polyphenols (Grenier et al., 2020), and antimicrobial peptides (Roshanak et al., 2020), highlighting the main advantages of synergy,

including increased antimicrobial activity through combination use, reduced required doses of compounds, and an expanded spectrum of activity (Bag & Chattopadhyay, 2017; Szabo et al., 2010). The time-kill assay revealed the antimicrobial potential of NIS and *S. platensis* extract, individually and in combination, on tested organisms on 2, 6, 12, and 24 hours (Figure 1). The results suggest that the combination of NIS/*S. platensis* extract is bactericidal, from the first 2 hours. These results support the data shown for the synergistic action (Table 2). They are significant in controlling the growth of foodborne pathogenic bacteria, as they enable reduced amounts of these antimicrobial compounds while achieving the desired food safety effect.

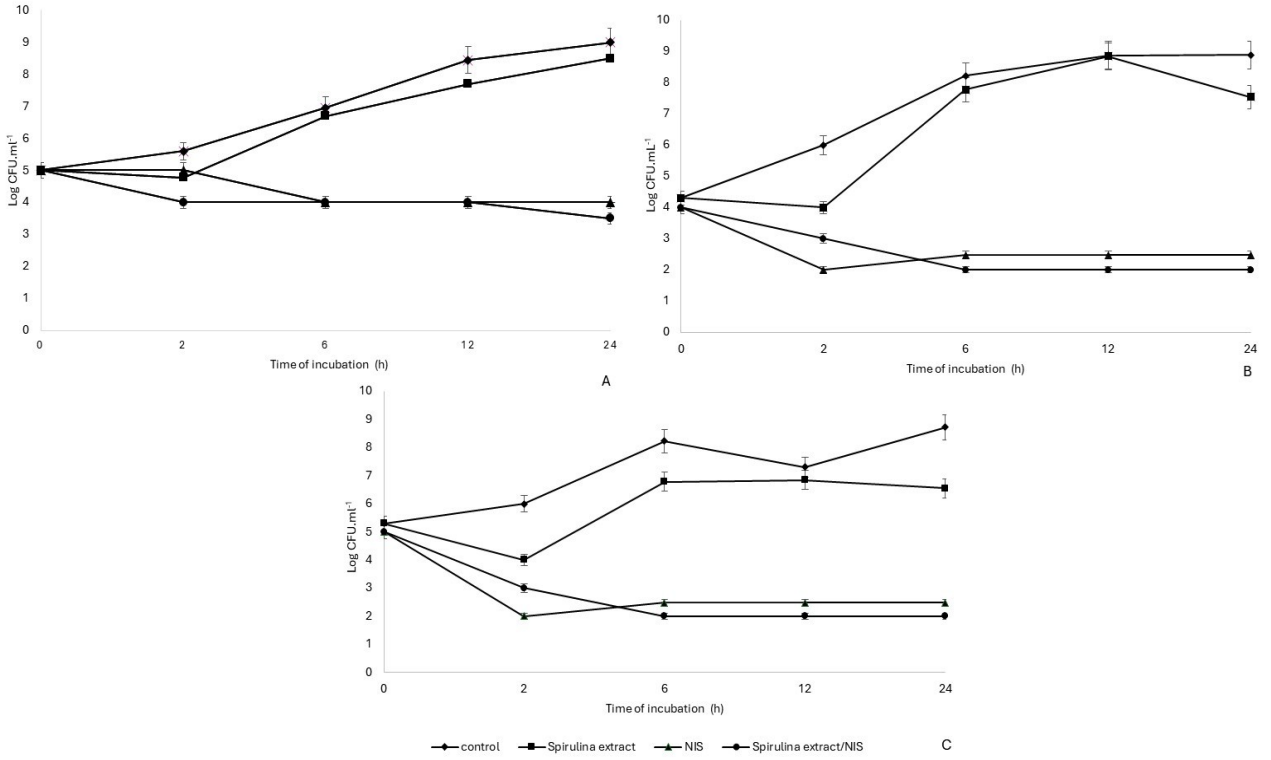

Figure 1. Time-kill curves of *Listeria monocytogenes* (A), *Staphylococcus aureus* (B), and *Escherichia coli* (C) exposed to *Spirulina platensis* extract and NIS, individually and in combination. Control indicates bacterial growth without antimicrobial compounds.

Scanning electron microscopy (SEM) and flow cytometry

The SEM technique was applied to assess the morphological changes induced by NIS and *S. platensis* extract in *L. monocytogenes* cells (Figure 2). Figure 2A represents the untreated control, demonstrating intact cellular integrity. After an 18-hour treatment, significant morphological alterations were observed, including numerous projections from the cell wall and liberation of membrane vesicles. Likewise, a significant amount of cytoplasmic material was observed in the surrounding environment, indicative of compromised cellular structure (Figures 2B, C, and D). These findings suggest that NIS and *S. platensis* extract disrupted bacterial cells, leading to cell death. According to Bouhdid et al. (2009), natural antimicrobials can affect the bacterial cell wall, causing the formation of numerous projections on the cell surface and the release of membrane vesicles, characterized as spherical structures derived from the cytoplasmic membrane or the cell wall. These vesicles are associated with the leakage of cytoplasmic contents into the extracellular medium, indicating structural damage and loss of bacterial cell integrity.

These data corroborate the results obtained in flow cytometry analysis (Figure 3). Two intervals of PI relative fluorescence were considered, depending on the intensity of fluorescence emitted by control cell (nontreated cells) and treated cells, we can distinguish two cell states: cells with intact membranes, which emit a low relative fluorescence (region R1), and cells with permeabilized membranes, which emit a high relative fluorescence (dead cells, region R2). The effect on membrane permeability was evaluated using PI, a cationic nucleic acid dye that is excluded by intact plasma membranes but can penetrate into cells with damaged membranes and intercalates with double-stranded DNA or RNA (Bouhdid et al., 2009). Our results suggest that after treatment, the cell membrane of the microorganism was altered, leading to increased permeability and permitting the entry of PI, indicating cell damage. It is known that nisin can interact directly with membrane lipids, increasing membrane permeability PI (Galván Márquez et al., 2020). Souza et al. (2024)

also observed the uptake of PI in bacterial cells treated with bacteriocin (as nisin), indicating membrane damage and compromised integrity of the bacterial cell envelope. NIS altered the cell membrane, resulting in cell disintegration into several small sections, explaining the observations of disrupted cell wall integrity (Zhou et al., 2014). The same effect was observed with the *S. platensis* extract regarding deleterious action. Therefore, it is suggested that, besides the damage to the cell wall and membrane, the extract may have caused cell rupture, which agrees with the data obtained by cytometry.

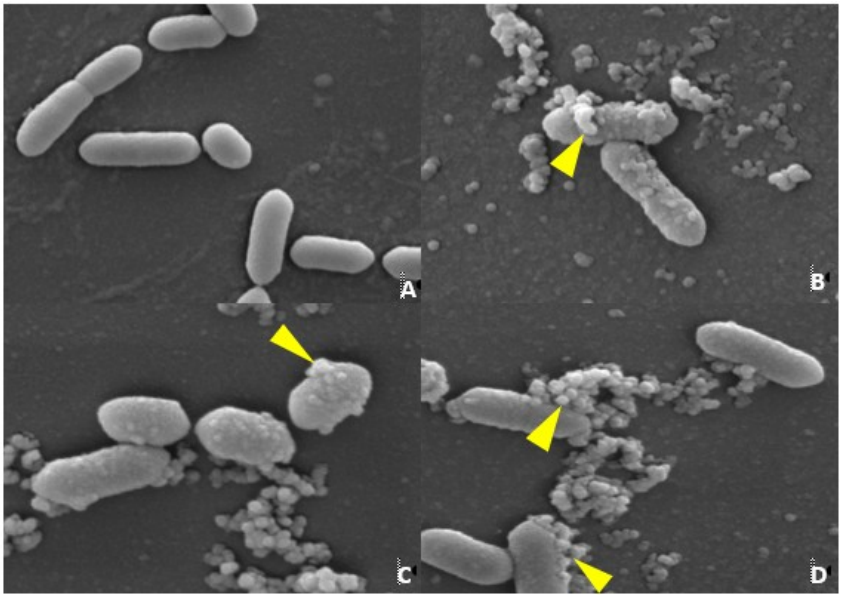

Figure 2. Scanning electron microscopy images of the effect of *Listeria monocytogenes* treatment; (A) Control cells/untreated; (B) Cells treated with nisin; (C) Cells treated with *Spirulina platensis* extract; (D) Cells treated with both nisin and *Spirulina platensis* extract; Arrow: amorphous mass; (magnification of 30,000x).

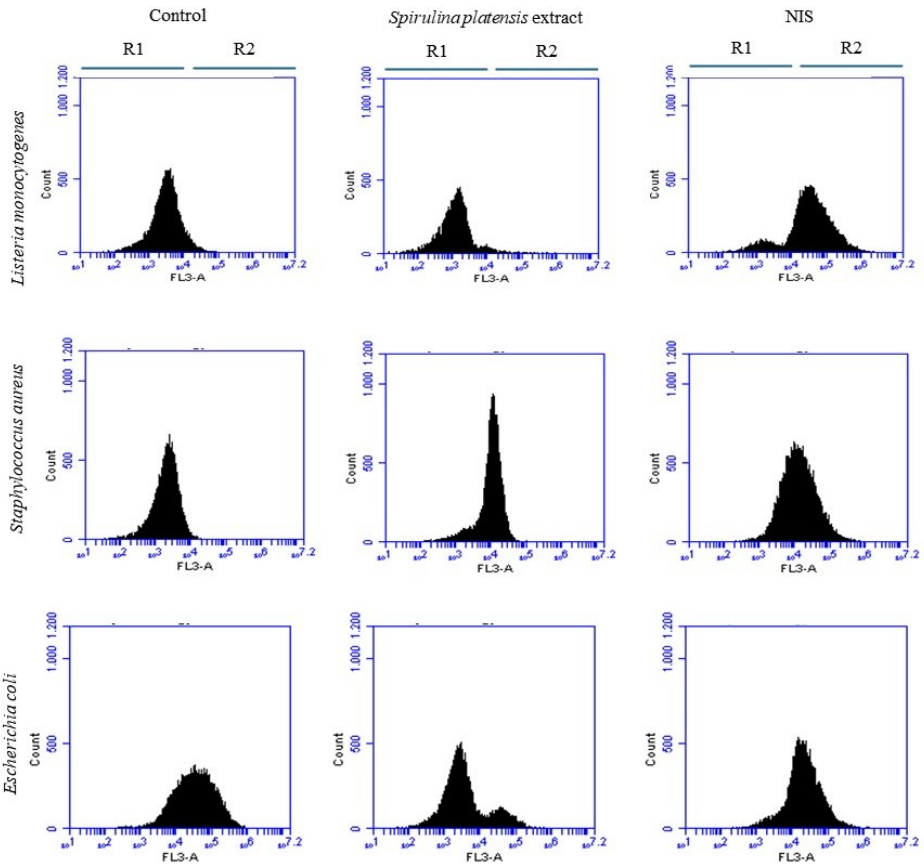

Figure 3. Fluorescence intensity graphs of *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*, stained with PI; (A) Control cells/untreated; (B) Cells treated with *Spirulina platensis* extract; (C) Cells treated with nisin.

Conclusion

In this study, it was possible to prove the synergistic antimicrobial action among NIS and *S. platensis* extract. Our findings show that the algal extract obtained by the alcohol/formic acid/sonication method has broad-spectrum activities comparable to NIS against different species of bacteria from food. The changes observed in SEM and flow cytometry suggest that the *Spirulina* extract's action was bactericidal due to the observed results of ruptures in the bacterial membrane of *L. monocytogenes*. It can be concluded that the combination of the antimicrobial agents studied can be used in the formulations of new food preservatives.

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