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Isolation and characterization of lactic acid bacteria from fermented quinoa (*Chenopodium quinoa*) for use in vegetable biopreservation

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Abstract. *Llaguno-sungey NS, Muñoz Guzmán JP, Neira Mosquera JA, Aldas Morejon JP, Revilla Escobar KY. 2025. Isolation and characterization of lactic acid bacteria from fermented quinoa* (Chenopodium quinoa) *for use in vegetable biopreservation. Biodiversitas 26: 3174-3182.* The objective of this study was to isolate and characterize Lactic Acid Bacteria (LAB) from fermented quinoa for biopreservation of broccoli (*Brassica oleracea* var. *italica*) and zucchini (*Cucurbita pepo*). A completely randomized design with bifactorial arrangement A×B was used, where factor A corresponded to the type of vegetable and factor B to the LAB concentration (1.0×10^7 and 2.0×10^7 CFU/mL). Analysis of the fermented musts showed a decrease in pH to 3.60 in white quinoa and 3.83 in red quinoa at 72 hours. Soluble solids also decreased to 8.2 and 8.6 °Brix, respectively. As for acidity, it was significantly higher in red quinoa (0.21%) compared to white quinoa (0.18%) at the end of fermentation. *Lactococcus lactis* subsp. *lactis* was identified as the predominant LAB (bootstrap of 92%). In the biopreservation application, broccoli treated with 2.0×10^7 CFU/mL showed a decrease in pH from 5.20 to 5.11, an increase in acidity from 0.19 to 0.23%, and soluble solids from 4.43 to 4.87 °Brix. For zucchini, pH decreased from 6.31 to 6.14, and acidity increased from 0.12 to 0.15%. Weight loss was greater in zucchini (from 0.42 to 0.82%) than in broccoli (from 0.31 to 1.46%). Microbiological analysis showed a reduction in mold and yeast counts in broccoli treated with 2.0×10^3 CFU/mL at day 10. In terms of sensory analysis, zucchini received better ratings. In conclusion, fermented quinoa musts represent an effective source of LAB with biotechnological potential for vegetable preservation, highlighting their positive effect on physicochemical, microbiological, and sensory parameters, as well as their viability as a natural alternative for postharvest preservation.

Keywords: Biopreservative, fermentation, inhibition, *Lactococcus lactis* subsp. *lactis*, postharvest

INTRODUCTION

Agriculture has historically played a key role in the economic and social development of developing countries (Armas-Vega et al. 2023). However, the Food and Agriculture Organization of the United Nations (FAO) reports that one-third of the food produced is lost or discarded during harvesting, storage, or preparation (Betancur and Giraldo 2023). These losses generate significant postharvest residues, posing challenges for sustainability and waste management. In economic terms, these losses represent up to 940 billion USD globally each year, and they also contribute to approximately 8% of global greenhouse gas emissions. Consequently, recent years have seen efforts to reduce such waste (Palumbo et al. 2022).

In this context, the search for alternative food preservation methods has become increasingly important, driven by concerns about food safety, environmental impact, and human health (Lisboa et al. 2024). Food preservation refers to strategies to prevent spoilage and extend shelf life (Sionek et al. 2024). Among the most promising techniques is biopreservation, particularly effective for highly perishable fresh produce (Muthuvelu et al. 2023). This method relies on the action of beneficial microorganisms, particularly Lactic Acid Bacteria (LAB), to inhibit the growth of spoilage and pathogenic organisms (Ibrahim et al. 2021). For example, LAB have been successfully used in the biopreservation of lettuce, carrots, and cucumbers, improving shelf life and microbial stability

LAB are widely used due to their ability to ferment sugars and produce lactic acid, which has antimicrobial properties (Mathur et al. 2020). Their application in vegetable biopreservation provides a protective barrier without compromising nutritional quality (Aguirre-Garcia et al. 2024). The process of isolating and characterizing LAB from fermentation environments is of special interest in food microbiology, as it allows the identification of strains with desirable features such as rapid acidification, resistance to stress, and antimicrobial compound production (Leal-Rojas and Amorocho-Cruz 2017).

These strains can be selected for vegetable preservation due to the high perishability of such products, which demands innovative methods to extend freshness (Tarlak et al. 2025). On a microbiological level, isolated LAB can be tested for activity against pathogens like *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*, which are major agents of foodborne illnesses (Lahiri et al. 2022). An interesting substrate for LAB fermentation is quinoa (*Chenopodium quinoa*), recognized for its prebiotic potential given its high soluble fiber content that supports the growth of beneficial microbes (Paucarchuco-Soto and De la Cruz 2024). Batch fermentation of quinoa provides a controlled environment to isolate LAB strains with specific properties suitable for biopreserving fresh vegetables (Franco et al. 2020).

The consumption of vegetables and fruits with disease-preventing properties is vital for public health. One commonly consumed vegetable is broccoli (*Brassica oleracea* var. *italica*), a member of the Brassicaceae family. Its edible head consists of clustered flower buds, and in Ecuador, it is cultivated on around 9,000 hectares, harvested within 90-100 days (Vélez-Duque and Álava-Murillo 2021). Broccoli is rich in vitamins C, E, K, and A, along with essential minerals like potassium, calcium, and iron (Barber et al. 2020). Moreover, it contains antioxidants, β-carotene, and flavonoids, which contribute to reducing oxidative stress (Syed et al. 2023).

Another widely consumed vegetable is zucchini (*Cucurbita pepo*), valued for its micronutrients such as minerals, carotenoids, vitamin C, and phenolic compounds (Martínez-Valdivieso et al. 2017). Zucchini also possesses antioxidant, anticancer, anti-inflammatory, antimicrobial, antiviral, and analgesic properties, in addition to water- and fat-soluble vitamins and mineral salts (Butnariu et al. 2022). In Ecuador, zucchini is mainly cultivated in the inter-Andean region, with an annual yield ranging from 5 to 101 hectares (Zea et al. 2020). Due to its high respiration and transpiration rates, it is particularly susceptible to rapid degradation after harvest, losing water and firmness (Grisales et al. 2020).

This biopreservation approach not only enhances food safety but also promotes sustainability by offering an ecological and natural alternative to conventional preservation methods, which often rely on chemicals or energy-intensive processes. Therefore, this study aimed to isolate and characterize lactic acid bacteria from quinoa (*Chenopodium quinoa*) fermentation and to evaluate their potential for the biopreservation of broccoli (*Brassica oleracea* var. *italica*) and zucchini (*Cucurbita pepo*), focusing on their effects on physicochemical, microbiological, and sensory attributes.

MATERIALS AND METHODS

Location

The present study was carried out at the facilities of the Universidad de las Fuerzas Armadas ESPE, Santo Domingo Extension, located at Hacienda Zoila Luz, on the Santo Domingo-Quevedo Road, kilometer 24, in the province of Santo Domingo de los Tsáchilas, Ecuador. The corresponding analyses were carried out in the specialized laboratories of Bromatology and Food Microbiology of the same institution, with the objective of guaranteeing rigorous control and quality in the investigation procedures.

Obtaining the raw material

The quinoa seeds (*Tunkahuan* variety) were acquired through a commercial distributor located at Avenida Pedro Vicente Maldonado S11-122, in the city of Quito, Ecuador. The vegetables were obtained from the Municipal Market of Santo Domingo de los Tsáchilas, Ecuador.

Quinoa fermentation

Weighing and soaking

The seeds of white and red quinoa were visually inspected to remove any that were damaged or in poor condition. After inspection, 150 g of each variety was weighed per replicate, with a total of three replicates per variety, resulting in 900 g of seeds in total. The soaking process was then carried out by submerging the seeds in distilled water at a 1:3 ratio (seeds:water) for 4 hours at 20°C.

Germination and drying

The previously soaked seeds were filtered through a strainer in order to eliminate excess water, and then placed in metal trays for incubation for 24 h at 30°C. On the other hand, the drying stage consisted of exposing the germinated seeds to the sun until they were free of moisture.

Grinding and cooking

The seeds were crushed in a mortar to obtain smaller particles and then placed in an electric mill until a fine powder (flour) was obtained. For the cooking stage, 100 g of flour was weighed and mixed with 1 L of distilled water, then heated for 20 minutes at 70°C with constant stirring. At the end of the heating process, the soluble solids concentration (°Brix) was adjusted to 15°Brix, thus obtaining the quinoa must.

Fermentation

The fermentation had two processes, where the first consisted of activating 1 g of dry yeast (*Saccharomyces cerevisiae*) in 100 mL of quinoa must, after which the mixture was left to stand for 15 min at 30°C. The second stage involved inoculating the yeast in plastic bottles covered with aluminum foil with water trap couplings, pouring 900 mL of the must and 100 mL of must with the activated yeast. Finally, fermentation was carried out for 72 h at 20°C.

Seeding and isolation of Lactic Acid Bacteria (LAB)

Serial dilutions up to 10-6 were prepared using 1 mL of the quinoa fermentates in test tubes with 9 mL of peptone water (1.5%), then 0.2 mL of the 10-4 and 10-6 dilutions were seeded in duplicate on MRS agar using the extension method. The inoculated samples were incubated for 48 h at a temperature range of 35 to 37°C. Meanwhile, to obtain pure colonies, the colonies formed were subcultured on a new MRS agar and seeded using the streaking method (Morales-Nolasco et al. 2020).

Microbial identification

Gram staining

The inoculum of a bacterial colony was collected with the help of a bacteriological loop and placed in a drop of distilled water previously deposited on a slide. The slide was then heat-fixed. Crystal violet was applied for 1 minute, followed by Lugol's iodine for 30 seconds, ketone alcohol for 15 seconds, and finally safranin for 1 minute. After staining, excess dye and water were removed, and the sample was observed under a light microscope (e.g., Leica DM500) using the oil immersion objective at 1000× total magnification (Bartholomew and Mittwer 1952).

Catalase test

The catalase test was performed using a Pasteur pipette and a drop of hydrogen peroxide (3%) was placed on a slide, then a bacterial colony was collected with the bacteriological loop and the sample was spread, observing the reaction generated. It is emphasized that bubble formation before 20 seconds suggests that the test is positive (Khatoon et al. 2022).

Sequencing and phylogenetic analysis

Sequencing analysis of the isolated bacteria was performed in Petri dish with MRS agar medium and were sent to Macrogen Inc. of South Korea for 16S rRNA sequencing. The sequences obtained were compared with BLASTn program (Saeedi et al. 2015). Whereas, phylogenetic analysis was performed by using the MEGA X program, using an alignment of the sequences obtained with the ClustalW algorithm. A phylogenetic tree was also performed by the Neighbor-Joining method using the Tamura-Nei genetic distance based on 1000 Bootstrap replicates. The bacterium used as outgroup was *Staphylococcus aureus* (Sohpal et al. 2010).

Biopreservation of vegetables

Bacterial solution

MRS agar with 27.58 g in 500 mL of distilled water was used to inoculate the isolated bacterial colonies FOR 24 h at 37°C. Subsequently, the bacterial solutions were washed with citric acid - sodium citrate buffer (0.1M; pH 3.8), centrifuged at 5000 rpm for 15 minutes, this process was repeated 2 times. Subsequently, the washed solution was suspended in citric acid - sodium citrate buffer at a 1:1 volume ratio (Tuttle et al. 2021). It is emphasized that bacterial quantification in the probiotic solution was performed by density, and absorbance at 600 nm was determined with a spectrophotometer.

Preparation of broccoli and zucchini samples with the solution

Samples of broccoli and zucchini vegetables were carefully washed with distilled water and 2% citric acid solution, respectively. Then, the bacterial solution was sprayed on both samples, which were kept in airtight bags for 10 days.

Physicochemical analysis of broccoli and zucchini samples

pH determination

pH is a measure of acidity or alkalinity of an aqueous solution and indicates the concentration of hydrogen ions present in certain solutions. To determine the pH, 10 mL of the sample was mixed in a beaker with 100 ml of distilled water, where it was shaken gently, and then the preparation was filtered to remove the presence of particles in accordance with Ecuadorian Technical Standard NTE INE 389:1985.

Obtaining acidity

The determination of acidity was determined according to the methodology established by Ecuadorian Technical Standard 0381:1986. In this method, 25 mL of the sample was added to a 250 cm^3 volumetric flask and diluted to volume with distilled water, mixing the solution.

Determination of soluble solids and weight loss

Soluble solids were determined in accordance with Ecuadorian Technical Standard NTE INE 1083:1984, where several drops of the sample were placed on the prism of the refractometer for direct reading expressed in Brix. The weight loss was calculated using the following equation:

$$Weight\ loss\ \ (\%) = \frac{Initial\ weight - Final\ weight}{Final\ weight} * 100\ Ecu1$$

Microbial population finding

The determination of colony forming units (CFU/mL), was established according to the methodology established by the Ecuadorian Technical Standard INEN 1529: 2013. Five g of peptone water was homogenized in 1000 mL of distilled water, and 9 mL of the solution was placed in test tubes for the preparation of serial dilutions up to 10-8. Subsequently, the tubes with the solution were sterilized in the autoclave. Then, in the laminar flow chamber, dilutions were prepared with 1 g of the sample, and 1 mL of the solution was inoculated onto the Petrifilm slides avoiding the formation of air bubbles. The bacterial Petrifilms were incubated at a temperature range of 35 to 28°C for 48 hours, while the mold and yeast Petrifilms were left at room temperature for 72 hours. Finally, the colony content was measured using a Colony Counter ISOLAB colony counter and applying the following equation:

$$Count\ \left(\frac{UFC}{mL}\right) = \frac{Number\ of\ colonins\ *\ Inverse\ of\ the\ dilution\ factor}{Volume\ inoculated}\ Ecu\ 2$$

Statistical analysis

For the present study, a completely Randomized Block Design with bifactorial arrangement A*B was used, where factor A = Type of vegetables (broccoli: zucchini), and factor B = BAL concentrations (1.0E+07: 2.0E+07 CFU/mL), presenting 12 experimental units with 3 replicates. Statistical differences were measured by Tukey's test ($p<0.05$) using the InfoStat program. The combinations of treatments used in the study are summarized in Table 1.

Table 1. Treatments to be compared in the study of broccoli and zucchini biopreservation with lactic acid bacteria from quinoa

Treatments	Experimental units
T1	Brócoli + 1.0 × 10^7 UFC/mL
T2	Brócoli + 2.0 × 10^7 UFC/mL
T3	Zucchini + 1.0 × 10^7 UFC/mL
T4	Zucchini + 2.0 × 10^7 UFC/mL

RESULTS AND DISCUSSION

Physicochemical behavior of the quinoa musts

The statistical analysis of the pH variable showed no significant differences between the quinoa phenotypes used (p = 0.80>0.05), indicating that the type of quinoa (white or red) did not significantly influence the pH variation during the fermentation process. As shown in Figure 1.A, both pH curves present a progressive decreasing trend in the first 24 hours of fermentation. However, at 48 hours a more pronounced decrease was evident, reaching pH values of 3.69 for white quinoa and 3.93 for red quinoa. Subsequently, between 48 and 72 hours, the pH reduction was milder, culminating the fermentation process with final values of 3.60 and 3.83 for white and red quinoa, respectively. This decrease in pH, especially marked between 24 and 48 hours, is attributed to the production of organic acids, mainly lactic acid, generated by the metabolism of the yeasts and bacteria present during fermentation. In this context, the higher the concentration of microorganisms in the must, the greater the acid production, which leads to a reduction in pH (Morocho and Michel 2019). On the other hand, the results obtained in this study agree with those reported by Llacsa-Mamaní and Cucho-Arapa (2019), who also observed a greater decrease in pH in white quinoa compared to red quinoa after 72 hours, recording values of 3.49 and 3.59, respectively. Although a similar trend is identified in both studies, the differences between phenotypes are not statistically significant, which reinforces the idea that pH behavior during fermentation is more dependent on microbiological activity than on the type of quinoa used.

Figure 1.B shows a progressive decrease in soluble solids (°Brix) as fermentation time progresses, with no statistically significant differences between treatments (p = 0.92>0.05), indicating that the quinoa phenotype (white or red) does not influence the amount of soluble solids consumed during fermentation. The results show that, at 72 hours, the °Brix values were 8.2 and 8.6 for white and red quinoa, respectively, reflecting a reduction of nearly 50% of the initial sugar content at 48 hours of fermentation, due to the intense metabolism of the yeasts during the first 36 hours, when they transform the sugars into ethanol and carbon dioxide, compounds that gradually alter the concentration of soluble solids (Hoyos et al. 2010). These results differ from those obtained by Llacsa-Mamaní and Cucho-Arapa (2019) who reported final values of 11.30 and 11.02 °Brix for white and red quinoa, respectively; this discrepancy may be attributed to geographical differences between the two studies, since while the present work was developed in a tropical region, the comparative study was carried out in the Peruvian altiplano, where factors such as lower ambient temperature may influence a lower rate of yeast cell growth and, consequently, a slower reduction of soluble solids during fermentation (Henderson et al. 2013).

In relation to the acidity of the fermented must, a significant difference was found between quinoa phenotypes (p = 0.041<0.050), indicating that the type of quinoa has a significant influence on the acid behavior during fermentation. Figure 1.C shows the evolution of this variable, where a progressive increase in acidity is observed throughout the fermentation process. At the beginning, the white quinoa must present an acidity of 0.038%, while the red quinoa must have reached 0.048%; after 72 hours of fermentation, these values increased to 0.18% and 0.21%, respectively. This increase is associated with the formation of low molecular weight organic acids, common products of microbial metabolism, which can interfere with the quality of the process and decrease the efficiency of ethanol production. High acidity also shows an increased presence of lactic acid bacteria, which proliferate in acidic conditions and contribute to further elevate the acidity of the medium (Okoye et al. 2023). This acidified environment favors the growth of these microorganisms tolerant to pH ≤5, while limiting the presence of undesirable bacteria such as *Escherichia coli* and *Salmonella* spp. (Flores-Tixicuro et al. 2021). The values obtained in this study are consistent with those reported by Casas-Forero et al. (2016), who found an acidity of 0.19% for white quinoa, and by Onofre-Huamanchoque (2018) with 0.20% for red quinoa.

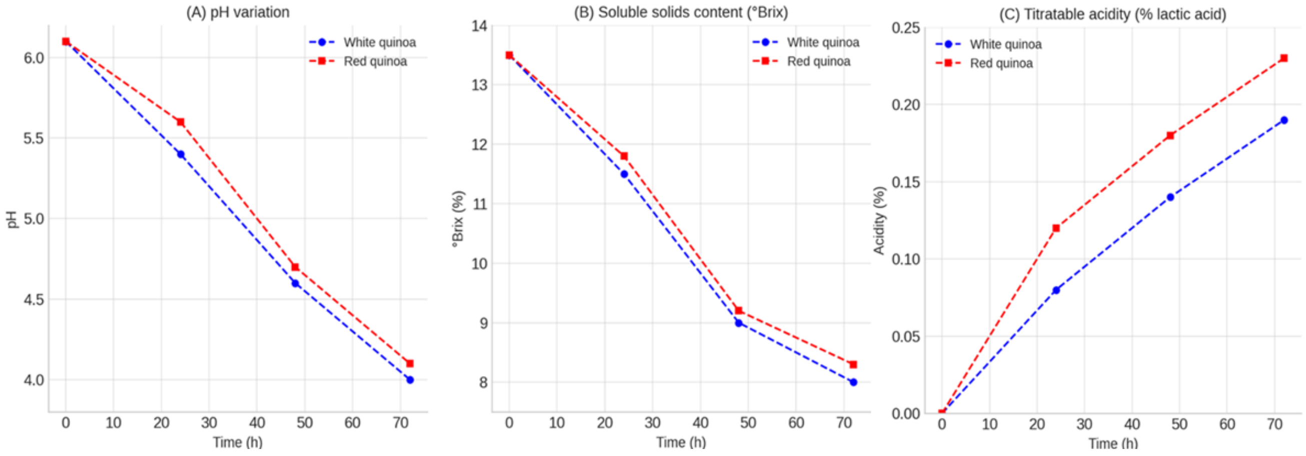

Figure 2. Physical-chemical behavior of *Chenopodium quinoa* musts

Microbiological analysis of quinoa fermentation

The initial microbial load and the load after 72 hours of storage were evaluated in two quinoa phenotypes (white and red), considering counts of molds and yeasts, as well as mesophilic aerobic microorganisms (Table 2). Regarding mold and yeast growth, both phenotypes showed a slight increase in colony-forming units (CFU/mL) after 72 hours of storage. White quinoa showed an increase from 3.1×10^3 to 4.1×10^3 CFU/mL, while red quinoa increased from 2.8×10^3 to 3.9×10^3 CFU/mL.

These values were lower than those reported by Franco et al. (2020), who observed concentrations of 5.45±0.3 log CFU/g in red quinoa after six days of storage. In the case of black quinoa, even higher values were obtained, reaching 7.21±0.3 log CFU/g, suggesting a greater susceptibility of this phenotype to yeast development. It is worth noting that the presence of these microorganisms was not detected in quinoa flour, which the authors attributed to the lower availability of water and nutrients necessary for microbial growth.

Similarly, the values obtained in this study were also lower than those reported by Sanchéz-García et al. (2023), who observed fungal and yeast counts in quinoa flour fermented and dried at different temperatures, with values of 3×10^2, 2×10^2, and $<10^2$ CFU/g for treatments at 50, 60, and 70°C, respectively. These findings demonstrate that both the fermentation process and thermal drying significantly influence the reduction of microbial load, with temperature being a determining factor in the elimination of undesirable microorganisms.

Phylogenetic tree based on the sequence of the 16s rRNA gene of BAL

Figure 2 shows the phylogenetic tree corresponding to the strains sequenced during the fermentation of white and red quinoa, which shows the formation of a clade that groups the bacteria isolated with *Lactococcus lactis* subsp. *lactis*, with a Bootstrap support value of 92%. According to Ruiz-Rodríguez et al. (2016), during quinoa fermentation a significant microbial diversity is present, in which Lactic Acid Bacteria (LAB) with both *cocciferous* and bacillary morphologies predominate, whose presence is influenced by factors such as pH and fermentation time. In the early stages of the fermentation process, it is common to detect LAB with *cocciferous* morphology belonging to the genus *Enterococcus*, which are usually associated with contamination from grain processing. However, the use of commercial grains significantly restricts the presence of this microbial group. On the other hand, in initial phases of fermentation (1-2 days), it has been possible to isolate strains of *Lactococcus lactis*, especially when the pH of the medium reaches values between 4.36 and 3.87. In this context, the strains isolated from the fermentation of white and red quinoa present a high phylogenetic similarity with *Lactococcus lactis* subsp. *lactis*, a species that according to Hamdaoui et al. (2024) emphasizes that it is.

Table 2. Microbiological results of bacterial identification

Quinoa phenotype	**Molds/yeasts**		**Aerobes**	
	0 hours	**72 hours**	**0 hours**	**72 hours**
White	3.1×10^3 UFC/mL	4.1×10^3UFC/mL	0	2.3×10^3 UFC/mL
Red	2.8×10^3 UFC/mL	3.9×10^3 UFC/mL	0	3.1×10^3 UFC/mL

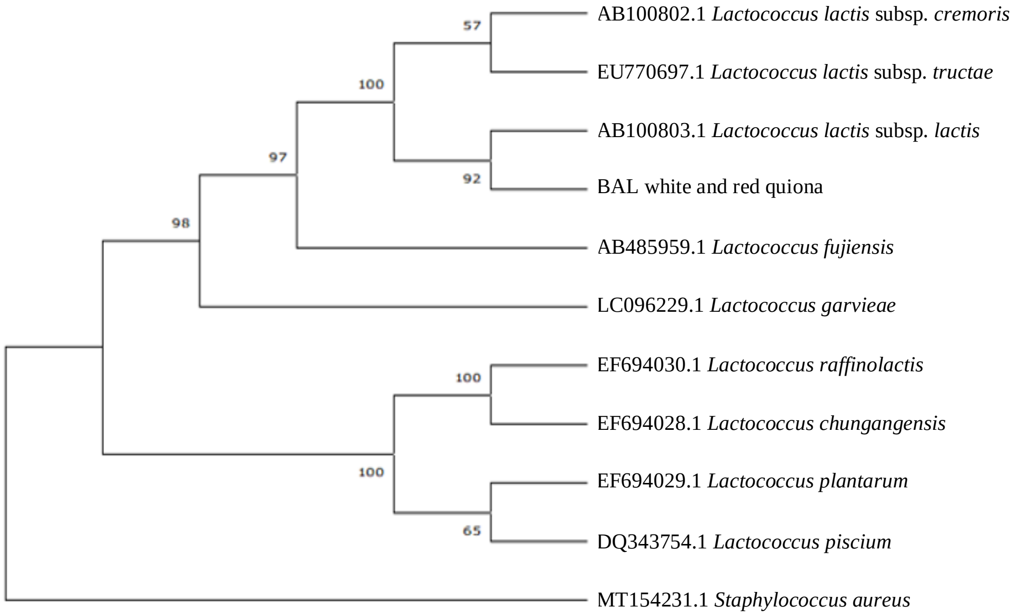

Figure 2. Phylogenetic tree corresponding to LAB isolated from white and red quinoa, with *Staphylococcus aureu*s as outgroup

Physicochemical results of the application of lactic acid bacteria obtained from white and red quinoa (*Chenopodium quinoa*) for the biopreservation of broccoli (*Brassica oleracea* var. *italica*) and zucchini (*Cucurbita pepo*)

Table 3 shows the results of the physicochemical variables evaluated (pH, titratable acidity, soluble solids and weight loss), considering the interaction between the type of vegetable (broccoli and zucchini) and the different concentrations of Lactic Acid Bacteria (LAB) applied in the biopreservation process. In broccoli, a progressive decrease in pH was observed between days 5 and 10, especially with the highest concentration of LAB (2.0 × 10^7 CFU/mL), suggesting higher microbial activity associated with increased acidity. This behavior was also evidenced in titratable acidity, which reached its highest values with the highest BAL concentrations, reinforcing the direct relationship between acidification and bacterial activity, in line with that reported by De Souza et al. (2022). However, this parameter decreased towards the end of the storage period, possibly due to physiological processes characteristic of vegetables, such as the utilization of organic acids as a source of energy (Anthon et al. 2011). On the other hand, soluble solids (°Brix) showed an initial increase in broccoli with high BAL concentrations, although, as in zucchini, they tended to decrease over time, which agrees with Godana et al. (2015), who considers this parameter a key quality indicator in fresh produce. However, the values obtained were lower than those reported by Vatansever et al. (2017) and Li et al. (2021) in studies with fruits and vegetables treated with vegetable coatings or salt. In terms of weight loss, broccoli treated with BAL presented higher levels of spoilage, while zucchini, although showing less acidification and a more stable soluble solids content, also experienced significant weight loss, possibly attributed to its higher water content. Previous studies indicate that BAL can reduce the respiratory rate of vegetables, which decreases water and nutrient loss (Tamang et al. 2016; Yang et al. 2023) however, the values observed in this study were lower than those reported by Dirapan et al. (2021) and Guo et al. (2024) and those who applied postharvest chilling techniques. In general, the results confirm that BAL concentration significantly influences physicochemical properties during preservation, where lower concentrations seem to be associated with lower metabolic activity and thus lower weight loss.

Microbiological parameters in vegetables

Table 4 shows the bacterial count results corresponding to days 5 and 10 of the biopreservation period. It is observed that the treatments sprayed with the probiotic solution of higher concentration (2.0 × 10^7 CFU/mL) present a higher bacterial load compared to those treated with the solution of lower concentration (1.0 × 10^7 CFU/mL), in which the lowest bacterial growth was recorded. This decrease in microbial count appears to be associated with the exposure time of the microorganisms to the antagonistic agents present in the solution. In agreement with previous research, up to a 40-fold reduction in bacterial concentration has been reported after 11 days of exposure to bioactive compounds, which reinforces the time-dependent effectiveness of the treatment.

The use of fermented products for preservation purposes is an ancestral practice in the food industry, although in recent years it has become more relevant due to the antagonistic capacity of certain strains of Lactic Acid Bacteria (LAB) against pathogenic microorganisms such as bacteria, molds and yeasts (Girma and Aemiro 2021; Zapaśnik et al. 2022). However, several studies have shown that an increase in LAB concentration does not always translate into a greater inhibition of undesirable microorganisms (López et al. 2021; Galo Insuati et al. 2023). According to Ibrahim et al. (2021), this could be due to the fact that, at high concentrations, LAB can form biofilms that affect their effectiveness, in addition to competing among themselves for the available nutrients, which could decrease their antimicrobial activity.

Table 4. Aerobic bacteria and mold/yeast counts during biopreservation in vegetables

Parameter	Treatments	Day 10
Color	Broccoli + 1.0 x10^7	1.33^{A}
	Broccoli + 2.0 x10^7	1.67^{A}
	Zucchini + 1.0 x10^7	3.33^{B}
	Zucchini + 2.0 x10^7	4.00^{B}
Texture	Broccoli + 1.0 x10^7	2.00^{A}
	Broccoli + 2.0 x10^7	2.33^{AB}
	Zucchini + 1.0 x10^7	3.33^{AB}
	Zucchini + 2.0 x10^7	3.67^{B}
Smell	Broccoli + 1.0 x10^7	1.33^{A}
	Broccoli + 2.0 x10^7	2.00^{A}
	Zucchini + 1.0 x10^7	3.67^{B}
	Zucchini + 2.0 x10^7	4.00^{B}

Note: The scale was Excellent (4), Good (3), Fair (2) and Poor (1)

Table 3. Results of Tukey's test for the A*B interaction in the physicochemical variables

Factor A (Vegetables)	Factor B (Concentration de BAL)	pH	Titratable acidity	°Brix	Weight loss	pH	Titratable acidity	°Brix	Weight loss
		Day 5				Day 10			
Broccoli	1.0 × 10^7 CFU/mL	5.20^{B}	0.19^{A}	4.43^{B}	0.17^{C}	7.79^{D}	0.14^{A}	3.77^{A}	0.42^{A}
Broccoli	2.0 × 10^7 CFU/mL	5.11^{A}	0.22^{C}	4.87^{C}	0.31^{C}	7.18^{C}	0.23^{B}	4.23^{B}	1.46^{D}
Zucchini	1.0 × 10^7 CFU/mL	6.31^{D}	0.14^{A}	3.93^{A}	0.42^{C}	6.23^{B}	0.12^{A}	3.80^{A}	0.50^{B}
Zucchini	2.0 × 10^7 CFU/mL	6.26^{C}	0.21^{BC}	4.00^{A}	0.43^{C}	6.11^{A}	0.15^{A}	3.73^{A}	0.82^{C}

Table 5. Sensory analysis of the vegetables on the tenth day of biopreservation

Treatment	**Day 5**		**Day 10**	
	Aerobes	**Molds/yeasts**	**Aerobes**	**Molds/yeasts**
Broccoli + 1 × 10^7 CFU/mL	1.9 × 10^5 CFU/mL	0	1.7 × 10^5 CFU/mL	2.7 × 10^5 CFU/mL
Broccoli + 2 × 10^7 CFU/mL	2.4 × 10^5 CFU/mL	0	2.1 × 10^5 CFU/mL	1.3 × 10^5 CFU/mL
Zucchini + 1 × 10^7 CFU/mL	1.8 × 10^5 CFU/mL	0	1.6 × 10^5 CFU/mL	0
Zucchini + 2 × 10^7 CFU/mL	2.1 × 10^5 CFU/mL	0	1.8 × 10^5 CFU/mL	0

As for the analysis of molds and yeasts, the results of day 5 of the bioconservation period showed no presence of these microorganisms in any of the treatments, which suggests an adequate microbiological control of the food matrix during the first stages of storage. However, at day 10, the presence of molds and yeasts was detected in the treatments applied to broccoli, regardless of the concentration of the probiotic solution used. The highest proliferation was recorded in the treatment with the lowest concentration (2.7 × 10^3 CFU/mL), while the treatment with the highest concentration managed to reduce their presence to 1.3 × 10^3 CFU/mL. These values are within the range reported by López et al. (2021) who indicates that the levels of molds and yeasts in broccoli can range between 1.4 × 10^4 and 1.7 × 10^5 CFU/mL, indicating that the treatments applied maintained microbial concentrations at acceptable levels.

Sensory analysis of the vegetables on day 10 of biopreservation

Table 5 shows the results of the sensory analysis, which revealed that rose zucchini treated with both concentrations (1.0 × 10^7 and 2.0 × 10^7 CFU/mL) received the highest scores from the panel across all three evaluated parameters. In contrast, broccoli samples were rated below 2 for color and odor, although texture received higher scores (≥2). Overall, based on the sensory analysis, it was observed that the application of the higher LAB concentration (2.0 × 10^7 CFU/mL) led to greater acceptance among respondents compared to the lower concentration (1.0 × 10^7 CFU/mL).

The use of LAB in the food industry extends beyond their functional and preservative properties, as they have also demonstrated potential to enhance the sensory characteristics of food products (Bangar et al. 2022). In this context, Zhu et al. (2022) reported that the incorporation of LAB isolated from smoked dairy products yielded favorable sensory outcomes, indicating strong potential for industrial-scale application in pasteurized milk. However, researchers such as Guney et al. (2025), who evaluated the effect of LAB combined with various spices in pickled gherkins over five weeks period, observed that sensory preferences varied significantly depending on storage duration.

Similarly, Zhu et al. (2022) found that the application of LAB in a traditional Chinese aryan-based beverage helped delay anthocyanin degradation, resulting in a marked improvement in color stability and visual appeal. As proposed by Di Cagno et al. (2013) and Wieczorek and Drabińska (2022), lactic fermentation generates aromatic compounds that enrich the sensory profile of foods, contributing to greater complexity and consumer acceptability, while also enhancing their functional attributes.

In conclusion, the study of the physicochemical and microbiological behavior of quinoa musts during fermentation revealed that, although no significant differences were observed in the pH and soluble solids variables between the white and red phenotypes, a significant difference in acidity was observed, indicating an effect of the type of quinoa on this variable. The decrease in pH and sugar consumption is attributed to the metabolic activity of fermentative microorganisms, particularly Lactic Acid Bacteria (LAB), among which *Lactococcus lactis* subsp. *lactis* was identified by phylogenetic analysis of the 16S rRNA gene. Likewise, the application of LAB cultures isolated from the fermented musts demonstrated efficacy in the biopreservation of *Brassica oleracea* var. *italica* and *Cucurbita pepo*, by inducing favorable physicochemical modifications (increased acidity, pH reduction and stabilization of soluble solids), decreasing microbial load and improving sensory acceptance in treatments with high BAL concentrations (2.0 × 10^7 CFU/mL). These results highlight the potential of quinoa fermented musts as functional matrices for the isolation of LAB with biotechnological applications in the postharvest preservation of vegetables.

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