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Quality and safety preservation of pineapple sticks using the cell-free supernatant of lactic acid bacteria

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ABSTRACT

The demand for clean-label and minimally processed foods has increased interest in natural biopreservation strategies. The present study evaluates the use of the cell-free supernatants (CFSs) of three lactic acid bacteria (LAB) strains (*Pediococcus acidilactici* CNTA 1059, *Levilactobacillus brevis* CNTA 1374, and *Lactiplantibacillus plantarum* CNTA 600) as potential bioprotective agents to preserve fresh-cut pineapple sticks. Two spoilage yeasts, *Meyerozyma* spp. and *Rhodotorula toruloides*, were identified as the predominant microbiota responsible for deterioration. The CFSs of the three LAB strains were then tested in combination with passive modified atmosphere packaging for their ability to inhibit microbial growth and preserve physicochemical and sensory properties during refrigerated storage. Among the treatments, the *P. acidilactici* CNTA 1059 CFS effectively inhibited yeast proliferation while preserving the colour, texture, and flavour of the pineapple sticks for up to 12 days. Consumer-based evaluations indicated higher acceptability scores for treated samples compared to controls, consistent with their better sensory quality over storage. The suitability of *P. acidilactici* CNTA 1059 as a bioprotective agent was further supported by whole-genome sequencing analysis, which showed that the strain does not carry transmissible antibiotic resistance genes and lacks pathogenic markers. The genome analysis revealed the presence of the pediocin PA-1 biosynthetic gene cluster, and mass spectrometry confirmed its actual production in the CFS. These results present *P. acidilactici* CNTA 1059 as a safe, effective, and multifunctional LAB strain, whose CFS can extend the shelf life of minimally processed pineapple sticks, with potential applications for other minimally processed fruits to meet clean-label demands.

1. Introduction

Contemporary consumer trends are increasingly moving towards healthy, chemical-free, sustainable, and clean-label products, as well as ready-to-eat (RTE) products (Alegbeleye & Sant'Ana, 2022). According to the European Community standards, RTE foods are those intended for direct consumption with minimal processing and handling (Botondi et al., 2021). Despite a decline in production during the pandemic years, a compound annual growth rate of 6.5% is expected for RTE products through 2032 (DataIntelo, 2023), a period during which the fresh-cut fruit market will continue to grow (Testa et al., 2021).

RTE fruit and vegetables are common products whose processing involves peeling, coring, and cutting. These operations trigger metabolic and enzymatic processes, affecting colour, odour, flavour (Botondi et al., 2021; Iturralde-García et al., 2022), and firmness (Giannakourou & Tsironi, 2021; Iturralde-García et al., 2022). Additionally, processing and cold storage increase nutrient and water release, making them more accessible to microorganisms (Collu et al., 2021). This includes spoilage microorganisms such as lactic acid bacteria (LAB), moulds and yeasts, and pathogen microorganisms such as *Pseudomonas* spp. and members of the *Enterobacteriaceae* family (Giannakourou & Tsironi, 2021; Iturralde-García et al., 2022). These challenges are especially pronounced in

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RTE perishable fruits such as fresh-cut pineapple, whose quality quickly declines even at refrigeration temperatures (O'Connor-Shaw et al., 1994).

The food industry addresses this issue through meticulous handling, disinfection, and preservation treatments (Paulsen et al., 2023), such as the use of modified atmosphere packaging (MAP) to preserve the product's freshness (Giannakourou & Tsironi, 2021). Disinfection of RTE fruit and vegetables with sodium hypochlorite is commonly carried out in the food industry (Arbor, 2008), but its application is strictly regulated and discouraged in the European Union due to the potential formation of chlorate and other halogenated by-products with possible harmful effects (Commission Regulation (EU) 2023/915; EFSA CONTAM Panel, 2014).

To avoid the negative effects of hypochlorite and in response to consumer demands, alternative strategies aligned with the clean-label concept are being explored. One material-based approach involves the use of edible coatings derived from natural biopolymers with intrinsic antimicrobial properties, such as chitosan (Basumatary et al., 2022; Wang et al., 2024; Zhao et al., 2021). A separate, very promising biological approach is based on the use of protective cultures, that is, microorganisms added to RTE fruits and vegetables to control the presence of pathogenic and spoilage bacteria and fungi (Doukaki et al., 2024; Ramos et al., 2020). Additionally, some of these protective cultures can act as probiotics, providing health benefits to the consumer, such as through the addition of live bacteria in fruits (Hashemi & Jafarpour, 2021; Tenea et al., 2020).

In contrast with protective cultures, which require the presence of live microorganisms, cell-free supernatants (CFSs) obtained after the removal of viable cells in cultures provide antimicrobial activity via the organic acids, bacteriocins, and other metabolites accumulated in the medium (Barbosa et al., 2017). Previous studies have already demonstrated the antimicrobial efficacy of LAB-derived CFSs in fresh-cut fruits, supporting their potential as clean-label preservation tools (Collazo et al., 2017; De Simone et al., 2021; Tenea et al., 2020; Islam et al., 2023; Wong & Li, 2023). For this purpose, LAB are particularly suitable due to their well-documented antimicrobial activity, widespread use in food (Hernández Figueroa et al., 2024), and the Qualified Presumption of Safety (QPS) status granted to most of their species (EFSA BIOHAZ Panel, 2024). In addition, many LAB are recognised by the U.S. Food and Drug Administration (2024) as Generally Recognised as Safe (GRAS) for use in foods, which further supports their safe application in food systems. Furthermore, this QPS status and their long history of safe use indicate that intentionally added, well-characterized LAB strains are not classified as biological hazards within a HACCP framework (*Codex Alimentarius* Commission, 2020).

The main objective of this study was to evaluate the potential of CFSs from three previously selected LAB strains (*Pediococcus acidilactici* CNTA 1059, *Levilactobacillus brevis* CNTA 1374, and *Lactiplantibacillus plantarum* CNTA 600) as natural antimicrobial agents for extending the shelf life of fresh-cut pineapple. The study followed a stepwise approach: (i) identification of the predominant spoilage microbiota associated with fresh-cut pineapple; (ii) assessment of the influence of CFS treatment on physicochemical parameters of minimally processed product through preliminary screening; (iii) evaluation of their antimicrobial potential to select the most effective treatment; and (iv) validation of the selected treatment through testing under abusive temperatures and a consumer-based sensory evaluation; and (v) exploration of the underlying mechanisms responsible for the antimicrobial activity of the selected CFS. For clarity, a graphical abstract summarising the overall workflow and main findings is provided in the Supplementary Material (Figure S0).

2. Material and methods

2.1. Isolation and identification of pineapple spoilage microorganisms

The fruit used in this experiment was pineapple (*Ananas comosus* (L.) Merr.), variety Golden Sweet. All the material was purchased at the wholesale market (Mercazaragoza, Zaragoza, Spain), which allowed us to work with fruit at a uniform stage of ripeness.

The first step involved storing the peeled and cut fruit under refrigeration until the spoilage microorganisms were visually observed. At that point, samples were taken for microbiological analysis. To simulate typical marketing conditions for perishable products, the cut fruit was stored at 4°C for 4 days (one-third of the shelf life) and then at 8°C for 8 days (two-thirds of the shelf life) in a cold room, following the approach described by de Andrade Cavalari et al. (2024). After these 12 days, the fruit was homogenized under sterile conditions (Biomérieux, Marcy-l'Étoile, France) with 0.1% buffered peptone water (BPW) (Merck KGaA, Darmstadt, Germany). The resulting homogenate was diluted to different concentrations and seeded on various agar media as previously described by Yousuf & Srivastava (2017) for subsequent enumeration and microbial identification (Table S1).

Following the isolation of the colonies, taxonomic identification was performed using 16S rRNA gene sequencing for bacteria and the D1/D2 region of the 26S rDNA gene sequencing for fungi. Genomic DNA was extracted from pure cultures using the silica-column commercial kit GenElute™ Bacterial Genomic DNA Kit (NA2110, Sigma-Aldrich, Massachusetts, United States of America) according to the manufacturer's instructions. The V1-V3 region of the 16S rRNA gene was amplified using the bacterial primers 27F-YM (5′-AGAGTTTGATYMTGGCTCAG-3′) and 515R (5′-ATCGTATTACCGCGGCTGCTGGCA-3′) (Stackebrandt and Goodfellow, 1991), while the region D1/D2 of the 26S rDNA was amplified using the fungal-specific universal primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGTGTTCCAAGACGG-3′) (O'Donnell, 1993). PCR products were purified using the mi-PCR Purification Kit (Cat. No. mi-PCR250) (Metabion International AG, Planegg, Germany) and sequenced via Sanger sequencing using an ABI 3730xl DNA Analyzer (Applied Biosystems). Sequences were trimmed, quality-checked, and compared against the NCBI nucleotide database using the BLASTn algorithm to assign taxonomic identity based on sequence similarity. Fungal isolates were identified at the species level based on a similarity greater than 97%.

2.2. Preparation of the cell-free supernatants

The selection of the strains to produce the CFSs was based on the results of a previous work (Garin-Murguialday et al., 2024). These microorganisms were *P. acidilactici* CNTA 1059, *L. brevis* CNTA 1374, and *L. plantarum* CNTA 600. Their CFSs were obtained following a methodology previously described (Garin-Murguialday et al., 2024). Strains cryopreserved at -80°C were streaked onto de Man, Rogosa and Sharpe (MRS) agar plates and incubated at 37 °C for 24–48 h. For each strain, a single colony was then transferred into 10 mL of MRS broth and incubated at 37 °C for 18–24 h under anaerobic conditions. This pre-culture was subsequently used to inoculate 1 L of MRS broth, which was then incubated at 37 °C for 24 h under anaerobic conditions, and then centrifuged at 4347 × g for 10 min at 4 °C and filtered through 0.22 μm filters (VWR, Radnor, PA, USA).

2.3. Pineapple sticks preparation and storage

Different batches were acquired throughout a one-month period for the evaluation of the preservation treatments under realistic commercial conditions. The pineapples were disinfected with running tap water at 4°C containing 100 ppm sodium hypochlorite for 3 min, then rinsed in tap water at 4°C for 5 min and drained. Both coring and peeling were performed manually. The fruit was cut into 16 sticks (60 g) with a sterile steel knife and subsequently immersed in refrigerated running tap water (4° C) or in the corresponding pure cell-free supernatant (CFS) of *P. acidilactici* CNTA 1059, *L. brevis* CNTA 1374, or *L. plantarum* CNTA 600 for treated batches, which constituted 5% v/w. Excess solution was drained for 15 min at 4°C.

A portion of the untreated sticks was packaged in TS250 polypropylene trays (Linpac, Featherstone, England) with a capacity of 250 mL, containing one stick per package, and these were packed with a polyethylene macroperforated (Amcor Flexible; Victoria, Australia) film. Another portion of the untreated and all the treated pineapple sticks were packaged in the same type of trays, also individually, but using Passive Modified Atmosphere Packaging (MAP). More specifically, they were heat-sealed using a 40 μm thickness polyethylene microperforated film with gas permeabilities of 10.544 mL O_2/h and 9.178 mL CO_2/h at 21 °C (Amcor Flexible) in a B160A semi-automatic packaging machine (ORA, Trevoux, France).

Therefore, five working batches were defined: control-air (C1) (water + air), control-MAP (C2) (water + passive MAP), *P. acidilactici* CNTA 1059 CFS + passive MAP, *L. brevis* CNTA 1374 CFS + passive MAP and *L. plantarum* CNTA 600 CFS + passive MAP.

As stated before, the sticks were stored for one-third of the time at 4°C and two-thirds at 8°C. Trays were randomly selected throughout storage for the different analyses. Headspace gas composition and physicochemical parameters (texture, °Brix, acidity, maturity index, and CIEL*a*b* colour coordinates) were assessed only at days 0 and 12. Microbial counts were determined at days 0 and 12 under standard storage conditions, and at days 0, 5, and 10 in the parallel study simulating abusive temperatures, following the conditions described by Iglesias et al. (2018). Sensory evaluation was performed on days 0, 8, and 12.

2.4. Headspace atmosphere determination and physicochemical analyses

2.4.1. Headspace atmosphere determination

An automatic gas analyser (PBI, Dansensor; Barcelona, Spain) was used to determine the headspace gas composition before the physicochemical analyses at each sampling time in five random trays for each batch. A syringe was inserted through an adhesive septum affixed to the film cover to measure the carbon dioxide and oxygen levels. The results are presented as percentages.

2.4.2. Texture evaluation

The texture analysis of fresh-cut pineapple was conducted over a 12-day period of cold storage using a penetration test. Prior to texture analysis, the pineapples were tempered to 21°C. Texture analysis was carried out on the whole pineapple stick (approximately 10 cm long), and each measurement was taken at the central part of the pineapple stick. Firmness was determined as the peak value on the Y-axis and expressed per unit of force (N) (Russo et al., 2014). It was assessed using a TA-XT PLUS texture analyzer (Stable Micro Systems, Godalming, England) equipped with a Magness-Taylor penetration probe. The methodology was adapted from Frisón et al. (2021), involving the insertion of a 2 mm diameter stem into three distinct points on each pineapple stick at a velocity of 0.005 m/s, penetrating to a depth of 10 mm. Measurements were taken at each sample time on five samples from each batch.

2.4.3. Maturity index

The maturity index (MI) is defined as the ratio of soluble solids content to titratable acidity (MI = soluble solids / acidity).

2.4.3.1. Measurement of soluble solids content (°Brix). The total soluble solids content (°Brix) was determined using the method described in the Official Methods of Analysis of Fruit Juices (Association of Official Analytical Chemists, 1984). At each sampling time, five sticks were divided into three batches, and a juice was made from each to have three replicates. Two drops of these juices were analyzed using a digital refractometer (LLG-Labware, Germany) equipped with an automatic temperature corrector feature. The results were expressed as °Brix at 20°C.

2.4.3.2. Determination of titrable acidity. The total content of natural acids was quantified using the potentiometric method number 942.15 (Association of Official Analytical Chemists, 1990) through titration with a sodium hydroxide solution. Each analysis was performed in triplicate on five pineapple sticks. A 10 mL aliquot of juice from each stick was diluted to 100 mL with distilled water and titrated with 0.1 N sodium hydroxide to reach a pH of 8.10. An automatic titrator (CRISON) was employed for the titration. The total acidity, considering the predominant acid in the studied fruits, was expressed as g of citric acid per 100 mL of pineapple juice.

2.4.4. Colour measurement

2.4.4.1. Instrumental colour determination. CROMA METER CR-400 colorimeter (Konika Minolta, Japan) was employed to measure colour at three distinct points on the surface of five pineapple sticks/batch, with each measurement lasting three seconds. Reflection measurements were taken across both the visible and infrared spectra, with a 1 nm interval for the former and a measurement range from 360 nm to 900 nm for the latter. The resulting data were expressed as ΔE, derived from the L*, a*, and b* coordinates in the CIELAB colour space, a standard established by the International Commission on Illumination (CIE):

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔE: colour difference between day 0 and day 12; ΔL: difference in lightness between day 0 and day 12; Δa: difference in the red-green coordinate between day 0 and day 12; Δb: difference in the yellow-blue coordinate between day 0 and day 12.

2.4.4.2. Colour determination via digital image analysis. In addition to instrumental colour determination, a digital image analysis (Arias et al., 2018) was performed in five sticks of each batch per sampling time to assess whether the application of the CFS treatment caused changes in the product's characteristic colour. The vision system included the lighting system, a digital camera and image processing software.

Regarding the lighting system, to ensure homogeneous conditions, all sticks were illuminated using two halogen lamps (Philips, Eindhoven, The Netherlands) positioned at a 45° angle to the samples and 40 cm above them. The lamps had a colour temperature of 3000 K.

The camera used to take the pictures was a Canon EOS 10d (Amsterdam, The Netherlands) colour digital camera with a 6.3 Mp CMOS sensor and equipped with a 50 mm-lens. The camera was placed 40 cm in front of each sample with an angle between the camera lens and the axis of the light source of approximately 45°. The images were captured manually using a lens aperture of f = 2.8 and a shutter speed of 1/2000. No flash or zoom was used, and the images were taken with autofocus, maximum resolution, and stored in JPEG format. The captured images focused on one side of the stick against a white background.

The images were processed consistently with the CellProfiler version 4.2.6. It was employed to enhance image quality, reduce noise, and determine browning area (%) of the pineapple sticks. Through this process, we determined whether the points in the segmented image belonged to the same object. Each object was assigned a colour, and morphometric measurements were obtained separately for each stick. Finally, a calibration was performed to measure the length of elements in pixels across all images, using known dimensions. The area of each identified object (sections of the stick based on colour) was calculated in square pixels. These results were then converted into real units to determine the percentage of browning: % browning area = (brown colour pixels / total object pixels) × 100.

2.5. Determination of yeast populations on pineapple sticks

To evaluate the efficacy of the treatments against microbial growth on the developed product and to establish the conditions for its commercial application.

The sticks from the applied treatments were homogenized with a stomacher homogenizer blender AES Easy Mix (QuestPair, The Netherlands) with 0.1% BPW, and 1:10 serial dilutions were prepared for subsequent surface spreading on Potato Dextrose Agar (PDA) plates. This medium was selected to allow for the growth of the yeasts identified during the preliminary characterization of spoilage microorganisms.

To evaluate the outcomes, the spoilage population (CFU/g) of the control and treated sticks with the CFS at the initial time point and after 12 days was compared. The difference in the population was calculated as the reduction in the population of the spoilage microorganisms (in log CFU/g) between the treated sticks after 12 days of storage (4°C/ 4 days and 8°C/ 8 days) and the treated sticks immediately after the CFS treatment (day 0). This same methodology was applied in the shelf-life study that simulated storage or distribution under abusive temperatures, sampling them at 0, 5, and 10 days for microbial analysis.

2.6. In vitro antimicrobial activity of the selected CFS by MIC (minimum inhibitory concentration) determination assay

To assess the antimicrobial activity of the selected CFS against *Rhodotorula toruloides* over time, a MIC determination assay was performed. Sterile 96-well flat-bottom microtiter plates (DELTALAB) were prepared with increasing concentrations of CFS (0%, 0.15%, 2.5%, and 5%) in potato dextrose broth. Each well was inoculated with *R. toruloides* to a final concentration of 10^5 CFU/mL. Plates were incubated under the optimal growth conditions for the yeast, and microbial growth was monitored both visually and spectrophotometrically. The optical density readings at 620 nm were recorded every 2 hours using a microplate reader (EPOCH 2 NS, Agilent Technologies, Santa Clara, CA, USA).

2.7. Evaluation of consumer ratings for the pineapple sticks

Once the optimal preservation treatment was identified, a subsequent experiment was performed with pineapple under ideal conditions to evaluate consumer acceptance of the new product.

For that purpose, a sensory evaluation was conducted using a panel of trained consumers (n = 10) to assess the organoleptic characteristics of pineapple sticks from two different batches: a control batch and an optimized treated batch (*P. acidilactici* CNTA 1059 CFS + Passive MAP). Evaluations were performed on days 0, 8, and 12 of shelf life. Participants assessed key sensory attributes (colour, odour, flavour, and texture) and provided an overall product evaluation. A structured, quantitative sensory analysis was employed, using a questionnaire with defined scales: colour was rated on a 0–5 scale (Figure S1), while odour, flavour, and texture were evaluated on a 1–7 intensity scale, ranging from absence to extremely intense. Within the same questionnaire, participants also responded to a hedonic acceptability question, using a 1–10 scale in which scores below 5 indicated product rejection. Figure S1 presents the translated sensory questionnaire used in this study.

2.8. Statistical analysis

The tables were generated using GraphPad Prism software (version 10.3.0). Statistical comparisons were performed assuming normal distribution within each group. For physicochemical parameters, independent-samples *t*-tests were performed to assess significant differences for each variable between 0 and 12 days within each preservation treatment, with significance set at $p \leq 0.05$. One-way ANOVA tests were applied at each time point (for physicochemical parameters), and across all time points (for microbial counts and sensory attributes) to assess significant differences ($p \leq 0.05$) between treatments. When a significant main effect was detected, pairwise comparisons were subsequently performed using Fisher's least significant difference (LSD) test, and significance was accepted at $p \leq 0.05$.

2.9. Whole genome sequencing (WGS) of the selected LAB strain and post-sequencing analysis

The genome of the selected LAB strain *P. acidilactici* CNTA 1059 was sequenced using the Illumina iSeq100 platform (KIMITEC, Almeria, Spain). Genomic DNA was extracted from morphologically and microscopically confirmed pure cultures using the Zymo Quick-DNA Fungal/Bacterial Miniprep Kit. DNA quantity was determined using the Quant-iT™ 1X dsDNA Assay Kit (Thermo Fisher, MA, USA), and quality was evaluated by spectrophotometry (Multiscan Sky, Thermo Fisher) and 0.6% agarose gel electrophoresis. Sequencing libraries were prepared using the Illumina DNA Prep Kit and analyzed with a Bioanalyzer 2100 (Agilent Technologies, CA, USA).

Raw 2 × 150 bp paired-end reads were quality-checked using FastQC v.0.12.1 (Andrews, 2010) and adapters and low-quality reads were filtered with Trim Galore v.0.6.10. *De novo* genome assemblies were generated using shovill v0.9.0 (Seeman, 2017) and the assembly quality (contiguity and completeness) was checked with QUAST v5.2.0 (Gurevich et al., 2013).

The previous taxonomic assignment performed via Sanger sequencing of the 16S rRNA gene was confirmed by analyzing clean reads using Kraken2 v.2.1.3 (Wood et al., 2019), by submitting the assembled genome to the Type Strain Genome Server (Meier-Kolthoff & Goker, 2019) for whole-genome-based classification, and by comparison of the assembled genome to a closely related reference genome (NCBI RefSeq assembly GCF_013127755.1) via calculation of the Average Nucleotide Identity using FastANI v1.33 (Jain et al., 2018).

Antimicrobial resistance genes were detected using AMRFinderPlus v3.12.8 (Feldgarden et al., 2021), BacAnt (Hua et al., 2021), and ABRicate v1.0.1 (Seeman, 2016) with comprehensive screening across the CARD, MEGARes 2.0, NCBI Bacterial Antimicrobial Resistance Reference Gene Database, ResFinder v4, and ARG-ANNOT databases. To maximize sensitivity, an initial relaxed threshold of 5% identity and coverage was applied to capture all potential hits, followed by manual filtering to retain only high-confidence matches with ≥80% identity and ≥70% coverage (EFSA, 2024). PathogenFinder v1.1 (Cosentino et al., 2013) was used to predict the pathogenic potential of the isolate based on genomic features. Finally, antiSMASH v6.1.1 was used to predict the type and location of secondary metabolite biosynthesis gene clusters within the assembled genome. In parallel, genome annotation was performed with Prokka v.1.14.6 (Seeman, 2014). The resulting annotated sequences were queried against the BAGEL4 database (Van Heel et al., 2018) and the APD3 (Antimicrobial Peptide Database) (Wang et al., 2016) using BLAST+ (Camacho et al., 2009) with a minimum identity threshold of 80% to identify genes predicted to encode antimicrobial peptides.

3. Results and discussion

3.1. Identification of the predominant spoilage microbiota associated with fresh-cut pineapple

There was no growth on MRSa and TSA-YE media, but colony growth was observed only on PDA medium, where two morphologically distinct colonies grew, being responsible for the alteration of the product. They were identified as *Meyerozyma* spp. (>99% BLAST identity to NCBI RefSeq NR_111247.1) and *Rhodotorula toruloides* (>99% BLAST identity to NCBI RefSeq NR_153295.1), both of which have been described in the literature as potential spoilage microorganisms of post-harvest pineapples (Manthou et al., 2021).

3.2. Study of the influence of CFS treatment on physicochemical parameters of minimally processed product through preliminary screening

After 12 days of storage, the evaluation of the impact of the addition of the three CFSs on the physicochemical parameters of the pineapple sticks, through both visual and objective analysis, was essential in selecting and/or discarding potential protective agents as a preservation option for this RTE product. The findings of this study are detailed below.

The evolution of the headspace concentrations of oxygen and carbon dioxide exhibited no significant differences between the untreated and CFS-treated sticks (data not shown). In all cases, the equilibrium atmosphere was 15.2% ± 1.3 O_2 and 5.1% ± 0.9 CO_2, considered an adequate atmosphere for the commercialization of this type of product. Therefore, these were the factors that proved decisive, and this merely served to ensure that no anomalous changes occurred.

3.2.1. Texture

The results of the firmness of the sticks at day 0 and after 12 days under refrigeration conditions are presented in Table 1.

At the beginning of the study, there were no significant differences ($p > 0.05$) in the firmness of the sticks between the C1 and C2 control groups and the CFS-treated batches, indicating that immersion in the CFS of LAB did not affect the sticks' firmness. In the sticks from batches C1 and those treated with the CFS of *L. brevis* CNTA 1374 and *L. plantarum* CNTA 600, no significant differences were observed when comparing the values obtained after 12 days of storage to the initial ones. Similar results were reported by Saravanakumar et al. (2020) in the case of the use of *L. rhamnosus* GG in red and yellow fresh-cut peppers. However, the sticks from the C2 batch showed a significant decrease ($p = 0.036$) in firmness, from 11.44 ± 0.70 N to 10.46 ± 0.52 N, aligning with the findings of Gomez et al. (2020), who reported a decrease in the firmness of pineapple slices packed in MAP conditions and stored at 8°C when the O_2 concentration was between 14% and 17%. The batch treated with the CFS of *P. acidilactici* CNTA 1059 exhibited a significant ($p = 0.011$) increasing trend in firmness over the 12 days of storage, with values of 12.10 ± 0.61 N. This value is slightly above the initial firmness, which is attributable to normal sample-to-sample variability rather than a true increase. Furthermore, the samples treated with this CFS showed significantly higher firmness than those treated with MAP + water, suggesting that *P. acidilactici* CNTA 1059 CFS helped maintain firmness and prevented the softening typically observed in samples packed in MAP alone.

Table 1
Evolution of physicochemical parameters (firmness, maturity index, colour and browning) of CFS *P. acidilactici* CNTA 1059, *L. plantarum* CNTA 600 and *L. brevis* CNTA 1374-treated pineapple sticks during 12 days at 4°C. For each parameter, an asterisk indicates a significant difference ($p \leq 0.05$) for that parameter within each treatment between 0 and 12 days (*t*-test), while superscript letters indicate significant differences between treatments at day 12 (based on one-way ANOVA followed by Fisher's LSD test); means not sharing a letter differ significantly ($p \leq 0.05$). Data expressed as mean ± standard deviation of 5 independent replicates.

Preservation treatment	Time (days)	Firmness (N)	Maturity index	Colour (ΔE)	Browning (%)
C1 (air + water)	0	11.44 ± 0.70	1.37 ± 0.24	-	-
	12	11.24^{abc} ± 1.29	1.64^{a} ± 0.16	12.75^{abc} ± 1.25	32.63^{a} ± 7.65
C2 (passive MAP + water)	0	11.44 ± 0.70	1.37 ± 0.24	-	-
	12	10.46^{b*} ± 0.52	1.48^{b} ± 0.05	12.22^{abc} ± 0.95	39.34^{ac} ± 8.16
passive MAP + CFS *P. acidilactici* CNTA 1059	0	11.03 ± 0.40	1.29 ± 0.11	-	-
	12	12.10^{c*} ± 0.61	1.93^{c*} ± 0.11	12.11^{abc} ± 0.49	9.46^{b} ± 5.55
passive MAP + CFS *L. brevis* CNTA 1374	0	11.44 ± 0.70	1.37 ± 0.24	-	-
	12	11.41^{abc} ± 0.82	1.43^{b} ± 0.12	11.14^{b} ± 0.75	11.54^{b} ± 4.19
passive MAP + CFS *L. plantarum* CNTA 600	0	11.03 ± 0.40	1.29 ± 0.11	-	-
	12	11.80^{ac} ± 0.83	1.92^{c*} ± 0.15	14.20^{c} ± 2.40	46.42^{c} ± 6.64

3.2.2. Maturity index

The effect of the different treatments on the maturity index of the pineapple sticks is detailed in Table 1. No significant differences were observed between the batches at the initial time. Both control batches and those packed with MAP and treated with *L. brevis* CFS CNTA 1374 had values of 1.37 ± 0.24, while the batches packed in MAP and treated with the CFS of *P. acidilactici* CNTA 1059 or *L. plantarum* CNTA 600 had values of 1.29 ± 0.11. Over the 12-day refrigerated storage period, both the controls and CFS-treated sticks showed an increase in maturity index, likely due to ripening or water loss (Martínez-Castellanos et al., 2011; Temiz & Özdemir, 2021). Batches with a lower initial maturity index exhibited a significant increase ($p < 0.001$), with values of 1.93 ± 0.11 (*P. acidilactici* CNTA 1059) and 1.92 ± 0.15 (*L. plantarum* CNTA 600), which were also significantly higher than those observed in the other batches at this time point. These increases may be reflect not only ripening effects but also some contributions from metabolites present in the CFSs affecting the sugar–acid balance. Importantly, a higher maturity index is generally desirable for fresh-cut pineapple, as it is associated with a sweeter flavour profile (Siti Rashima et al., 2021).

3.2.3. Colour

The colour evolution (ΔE) and the % of browning during the storage period varied depending on the combined treatment applied. Table 1 summarizes the ΔE results and the browning percentage obtained with the digital image analyzer after 12 days of storage. These results are presented for C1 and C2 and the sticks treated with different CFSs packed in passive MAP. Additionally, Figure S2 shows, shown as an example, representative images overlaid with CellProfiler segmentation outputs, with red areas identified as browning on the pineapple sticks.

The initial colour of the sticks from different batches was not affected (data not shown) by immersion in the CFS of LAB. This observation indicates that neither the CFS nor the culture medium used to obtain it caused any visible colour modification of the pineapple surface at time 0. These results align with Russo et al. (2014), who did not detect significant differences when immersing pineapple pieces in suspensions of *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum*, and De Simone et al. (2021) similarly observed that CFSs applied to fresh-cut kiwifruit did not alter initial colour parameters.

The ΔE values of the control sticks packaged in air (C1) were very similar to those of the control sticks packed under passive MAP (C2), with values of 12.75 ± 1.25 and 12.22 ± 0.49, respectively. Similarly, the CellProfiler recorded the percentage of browning for each batch as 32.63% and 39.34%, respectively.

However, the sticks immersed in the CFSs showed a markedly different colour evolution compared to the control and to each other. The CFS of *L. plantarum* CNTA 600 altered the colour of the sticks, resulting in a higher colour difference (ΔE = 14.20 ± 2.40) and browning percentage (46.42%) compared to other treatments. In contrast, the CFSs from the other two LAB (*P. acidilactici* CNTA 1059 and *L. brevis* CNTA 1374) showed the ability to inhibit the browning of pineapples compared to the controls and CFS *L. plantarum*-treated sticks, as indicated by lower ΔE values of 12.11 ± 0.49 and 11.14 ± 0.75, respectively. The lesser change in initial colour was also reflected in the CellProfiler analysis, where the browning percentages were 9.46% for the sticks treated with *P. acidilactici* and 11.54% for those treated with *L. brevis* CNTA 1374 CFS. In agreement with this, most reports highlight the anti-browning potential of LAB-derived metabolites, including bacteriocin-rich CFSs, in fresh-cut fruits. For instance, Islam et al. (2023) reported that apples immersed in the CFS of *L. plantarum* DMR14

retained their original colour and freshness, and De Simone et al. (2021) observed that kiwifruit pieces dipped in the CFS of *L. plantarum* UFG 121 retained acceptable sensory quality for up to 10 days. Our findings suggest that the effect of LAB CFSs on fruit appearance is strain-dependent and should be individually evaluated.

Enzymatic browning reactions, mostly catalyzed by polyphenol oxidase (PPO) isoenzymes, are known to be slowed by a combination of low temperatures and the use of specific enzyme or browning inhibitors. Various compounds (such as ascorbic acid, citric acid, melatonin, and others) have demonstrated anti-browning activity in PPO systems from diverse biological sources, acting through different regulatory mechanisms (Arias et al., 2007a; Arias et al., 2007b; Zheng et al., 2019). These mechanisms may include antioxidant activity, which enables the compound to react with oxidized phenolic intermediates, or a direct interaction with the PPO enzyme that interferes with its catalytic function. Such evidence supports the idea that the metabolites naturally present in certain LAB-derived CFSs could similarly contribute to delaying browning in fresh-cut fruits, as observed for the CFSs of *P. acidilactici* CNTA 1059 and *L. brevis* CNTA 1374 in the present study.

After evaluating the impact of the three CFSs on the colour of the pineapple sticks, it can be concluded that the combined treatment of passive MAP packaging and immersion in *L. plantarum* CNTA 600 CFS was not suitable and was discarded for application because it negatively affected the characteristic colour of the product. For this reason, the following sections will only discuss the CFS of the two remaining LAB strains.

3.3. Evaluation of the antimicrobial potential of the CFSs to select the most effective treatment

Monitoring the natural microbial evolution of the pineapple sticks was crucial for selecting the optimal combination treatment to extend the product's shelf life. Consequently, the yeasts responsible for the spoilage of the sticks were tracked over the 12 days of storage.

The results of the microbial counts for the different batches can be seen in Table 2. Initial values ranged from around 1 to 3 log CFU/g, depending on the initial batch, highlighting the heterogeneity of the product (Gomez et al., 2020). Microbial growth remained stable for the first days of the study, showing similar growth across all samples up to 8 days (not shown), consistent with the findings reported by Russo et al. (2014). However, between days 8 and 12, the evolution of the yeasts varied depending on the batch. At day 12, the sticks from control batches C1 and C2 showed the highest yeast concentrations (8.94 ± 0.54 and 8.02 ± 0.24 log CFU/g, respectively).

For the CFS-treated sticks, a reduction in the growth rate was observed, attributed to the synergistic effect of both the CFS and the MAP packaging, resulting in an extension of the product's shelf life (Hashemi & Jafarpour, 2021). These batches exhibited counts of around 6 log CFU/g. The batch treated with *L. brevis* CNTA 1374 CFS showed a growth of 4.31 ± 1.94 log CFU/g from time 0 to 12 days. This species is known for its antifungal activity against *Fusarium verticillioides* (Somashekaraiah et al., 2021).

Table 2
Total yeast counts (log CFU/g) of the different stick batches under refrigerated storage (4 days at 4°C followed by 8 days at 8°C). Superscript letters indicate significant differences between treatments (based on one-way ANOVA followed by Fisher's LSD test); means not sharing a letter differ significantly ($p \leq 0.05$). Data expressed as mean ± standard deviation of 5 independent replicates.

Preservation treatment	Time (days)	
	0	12
C1 (air + water)	$2.70^{a} \pm 0.02$	$8.94^{d} \pm 0.54$
C2 (passive MAP + water)	$3.12^{b} \pm 0.34$	$8.02^{e} \pm 0.24$
passive MAP + CFS *P. acidilactici* CNTA 1059	$3.14^{b} \pm 0.20$	$6.07^{f} \pm 0.10$
passive MAP + CFS *L. brevis* CNTA 1374	$1.61^{c} \pm 0.08$	$6.00^{f} \pm 0.20$

The combined treatment of *P. acidilactici* CNTA 1059 CFS and MAP demonstrated the lowest microbial growth, with an overall increase of only 2.93 ± 0.15 log CFU/g from the initial time point. This effect is likely due to the antifungal capacity of *P. acidilactici* strains as highlighted by Das et al. (2020) and Fugaban et al. (2022). This finding suggests that the CFS helped reduce the growth of spoilage microorganisms by acting as an additional protective barrier, in line with the findings of Tenea et al. (2020), who reported microbial reduction after the application of an edible coating with LAB on pineapple slices.

Therefore, considering the physicochemical and microbial characteristics, it was determined that the optimal combined treatment for this product involves passive MAP packaging and immersion in the CFS of *P. acidilactici* CNTA 1059. Fig. 1 shows the flowchart used to obtain the CFS-treated and control batches of pineapple sticks after completing the

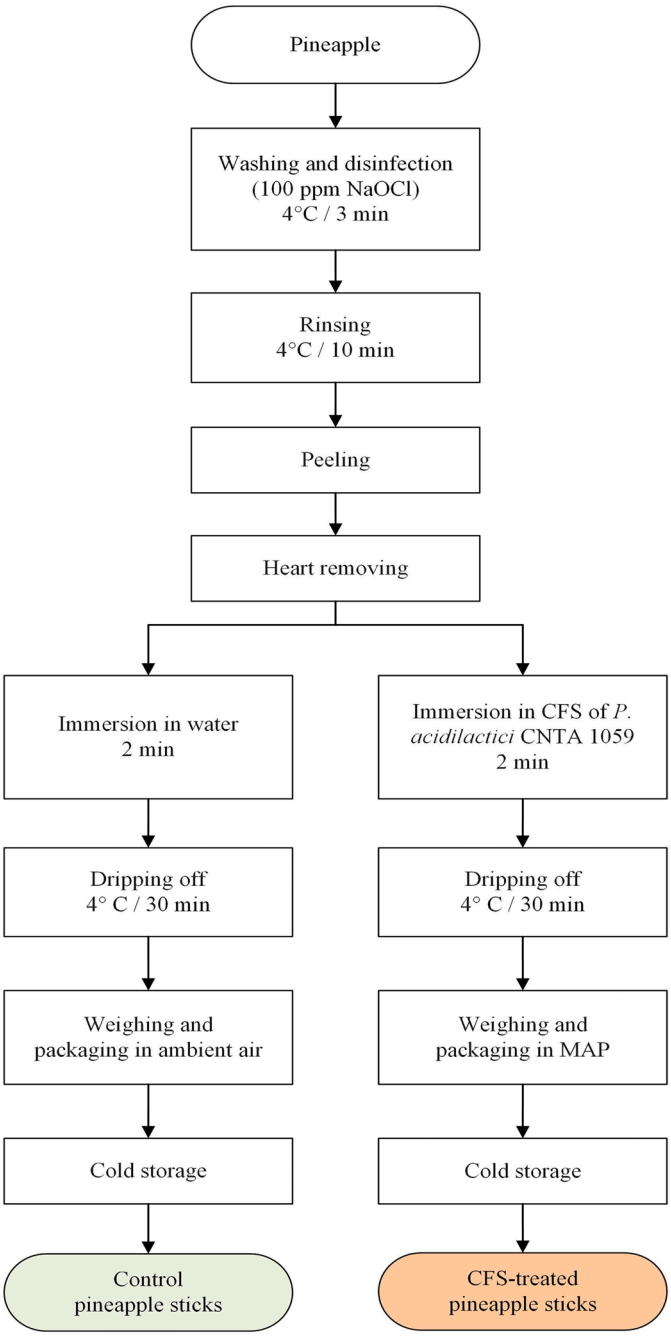

Fig. 1. Flowchart for the production of pineapple sticks treated with cell-free supernatant (CFS) and their respective control.

stepwise evaluation of the different treatments.

3.4. Validation of the selected treatment through testing under abusive temperatures and a consumer-based sensory evaluation

3.4.1. Microbial analysis under abusive temperatures

Given the promising results obtained with the *P. acidilactici* CNTA 1059 CFS from physicochemical and microbial results, a follow-up microbial analysis was conducted comparing the air + water control treatment and the combined MAP + *P. acidilactici* CNTA 1059 CFS treatment under temperature abuse conditions (10 days/ 10 °C). As shown in Table 3, yeast concentrations in the control samples (air + water) reached 8.26 ± 0.50 log CFU/g after 5 days and 9.10 ± 0.18 log CFU/g after 10 days at 10 °C. Compared to the control samples, those treated with *P. acidilactici* CFS and packed under MAP exhibited significantly ($p < 0.001$) lower yeast levels after 5 days (4.32 ± 0.36 log CFU/g) and 8.80 ± 0.51 log CFU/g after 10 days. Thus, the combined treatment effectively extended the shelf life by approximately 50%, despite potential breaks in the cold chain.

3.4.2. Evaluation of consumer ratings

Table 4 presents the evolution of selected sensory parameters of fresh-cut pineapple without any preservation treatment and with the selected combined treatment: CFS of *P. acidilactici* CNTA 1059 packed in MAP. The results from the global assessment indicated that the control pineapple sticks became inedible by day 8 of storage, so the evaluation of flavour and texture parameters in the control samples was discontinued after this period.

Over the storage period, the control batch of pineapple sticks significantly ($p = 0.001$) darkened in colour, while the CFS-treated batch maintained its typical colour (Figure S3). This observation is consistent with earlier reports demonstrating the positive effect of probiotic cultures and CFSs on fruit colour. De Simone et al. (2024) showed improved colour retention in strawberries treated with probiotic coatings, and De Simone et al. (2021) similarly reported higher colour scores in kiwifruits treated with LAB-derived CFSs compared with the control. In the same line, Mantilla et al. (2013) also observed better colour maintenance in products treated with viable LAB cells during storage at 4°C for 13 days.

Initially, testers noted significant differences in the odour and flavour profiles ($p < 0.001$ and $p = 0.017$, respectively) of the two batches, which were higher for the CFS-treated one. The control batch developed musty and rotten smells over time, consistent with findings by Montero-Calderón et al. (2008). In contrast, the CFS-treated batch retained a fresh pineapple odour and high flavour scores throughout the 12 days. This suggests that *P. acidilactici* CNTA 1059 CFS compounds did not introduce off-flavours into the sticks. Previous reports show that the CFS-treated fruits did not develop off-flavours (Islam et al., 2023; De Simone et al., 2021), which was attributed to the removal of viable cells during CFS preparation. This step eliminates the volatile compounds typically associated with LAB metabolism, while the remaining metabolites in the CFS (mainly organic acids and small antimicrobial compounds) are present at concentrations too low to negatively influence sensory attributes.

Table 3
Total yeast counts (log CFU/g) of the different stick batches under abusive temperatures (10°C). Superscript letters indicate significant differences between treatments (based on one-way ANOVA followed by Fisher's LSD test); means not sharing a letter differ significantly ($p \leq 0.05$). Data expressed as mean ± standard deviation of 5 independent replicates.

Preservation treatment	Time (days)		
	0	5	10
C1 (air + water)	3.72^{a} ± 0.60	8.26^{b} ± 0.50	9.10^{b} ± 0.18
passive MAP + CFS *P. acidilactici* CNTA 1059	3.92^{ac} ± 0.33	4.32^{c} ± 0.36	8.80^{b} ± 0.51

Table 4
Sensory analysis of pineapple sticks of control batch and combined treated batch of MAP + CFS *P. acidilactici* CNTA 1059 at 12 days. Controls were sticks without treatment with any CFS stored at 4 °C. Sensory attributes (colour, odour, flavour, texture, and overall acceptability) were evaluated using structured intensity and hedonic scales. For each attribute, statistically significant differences ($p \leq 0.05$) were determined using one-way ANOVA followed by Fisher's LSD test, and are indicated by different superscript letters. 'u.t.' = untested. Data expressed as mean t± standard deviation.

Time (days)	Control*	*P. acidilactici* CNTA 1059 CFS + passive MAP
Colour		
0	2.85^{a} ± 0.46	3.80^{c} ± 0.59
8	3.38^{b} ± 0.77	3.87^{c} ± 0.21
12	3.36^{b} ± 0.52	3.82^{c} ± 0.65
Odour		
0	2.31^{a} ± 0.37	2.92^{c} ± 0.19
8	2.16^{a} ± 0.34	3.30^{d} ± 0.39
12	1.78^{b} ± 0.14	3.37^{d} ± 0.18
Flavour		
0	3.78^{a} ± 0.62	4.53^{b} ± 0.28
8	u.t.	4.10^{ab} ± 0.41
12	u.t.	4.01^{ab} ± 0.51
Texture		
0	2.42^{b} ± 0.37	$2.75^{a,b}$ ± 0.46
8	u.t.	3.28^{a} ± 0.50
12	u.t.	$2.96^{a,b}$ ± 0.25
Global assessment		
0	6.81^{a} ± 1.04	7.60^{b} ± 0.39
8	<5	7.40^{b} ± 0.11
12	<5	7.13^{ab} ± 0.48

At the initial time point both batches showed similar texture, but the CFS-treated sticks maintained their texture over 12 days, while the control batch became softer, as observed but not formally tested by Mantilla et al. (2013). Finally, in terms of taster acceptance, the treated sticks consistently received higher scores throughout the storage period compared to the untreated ones. Initially, the first ones scored nearly one point higher (7.60 ± 0.39), with a slight decrease to 7.13 ± 0.48 after 12 days. In contrast, the control batch was rejected (score < 5) from day 8 onwards due to microbial growth on the surface. This earlier inedibility of control pineapples after a period of 8 days is consistent with previous findings by Russo et al. (2014) and slightly shorter than the 12 days reported by Basumatary et al. (2022). In these works, as well as others studying CFSs to preserve other fruits (De Simone et al., 2021; 2024), LAB-derived products consistently delayed the decline in overall acceptability according to sensory analyses.

In summary, the combined treatment with *P. acidilactici* CNTA 1059 CFS and passive MAP effectively preserved the sensory quality and extended the acceptability of fresh-cut pineapple sticks, maintaining colour, odour, texture, and overall acceptance for up to 12 days, in contrast to the rapid deterioration observed in the untreated control. Importantly, the efficacy of the treatment was also confirmed under temperature abuse conditions, simulating potential breaks in the cold chain during distribution. All these findings validate the designed preservation strategy and support its robustness and potential for commercial application.

3.5. Exploration of the underlying mechanisms responsible for the antimicrobial activity of the selected CFS

To explore the possible mechanism by which the CFS exhibits antimicrobial activity against the microorganisms present on the pineapple sticks, a sensitivity assay, Whole Genome Sequencing (WGS) of the

producing strain, and protein characterization of the CFS were performed. Details of the procedure for the protein characterization (SDS-PAGE, in-gel digestion, zymography and LC-MS/MS identification) are provided in the Supplemental Material and Methods.

3.5.1. *In vitro antimicrobial activity of the CFS*

A MIC determination assay was performed using increasing concentrations of the *P. acidilactici* CNTA 1059 CFS to evaluate its in vitro inhibitory activity against the two yeasts identified. As shown in Fig. 2, a dose-dependent inhibitory effect was observed, with 5% CFS inhibiting the growth of *R. toruloides*. This antifungal effect is consistent with previous studies showing that multiple *Pediococcus acidilactici* strains exhibit activity against *Candida* spp. (Das et al., 2020; Sookkhee et al., 2001).

3.5.2. *Whole genome sequencing (WGS)*

WGS was performed to confirm the taxonomic identity of the isolate, assess the absence of acquired antibiotic resistance and pathogenicity determinants, and detect biosynthetic cluster genes related to the production of bacteriocins or other secondary metabolites. This genomic evaluation aligns with EFSA recommendations (EFSA, 2005, 2024) for the use of microbial strains in food applications.

WGS-based taxonomic classification of the isolate was conducted using Kraken2, which identified it as *Pediococcus acidilactici*, a species included in the EFSA Qualified Presumption of Safety (QPS) list (EFSA BIOHAZ Panel, 2024). Taxonomic assignment was further validated using the Type Strain Genome Server (TYGS), which confirmed the isolate's affiliation with a well-supported *Pediococcus acidilactici* clade, showing digital DNA–DNA hybridization values ranging from 75% to 89% when compared with *P. acidilactici* type strains. In addition, FastANI analysis revealed an average nucleotide identity (ANI) of 98.56% with the reference genome of *P. acidilactici* strain PMC65, exceeding the species delineation threshold of 95%, thereby supporting its classification within the same species.

Regarding the search for antibiotic resistance genes, none of the low-confidence hits (Table S2) exceeded the high-confidence filtering threshold (≥80% identity, ≥70% coverage). These results, together with the previously performed phenotypic MIC testing (Garin-Murguialday et al., 2024), indicate that *P. acidilactici* CNTA 1059 does not appear to carry acquired antibiotic resistance determinants of concern. Furthermore, the strain was deemed non-pathogenic to humans after evaluation with PathogenFinder (prediction score of -24.564).

Functional genome mining using antiSMASH v6.1.1 only revealed a bacteriocin biosynthetic gene cluster sharing 80% similarity with the known coagulin cluster. Subsequent BLAST analysis against the BAGEL4 and APD3 databases identified this cluster as consistent with pediocin PA-1 biosynthesis, and key genes associated with the pediocin operon (*pedA, pedC*, and *pedD*)—were detected with 100% identity and coverage (Table S3).

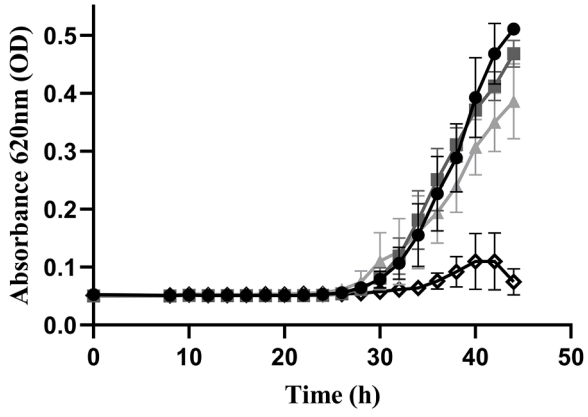

Fig. 2. Absorbance measurements of *Rhodotorula toruloides* in the presence of 0% (●), 0.15% (■), 2.5% (▲), and 5% (⋄) (v/v) of CFS. Data represent the mean ± standard deviation of three biological replicates.

3.5.3. *Protein characterization of the CFS by SDS-PAGE*

Figure S4 shows the results of the SDS-PAGE assay conducted with the CFS of *P. acidilactici* CNTA 1059. Various bands could be observed in the CFS; and a band was detected around 5 kDa, consistent with the expected size range of pediocin PA-1 (4.6 kDa), despite the resolution limitations of the gel preventing precise identification. This band would correspond to the size of pediocin PA-1, with a molecular weight of 4.6 kDa (Zhu et al., 2022).

3.5.4. *LC-MS/MS identification*

Mass spectrometry analysis of the 4.6 kDa band in the CFS enabled the detection of protein fragments consistent with known bacteriocins produced by *Pediococcus acidilactici*. Specifically, peptides corresponding to pediocin PA-1 and its associated biosynthetic components (PedA, PedC, and PedD) were identified in the CFS, confirming the presence of bacteriocin-related proteins (Table S4).

3.5.5. *Hypothetical mechanistic antimicrobial effect of the CFS*

Genome mining revealed a pediocin PA-1 biosynthetic gene cluster, suggesting the strain's potential to produce bacteriocins. This was further supported by mass spectrometry, which confirmed the presence of pediocin PA-1–related peptides in the CFS. Additionally, a zymography assay was performed using *Listeria monocytogenes* as an indicator strain, revealing an active antimicrobial band matching the expected molecular weight of pediocin PA-1 (Figure S5), thereby corroborating its production by *P. acidilactici* CNTA 1059 and supporting the antilisterial activity previously reported for this strain (Garín-Murguialday et al., 2024).

Pediocin is typically produced by *P. acidilactici* strains, and it specifically targets Gram-positive bacteria by forming pores in their membranes, leading to rapid cell death (Zhu et al., 2022). However, pediocin is not considered active against yeasts (Das et al., 2020; Rodríguez et al., 2002; Zhu et al., 2022), and therefore it cannot explain the antifungal activity observed in this study, both *in vitro* (Fig. 2) and in the fruit (Tables 2 and 3). Instead, a more plausible explanation lies in the action of other small metabolites with recognised antifungal properties. In fact, the CFS of *P. acidilactici* CNTA 1059 contains organic acids such as lactic and phenyllactic acid (Garin-Murguialday et al., 2024), which contribute to medium acidification and have well-established antifungal effects (Fugaban et al., 2022; Lindgren & Dobrogosz, 1990; Mandal et al., 2013). Moreover, Mandal et al. (2013) also reported that *P. acidilactici* strains can produce additional low-molecular-weight compounds with antifungal potential, supporting the idea that multiple metabolites may act synergistically to inhibit fungal growth.

Our findings indicate that *P. acidilactici* CNTA 1059 could exert antibacterial and antifungal inhibitory activity. This is consistent with previous work showing that *P. acidilactici* strains can suppress a wide range of pathogenic and spoilage microorganisms (including bacteria, yeasts, and filamentous fungi) in food products (Das et al., 2020). Such broad-spectrum activity, combined with the species' long-standing use in fermented foods, supports the potential of *P. acidilactici* or its CFS as a safe and effective bioprotective agent for enhancing food preservation.

To bring the product into the market, further tests are planned following this study. These assays will focus on optimizing and producing a medium suitable for human consumption that promotes the production of phenyllactic acid and bacteriocins. The main objective is to reduce the concentration of the CFS of *P. acidilactici* CNTA 1059 in the final product while maintaining the same organoleptic characteristics and achieving or improving the shelf-life results obtained with the 5 % of CFS.

4. Conclusions

The combined application of immersion in the CFS from *P. acidilactici* CNTA 1059 and passive modified atmosphere packaging (MAP) effectively reduced the proliferation of *Meyerozyma* spp. and *Rhodotorula toruloides* yeasts naturally present on pineapple sticks during 12 days of refrigerated storage (4°C for 4 days, then 8°C for 8 days), compared to the control batch. Furthermore, the synergistic effect of these two combined techniques preserved the physicochemical properties of the sticks, extending their shelf life from 8 to 12 days without compromising sensory quality. Notably, browning was greatly reduced in the treated samples.

Genomic analysis of the strain and molecular characterization of the CFS confirmed the production of pediocin PA-1. In compliance with EFSA's regulatory framework (EFSA, 2024), the strain was recognized as belonging to the QPS list (EFSA BIOHAZ Panel, 2024), harboured no transmissible antibiotic resistance genes, and was deemed non-pathogenic, further confirming previous phenotypic assays that supported the non-toxigenic characteristics of this microorganism (Garin-Murguialday et al., 2024).

This study identifies the CFS of *Pediococcus acidilactici* CNTA 1059 as an effective and safe bioprotective agent for minimally processed pineapple sticks. Its natural origin, proven antifungal activity, and ability to limit browning while maintaining sensory quality position this strain as a strong candidate for clean-label biopreservation approaches, including the reduction or replacement of E-number preservatives in fresh-cut fruits.

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Ethics statement

This study did not involve research on animals or identifiable human participants. A simple hedonic sensory test was carried out with adult volunteers to evaluate product acceptability. Participation was fully voluntary, informed consent was obtained from all participants, and no personal or identifying data were collected. According to institutional and national guidelines, this type of low-risk, non-invasive sensory evaluation does not require review or approval from a formal ethics committee; therefore, no ethics approval number is applicable.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the revision of this manuscript, the authors used ChatGPT to assist with improving the clarity and readability of certain sentences in English. Following this assistance, the authors reviewed, edited, and approved all content, and take full responsibility for the final work.

Ethics in Publishing Statement – Studies in humans and animals

I testify on behalf of all co-authors that our article submitted followed ethical principles in publishing.

Title: Quality and safety preservation of pineapple sticks using the cell-free supernatant of protective cultures

This study did not involve any research conducted on human participants or animals. A simple hedonic sensory test was conducted with adult volunteers to assess product acceptability. Participation was entirely voluntary, all individuals provided informed consent, and no personal or identifying data were collected. According to our institutional guidelines (CNTA and University of Zaragoza), this type of low-risk, non-invasive sensory evaluation does not require review or approval by a formal ethics committee. Informed consent forms signed by all participants have been submitted as supplementary files

All authors agree that:

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Nerea Garin-Murguialday: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Francisco Márquez-Urbano:** Visualization, Investigation. **Rafael Pagán:** Writing – review & editing, Funding acquisition. **Raquel Virto:** Supervision, Funding acquisition, Conceptualization. **Laura Espina:** Writing – review & editing, Formal analysis, Data curation. **Esther Arias:** Writing – review & editing, Supervision, Formal analysis, 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.101727.

Data availability

https://doi.org/10.5281/zenodo.15428149 (The raw sequencing reads were deposited in the European Nucleotide Archive (accession number of the project: PRJEB74905, sample SAMEA118005737). The raw research data are available at:)

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