Food Chemistry: X 17 (2023) 100563

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Influence of encapsulated *Lactobacillus plantarum* and eugenol on the physicochemical properties and microbial community of fresh-cut apples

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ARTICLE INFO

Keywords:
L. plantarum
Eugenol
The bacterial diversity
Browning

ABSTRACT

This study aimed to evaluate the application of encapsulated *L. plantarum* and eugenol as potential biocontrol agents in sliced apples. The combined encapsulated *L. plantarum* and eugenol treatment was more effective than separate encapsulated *L. plantarum* and eugenol treatments, with regards to browning inhibition and consumers panel test. The application of encapsulated *L. plantarum* and eugenol reduced the decline of the physicochemical qualities of the samples, and improved the ability of antioxidant enzymes to scavenge reactive oxygen species. Furthermore, reductions in the growth of *L. plantarum* of only 1.72 log CFU/g were observed after 15 days of storage at 4 °C for samples treated with encapsulated *L. plantarum* and eugenol. Results suggest the combined encapsulated *L. plantarum* and eugenol appears to be a promising method to protect fresh-cut apples from food-borne pathogens while maintaining the visual appearance.

Introduction

The consumption of fresh-cut fruit has increased significantly over recent years, as a result of increasing consumer demand for fresh, convenient, nutritious, and minimally processed fruit. Fresh-cut apples are healthy and available products with high levels of health-promoting bioactive compounds, such as dietary fibers, minerals, and polyphenols, and they are well accepted by consumers. However, fresh-cut products are susceptible to contamination with foodborne pathogens compared with whole apples (Russo et al., 2015). Additionally, enzymatic browning influences the overall quality, as well as consumer purchasing intention (Cavalcante Fai et al., 2016). Due to these limitations, there is a need for new methodologies that facilitate the elimination of pathogenic species and inhibit enzymatic browning. The use of natural microbiota and/or essential oils (EOs) as biopreservation agents has potential applications in fresh–cut products.

Eugenol is a major component of clove EO, and it has been recognized for its antibacterial, antioxidant, and antiviral properties. Furthermore, eugenol has been approved for use as a food additive by the Food and Drug Administration (FDA) (Teng et al., 2020). Recent studies have demonstrated excellent results with eugenol in fresh-cut fruit and vegetables. Teng et al. (2020) reported that fresh-cut Chinese water chestnuts were effectively retained in surface color and were significantly antibacterial after the treatment of eugenol emulsion. As a natural alternative to the use of commercial synthetic chemicals in controlling the most common postharvest diseases of fruits and vegetables, essential oils have shown significant activity against plant pathogens (El Khetabi, et al., 2022).

Bioprotective microorganisms, primarily lactic acid bacteria (LAB), have been widely used in the preservation of meat and fruit products (Corbo et al., 2016; Patrignani et al., 2017). The genera *Lactobacillus* and *Bifidobacterium* are types of LAB, and they are important probiotic microorganisms (Prado et al., 2008). Probiotics are live microorganisms that beneficial to the host when consumed in sufficient quantities (Thangrongthong et al., 2020). LAB not only provide health benefits but also prevent the growth of food-borne pathogens and spoilage microorganisms by competing for nutrients and producing organic acids and bacteriocins (Rydlo, Miltz, & Mor, 2006). Fresh-cut fruits and vegetables have been preserved with LAB, which is a promising antibacterial agent for controlling microbial growth on fresh-cut products. (Alegre et al.,

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https://doi.org/10.1016/j.fochx.2023.100563
Received 4 July 2022; Received in revised form 8 December 2022; Accepted 5 January 2023
Available online 7 January 2023

2011; Luo et al., 2015). The application of this culture could improve microbial safety and extend the shelf life of fruit products while reducing the use of chemically synthesized preservatives. The combination of probiotics and EOs has been shown to exhibit complementary antimicrobial effects as natural food biopreservatives (Shipradeep et al., 2012). Siroli et al. (2016) showed the potential of *Lactococcus lactis* CBM21 to increase the safety of fresh-cut apples via the inhibition of *Listeria monocytogenes*. The authors also reported that the combined *L. lactis* CBM21 and thyme EO better preserved the overall quality of fresh-cut lamb's lettuce, compared to the treatment of biocontrol agents or thyme EO individually (Siroli et al., 2017).

However, adverse conditions, including pH, heat, oxygen content, gastric acids, enzymes, and bile salts, can hinder the growth and survival of probiotics. Additionally, although EOs show antagonistic action against food-borne pathogens and spoilage microorganisms, their use may negatively affect probiotics in food (Feniman Moritz et al., 2012). The encapsulation of probiotics can improve their viability during the processing, storage, and digestion. In a recent study, an alginate-prebiotic coating based on *L. rhamnosus* and *Bifidobacterium animalis* subsp. *lactis*, exerted bactericidal effects on *L. monocytogenes* inoculated on fresh-cut apples, with a reduction of > 1.8 log after 8 days of storage (Victoria Alvarezet et al., 2021). Shigematsu et al. (2018) developed an alginate coating containing *Lactobacillus acidophilus* that reduced metabolism, conserved moisture, and minimized the change in color in minimally processed carrots.

To the authors' knowledge, there are no reports on the protective effect of combined probiotics microcapsules and essential oil treatment on fresh-cut apples. This study evaluated the effect of combined encapsulated *L. plantarum* and eugenol treatment on the physicochemical quality and microbiota composition of fresh-cut apple wedges throughout a refrigerated storage period.

Materials and methods

Materials

Plate Count Agar (PCA), Rose Bengal Agar (RBA), Man Rogosa Sharpe (MRS) broth, pepsin, bile extract, and dextran (DX) of 20 kDa molecular weight were purchased from Sinopharm Chemical Reagent Co., ltd. (Shanghai, China). Whey protein isolate (WPI) was purchased from Hilmar Ingredients (California, USA). Eugenol was purchased from Aladdin (Shanghai, China). Fresh mature 'Fuji' apples (harvested in the fall of 2021) were purchased from a supermarket in Shaanxi province.

Microorganism and cultivation

The *Lactobacillus plantarum* 21,805 was purchased from the China Center of Industrial Culture Collection. The strains were activated in MRS broth at 37 °C for 24 h.

Preparation of L. Plantarum 21,805 microcapsules

WPI and DX solution (5 %, w/v) were prepared in deionized water (at a weight ratio of 1:1). The pH was adjusted to 8.0 with 1 M NaOH and stored at 4 °C overnight. Subsequently, the solution was freeze–dried and incubated at 80 °C for 5 h. *L. plantarum* 21,805 was centrifugated, washed, and then resuspended in WPI–DX solutions. The final mixtures were prepared by using a laboratory scale spray dryer SP-1500 (Sunyi, Shanghai, China). The parameters were sets as follows: inlet temperature = 170 °C; feed flow rate = 300 mL/h; outlet temperature = 80 °C; fan frequency = 50 Hz.

Impregnation treatments

After sanitizing with NaClO (100 mg/L, 1 min), rinsing with tap water (1 min), and air drying, the apples were cut into eight skin-on wedges using a sterilized knife on a clean benchtop. Apple wedges were treated with different solutions for 3 min. The four different treatments were as follows: the T1 group was treated with water; the T2 group was treated with *L. plantarum* microcapsules (~7 log CFU/mL); the T3 group was treated with eugenol (125 mg/L; dissolved in 1 % ethanol), and the T4 group was treated with *L. plantarum* microcapsules (~7 log CFU/mL) + eugenol (125 mg/L; dissolved in 1 % ethanol). Subsequently, apple wedges were drained and placed in 8 μm polyethylene (PE) bags (32 × 20 cm, permeability characteristics: O^2 – 6000 cm^3/m^2 24 h bar; CO_2 – 45,000 cm^3/m^2 24 h bar; water vapor – 157 g/m^2 24 h bar). There were eight apple wedges in each bag and three replicated bags for each experiment. All samples were stored at 4 °C for 15 d. Samples from each treatment group was analyzed at 0, 5, 10 and 15 d of storage. The concentration of biocontrol agent and the level of natural antimicrobials are selected according to Siroli et al., (2016) and Mehdizadeh et al., (2018).

Color and physicochemical analyses

The physicochemical parameters of apple wedges were evaluated after 5, 10 and 15 d of storage. The untreated apples wedges were considered as day zero. The color parameters of the cut surface were measured with a colorimeter Ci7600 (X-Rite, MI, USA) (Wu et al., 2021). The firmness was measured with a texture analyzer GYJ-5 (Aipu Measuring Instrument Co., ltd., Quzhou, China). A 6.0 mm diameter cylindrical probe was pushed downward through the apple samples at a rate of 1 mm/s, and the results were expressed in Newtons (N). Apple juice was extracted by crushing the apple wedges with a JP351 Midea juicer (Midea Group Co., ltd, Shanghai, China) and analyzed for soluble solid content and pH. The total soluble solids (TSS) were measured with a digital refractometer PAL-1 (ATAGO ltd., Tokyo, Japan) according to (Sugri et al., 2019) and are expressed as %. All samples were measured using a pH meter FE28 (Mettler Toledo, Zurich, Switzerland). Folin–Ciocalteu assay was used to measure the total phenol content (TPC) (Benito Martinez-Hernandez, Castillejo, & Artes-Hernandez, 2019). The absorbance at 765 nm was determined by a spectrophotometer Victor Nivo (PerkinElmer, MA, USA) and calibrated with a gallic acid standard. Total phenolic content was expressed as gallic acid equivalents (GAE). A titration with 0.01 M NaOH to pH 8.1 was used to determine titratable acidity (TA) and the result was calculated as g malic acid L^{-1}. All measurements were done in triplicates.

Reactive oxygen species (ROS) and enzyme activity in apple samples

Hydrogen peroxide (H_2O_2) content was determined based on a previously published method with slight modifications (Prochazkova et al., 2001). Each ground fresh-cut apple sample (1 g) was homogenized with 10 mL of pre-cooled (4 °C) acetonitrile and centrifuged at 10000 × *g* for 15 min at 4 °C. Then, 2 mL of the supernatant was mixed with 0.1 mL of 5 % titanium sulfate and 0.2 mL of concentrated ammonia solution, and the mixture was centrifuged at 6000 × *g* for 15 min. The precipitate was washed three times with cold acetone and dissolved in 3 mL of 2 M H_2SO_4. The absorbance of the supernatant was measured at 410 nm. The H_2O_2 content is expressed as μM g^{-1}. The superoxide radical ($O_2^{\bullet}$) production rate was assayed according to the method of Elstner & Heupel, (1976) with some modifications. Each ground fresh-cut apple sample (1 g) was diluted to 5 mL with 65 mM potassium phosphate buffer solution (PBS, pH 7.8) and then centrifuged at 8000 × *g* for 10 min at 4 °C. Then, 1 mL of supernatant was added to a mixture containing 0.5 mL PBS and 0.5 mL 1 mM hydroxylamine hydrochloride and incubated at 25 °C for 0.5 h. Subsequently, 1 mL 17 mM p-aminobenzenesulphonic acid and 1 mM α-naphthylamine were added to the mixture and incubated an additional 30 min at 25 °C. Finally, 4 mL trichloromethane was added, and the mixture was centrifuged again. The absorbance of the trichloromethane phase was measured at 530 nm. The $O_2^{\bullet}$ production rate is expressed as nM g^{-1} min^{-1}.

The catalase (CAT) and superoxide dismutase (SOD) activity were detected by a commercial assay kit (Solarbio Science & Technology Co., ltd., Beijing, China) in accordance with the manufacturer's instructions. The enzyme activity is expressed as U g^{-1}.

Consumer panel scoring of apple quality parameters

The sensory evaluation panel comprised of 30 untrained panelists aging between 20 and 40 years (students and staff of College of Food Science and Engineering, Northwest Agriculture and Forestry University) was employed to evaluate the quality parameters of apples wedges. The quality parameters, including flavor, color, browning, texture, acceptability, and overall quality, were scored from 1 to 5, with 1 as extremely poor, 2 as poor (limit edibility), 3 as acceptable (limit of marketing), 4 as looks fresh and good, and 5 as excellent quality (Martinez-Hernandez, Amodio, & Colelli, 2017).

L. Plantarum viability

After 0, 5, 10 and 15 d of storage, the enumeration of *L. plantarum* was carried out to assess *L. plantarum* populations that attached to the apple tissue. 10 g of treated apples were homogenized with sterile peptone solution (0.1 %, w/v). Subsequently, serial dilutions were spread onto MRS agar and incubated at 37 °C for 48 h. The survival of *L. plantarum* was evaluated using the standard plate count (SPC) method.

L. Plantarum survival under simulated gastrointestinal conditions

The viability of *L. plantarum* was determined under simulated gastrointestinal digestion according to the method described by Victoria Alvarez et al., (2021). The simulated gastric juice (SGJ) consisted of 3 g/L pepsin, 7.2 mM $CaCl_2$, 98 mM NaCl, 13.6 mM KCl, and 0.82 mM KH_2PO_4, and the pH was adjusted to 2.0. Apple sample (4 g) were added to 36 mL of SGJ and incubated for 120 min at 37 °C. Then, bile salts and pancreatic solution (0.1 % w/v pancreatin, 0.15 % w/v bovine bile salts, 22 mM NaCl, 3.2 mM KCl, 7.6 mM $NaHCO_3$, pH 8.0) were added to the gastric digested samples and incubated for 90 min at 37 °C. Enumeration was performed as described previously in section 2.8.

Microbiological analyses

Treated apples were homogenized with sterile peptone solution. Tenfold dilutions of the homogenized apple solution were plated on MRS agar as described in section 2.8. The following media and incubation conditions were used: Plate Count Agar (PCA) for psychrophilic bacteria, with incubations of 4 °C for 2–3 d; Rose Bengal Agar (RBA) for yeasts and molds, with incubations of 28 °C for 2–3 d. All the microbiological analyses were performed in duplicate on three independent samples.

Bacterial diversity analyses

Amplicon-based sequencing was used to evaluate the microbiome composition of all treated apple wedges after storage for 15 d. Apple samples were ground in a sterile mortar, filtered with gauze, and centrifuged at 8000 × *g* for 10 min. DNA extraction, PCR amplification, sequencing, and data analyses were conducted on the sediment as previously described by Du et al., (2021); Gao & Zhang, (2019). The detailed method is presented in the Supplementary Information.

Statistical analysis

The data were processed with Origin v8.5 software (Origin Lab Corp., Northampton, MA, USA). T-tests and ANOVA tests were performed using the SPSS 19.0 software package (IBM Corp., Armonk, NY, USA). Significant differences between the samples were identified by Tukey's test, with a confidence level of 95 % ($P < 0.05$).

Results and discussion

Color changes of fresh-cut apples from four treatment groups

Total color change (ΔE) is closely related to customer perception of product freshness and is often used as a parameter to evaluate the degree of enzymatic browning. The L* value indicates luminosity. As shown in Fig. 1S and 1A, L* value decreased and ΔE value increased sharply after cutting, and then the rate of change decreased slightly after 5 days. L* and ΔE decreased significantly ($P < 0.05$) for T3 samples as storage time increased. Therefore, the direct application of eugenol in fresh-cut apples was not appropriate, as the effective dose may exceed the acceptable sensory limits and adversely affect the quality (Yousuf, Wu, & Siddiqui, 2021). However, the L* and ΔE values of the T4 samples presented smaller variations than in those treated with the encapsulated strain or eugenol (T2 and T3 samples) alone, which indicates superior anti-browning effects of the combined treatment. The WPI-DX coatings are efficient moisture and physical barriers that retard evaporation and, consequently, prevent color changes. Moreover, eugenol has been shown to inhibit the growth of fungi that cause decay and discoloration in fruit (Peretto et al., 2014), which also leads to color retention of fresh-cut apples.

Physicochemical qualities

According to Fig. 1B, the firmness of fresh-cut apples decreased with time in all treated groups. Pectin hydrolysis and depolymerization, cell wall degradation, and cell damage due to senescence are direct causes of fruit softening and decreased firmness (Paniagua et al., 2014). After treatment with *L. plantarum*, the hardness of the T2 sample reduced rapidly during the first 5 d because of the decomposition of pectin in apples caused by bacteria. Li et al. (2020) sprayed LAB on the surface of lotus skin and found similar results. There was significant difference ($P < 0.05$) between the firmness of the T1 and T4 samples after 5 d and 10 d of storage, and no significant difference ($P > 0.05$) was observed between all samples after 15 d. Slightly higher firmness was achieved for T4 samples when compared apples without treatments in the final storage period. It can be attributed to the additional effect of on the surface of eugenol on the fresh-cut apples. Alves, Goncalves, & Rocha, (2017) reported that antioxidant can act as cross-linker with polysaccharides present on the surface of apples, promoting a more rigid structure.

TSS and TPC of fresh-cut apples during storage at 4 °C are shown in Fig. 1C and 1D. TSS of all samples decreased during the first 10 d of storage, which was associated with the continuing respiration of the apple samples. *L. plantarum* inoculated samples (T2 and T4) showed lower values than other groups, especially the T4 samples. The TSS of fresh-cut apples was considerably influenced by the treatment with *L. plantarum*, which may be related to the consumption of apple nutrients via bacterial growth. The change in TSS could be detrimental to maintaining product integrity, yield and quality (Huang et al., 2021). The additional addition of eugenol can reduce the adverse effects of probiotics on TSS. Similarly, the inoculation of bacteria negatively affected the TPC content in fresh-cut apples. The TPC values of the T2 and T4 samples were lower than that in the control samples from day 10 to 15. The decrease in phenolic compounds may be a consequence of fruit senesced, leading to the destruction of cellular structure (Khodaei & Hamidi-Esfahani, 2019). The application of eugenol had little effect on the quality of fresh-cut apples, and TSS and TPC contents were maintained. These results indicate the addition of *L. plantarum* can promote the aging process of fresh-cut apples, which is inconsistent with previously reported results of fresh-cut apples enriched with the probiotic strain *L. rhamnosus* GG (Alegre et al., 2011).

Additionally, pH and titratable acidity (TA) were investigated and

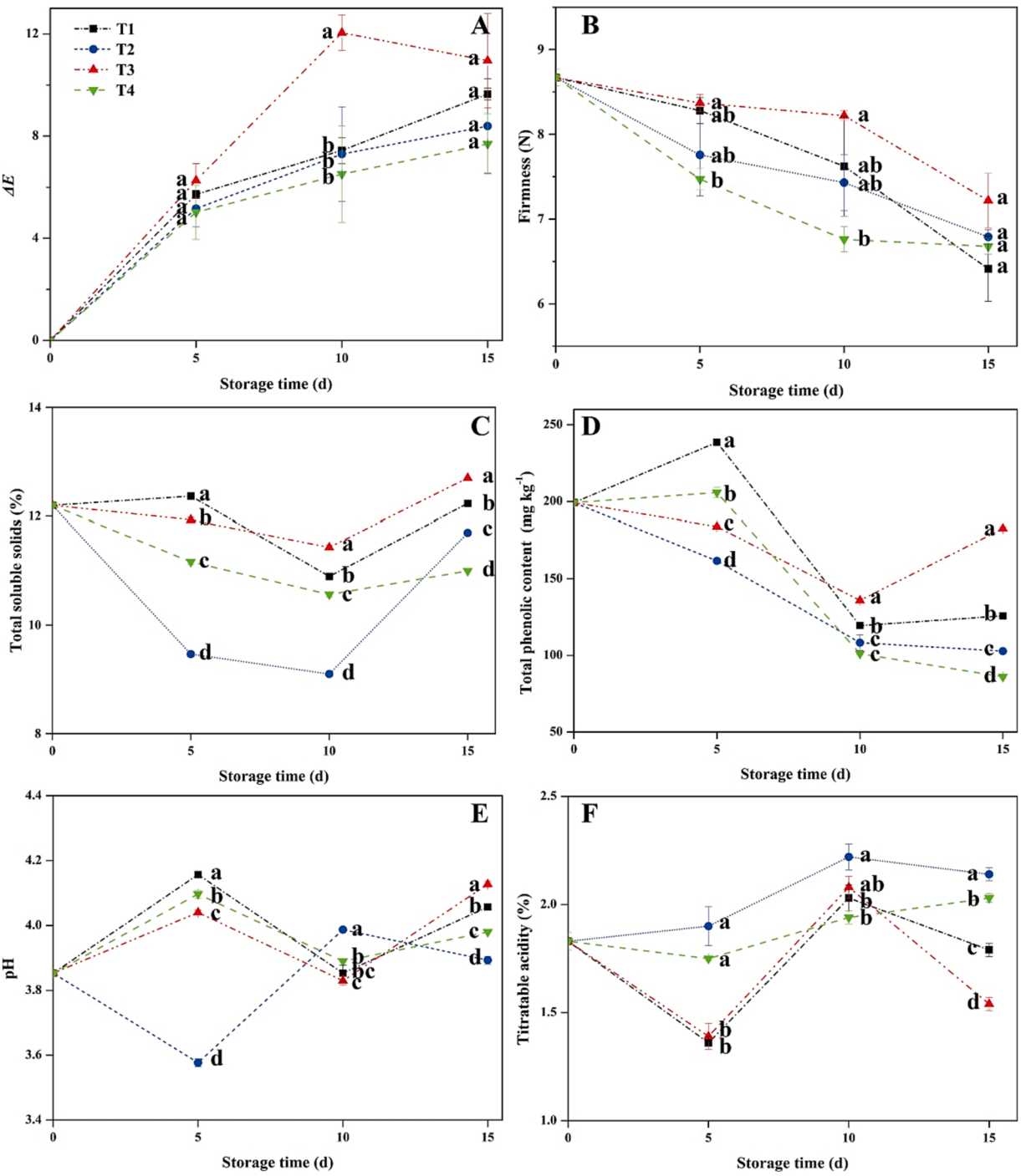

Fig. 1. Effect of encapsulated *L. plantarum*, eugenol, and the combination of encapsulated *L. plantarum* and eugenol treatments on ΔE (A), firmness (B), TSS (C), TPC (D), pH (E), and TA (F) of fresh-cut apples over 15 d of storage at 4 °C. Different lowercase letters indicate significant differences at the same storage time ($P < 0.05$).

are shown in Fig. 1E and 1F. For the T1 and T3 samples, the TA content gradually decreased during the first 5 d, followed by an increase from day 5 to day 10, and then a decrease in the later period. However, the T2 and T4 samples, treated with probiotics, showed significantly higher TA values ($P < 0.05$) throughout the entire storage period. This can be attributed to the metabolic processes of apples and *L. plantarum* during storage, resulting in an increased concentration of organic acids (Shigematsu et al., 2018). The initial pH value of fresh-cut apples was 3.85 ± 0.01, which varied between 3.58 ± 0.01 and 4.16 ± 0.01 during the storage period. Similar change trends in pH were observed for the T1, T3, and T4 samples. However, opposite trends were observed for the T2 sample, compared to other three treatments. pH values of T2 samples decreased in the first 5 d, and this may be due to the colonization of probiotics and the production of organic acids. TA values are related to the presence of organic acids and the growth of microorganisms on fruit (Yousuf, Wu, & Siddiqui, 2021). The microencapsulation of probiotics using edible coating could improve the cell viability of microorganism in adverse conditions (de la Cruz Pech-Canul et al., 2020). The direct application of eugenol had a positive impact on fresh-cut apples by maintaining the chemical attributes but negatively affected the color parameters. The color decline in fresh-cut apples may be controlled by WPI-DX coatings, which provide good protection against oxidation.

Reactive oxygen species (ROS) and enzyme activity in apple samples

As shown in Fig. 2A and 2B, the H_2O_2 content and $O_2^{\bullet}$ production rate in the T2, T3, and T4 groups first decreased and then plateaued during the 15 days storage period. The change in H_2O_2 content in the negative control was similar to the $O_2^{\bullet}$ production rate and decreased with prolonged storage time. After 5 d of storage, significant differences ($P < 0.05$) were observed between treated samples and the control in terms of the H_2O_2 content and $O_2^{\bullet}$ production rate. The H_2O_2 content in T1, T2, and T4 samples was significantly lower ($P < 0.05$) than that in T3 samples after 15 d. Moreover, significant differences ($P < 0.05$) were observed between T1 and T4 for the $O_2^{\bullet}$ production rate after 15 d. ROS may induce oxidative reactions with lipids, thus aggravating the browning of cut apples (Gao et al., 2017). These data demonstrate that the encapsulated probiotics and eugenol treatment significantly decrease the accumulation of $O_2^{\bullet}$ and H_2O_2 during the first 5 d of storage.

Antioxidant enzymes, such as CAT and SOD, are produced by fresh-cut apples and can scavenge redundant ROS, thereby protecting cell membranes from damage. The CAT and SOD activities are shown in Fig. 2CD. The CAT activity in the T2 and T4 groups first increased and then decreased during the storage period. CAT activity of T4 samples reached a maximum value of 300.74 ± 14.42 U g^{-1} at day 10 and decreased to 290.54 ± 12.49 U g^{-1} at the end of the storage period, which was higher than that in other samples. In Fig. 2D, the SOD activity in all groups first increased and then decreased during storage. The SOD activity in T3 reached a maximum (48.41 ± 2.05) on day 5, and the maximum value of 51.24 ± 1.52 in T4 was reached on day 10. In this study, encapsulated *L. plantarum*, alone or in combination with eugenol, activated CAT and SOD, which resulted in considerably lower levels of $O_2^{\bullet\,-}$ and H_2O_2. The CAT and SOD activity in the *L. plantarum* and eugenol treatment group was higher than that in the other three groups on day 15. CAT and SOD act synergistically in antioxidant systems. A high level of activity of these antioxidant enzymes were beneficial in reducing lipid peroxidation and slowing senescence in many fruit and vegetables (Sun, et al., 2020). A similar result was reported by Gao et al. (2018) who found CAT and SOD enzyme activities were increased after treatment with γ-aminobutyric acid on fresh-cut apples, as well as the scavenging of redundant ROS. In our study, the combination of encapsulated *L. plantarum* and eugenol effectively activated CAT and SOD to limit oxidative stress by scavenging accumulated ROS in tissue and, thereby, extending the shelf-life of fresh-cut apples.

Consumer panel results

The fresh-cut apples of each treatment group were scored and judged by a panel of 30 untrained consumers. Fig. 3 illustrates the overall acceptance of apple samples treated with eugenol and encapsulated *L. plantarum* individually and in combination. After 5 d of storage, the samples treated with eugenol showed considerably lower overall acceptability and color intensity, compared to the other groups. This is likely due to the loss of natural color induced by the browning effect, which reduced the evaluation scores of the T3 samples. The overall acceptability scores were similar between T1, T2, and T4 samples on day 10. In addition, panelists perceived good flavor of samples treated with

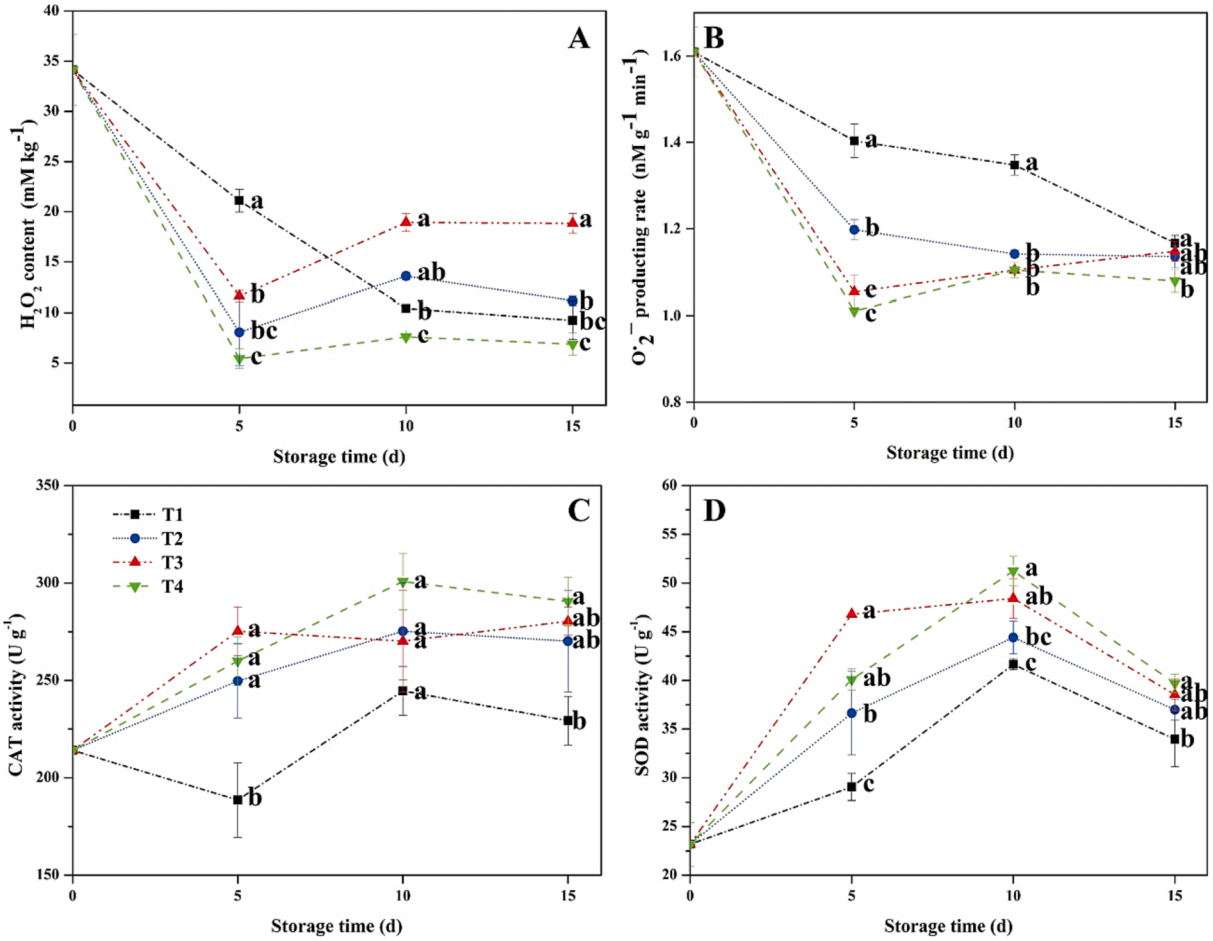

Fig. 2. Effect of encapsulated *L. plantarum*, eugenol, and the combination of encapsulated *L. plantarum* and eugenol treatments on H_2O_2 content (A), $O_2^{\bullet}$ production rate (B), CAT (C), and SOD (D) of fresh-cut apples over 15 d of storage at 4 °C. Different lowercase letters indicate significant differences at the same storage time ($P < 0.05$).

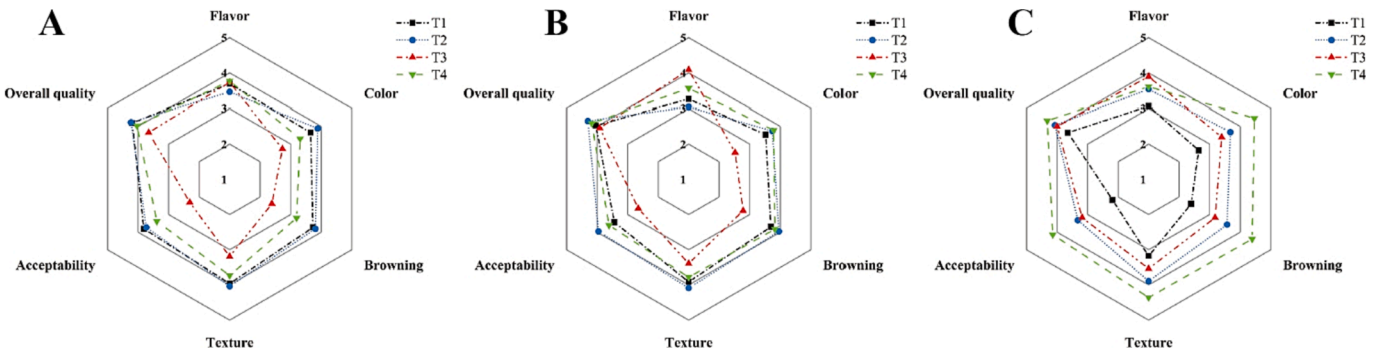

Fig. 3. Effect of encapsulated *L. plantarum*, eugenol, and the combination of encapsulated *L. plantarum* and eugenol treatments on the sensory properties of fresh-cut apples at 5, 10, and 15 d of storage.

eugenol, but the T3 samples received the lowest scores for quality parameters, in particular the color, browning, texture, and acceptability. On day 15, there were significant differences in the panelist scores between the control and treated samples. The quality of fresh-cut apples treated with water was very poor, and the panelists rejected them. The efficacy of the encapsulated *L. plantarum* and eugenol treatment in inhibiting browning and decay was evident, and samples in the T4 group received good overall acceptance scores. In a previous study, probiotics were shown to have positive impacts on the quality of fresh-cut fruit (Hashemi & Jafarpour, 2021). Siroli et al., (2017) found that inoculating lettuce with combined thyme essential oil and *Lactococcus lactis* maintained lettuce quality via a shift in the microbiota towards genera and species that had fewer impacts on the sensory properties of lettuce.

Viability of L. Plantarum during storage and under simulated gastrointestinal conditions

The viability of *L. plantarum* in the fresh-cut apples during storage is shown in Fig. 4A. T2 samples presented more viable *L. plantarum* cells than T4 samples throughout the storage period. Over the entire storage period, a 1.51 and 1.72 log CFU/g reduction in viability was observed for T2 and T4 samples, respectively. It is known that survival of bacteria is affected by low pH and complex environmental conditions (Guo, et al., 2022).

Fig. 4C–F show the variation in the viability of *L. plantarum* along simulated gastrointestinal digestion stages for each storage time. There were reductions of 0.34, 0.20, 0.22, and 0.19 log CFU/g for T2 samples after storage for 0, 5, 10, and 15 d, respectively, over the gastric phase of digestion. For T4 samples, smaller reductions in viability upon exposure to SGF (pH 2.0) were observed, with reductions of 0.05, 0.05, 0.05, and 0.15 log CFU/g for storage times of 0 d, 5 d, 10 d, and 15 d, respectively.

The subsequent intestinal stage caused additional reductions in probiotic counts (0.49–1.25 log and 0.61–1.45 log, respectively) for T2 and T4 samples. After in vitro gastrointestinal digestion, *L. plantarum* in T2 and T4 samples maintained 76 %–86 % and 74 %–87 % viability, respectively. Furthermore, the survival of *L. plantarum* in T2 and T4 samples after storage for 5 and 10 d was higher than that in samples at day 0 throughout digestion. Bacteria is able to adapt to sudden environmental changes, such as temperature, pH, and osmotic pressure, by regulating metabolic flow and genetic expression (Santivarangkna, Kulozik, & Foerst, 2008). *L. plantarum* produced acidic stress conditions on the apple surface (about pH 4.0) and increased the acid tolerance response mechanism to improve cell viability in the acidic environment. Gandomi et al. (2016) added *L. rhamnosus* GG encapsulated with chitosan-alginate to apple juice, stored the juice for 1 d at 4 °C, and then performed in vitro gastric digestion tests. The results show the probiotics maintained approximately 80 % viability. The slight discrepancies in the results obtained in our study can be explained by the differences in fruit types, processing operations, storage temperature, storage period, inoculation strains, and inoculum level. Several static in vitro digestion models are available in the literatures, but they exhibit different characteristics (e.g., pH, duration of each step, ratio of enzymes to substrate) (Brodkorb, et al., 2019). According to in vitro digestion tests in this study, the application of probiotics, such as *L. plantarum*, to fruit is a viable alternative to chemical preservatives, and the addition of eugenol had no significant effect on the survival of *L. plantarum*.

Native microbiota

The effects of encapsulated *L. plantarum* and eugenol alone or in combination on the growth of yeasts and molds on fresh-cut apples during storage at 4 °C are shown in Fig. 4B. After 5 d of storage at 4 °C, the use of *L. plantarum* and eugenol, alone or in combination, significantly inhibited the growth of yeasts and molds ($P < 0.05$), compared with control. In particular, encapsulated *L. plantarum* completely inhibited the growth of mold. However, for T2 samples, yeast and mold counts increased by 5.01 log CFU/g from day 5 to day 10. The yeast and mold counts in T3 and T4 samples were significantly lower ($P < 0.05$) than T1 and T2 samples after 10 d.

As for psychrophilic bacteria (Table S1), only the colony counts on days 10 and 15 for the T1 sample and day 15 for the T2 sample were above the detection limit (2 log CFU/g). Psychrophilic bacteria in T3 and T4 samples remained at an undetectable level (<2 log CFU/g) throughout the storage period. The inoculation of probiotics on fresh-cut apples increased the safety of the product at the beginning of storage due to the competitive inhibition of potentially pathogenic bacteria by the dominant bacteria. Competitive inhibition proceeds mainly through the consumption of nutrients and the production of organic acids by the dominant bacteria *L. plantarum* (Alegre et al., 2011). The probiotic counts gradually decreased, and spoilage and pathogenic species counts increased with the extension of storage time, resulting in a decline in microbial quality on fresh-cut apples. This may be associated with the competition between pathogenic microorganisms and LABs for foods and nutrients (Guimaraes et al., 2018).

Bacterial diversity associated with treatment type

16S rRNA gene sequencing was performed to investigate the effect of encapsulated *L. plantarum,* alone or in combination with eugenol, on the microbiota of fresh-cut apples after 15 d of storage. A total of 25,512 ASVs were observed after rarefying to the lowest number of sequences per sample. The above datasets were assigned to 4 represented phyla, including Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidota. These phyla are also reported as the dominant ones in peaches by Grande Burgos et al., (2017). The α diversity analyses are shown in Fig. 5A–D. The diversity is expressed as the richness (Chao1 and Ace indexes) and diversity (Shannon and Simpson indexes). Compared to the T1, T2, and T4 groups, the application of eugenol significantly ($P <$

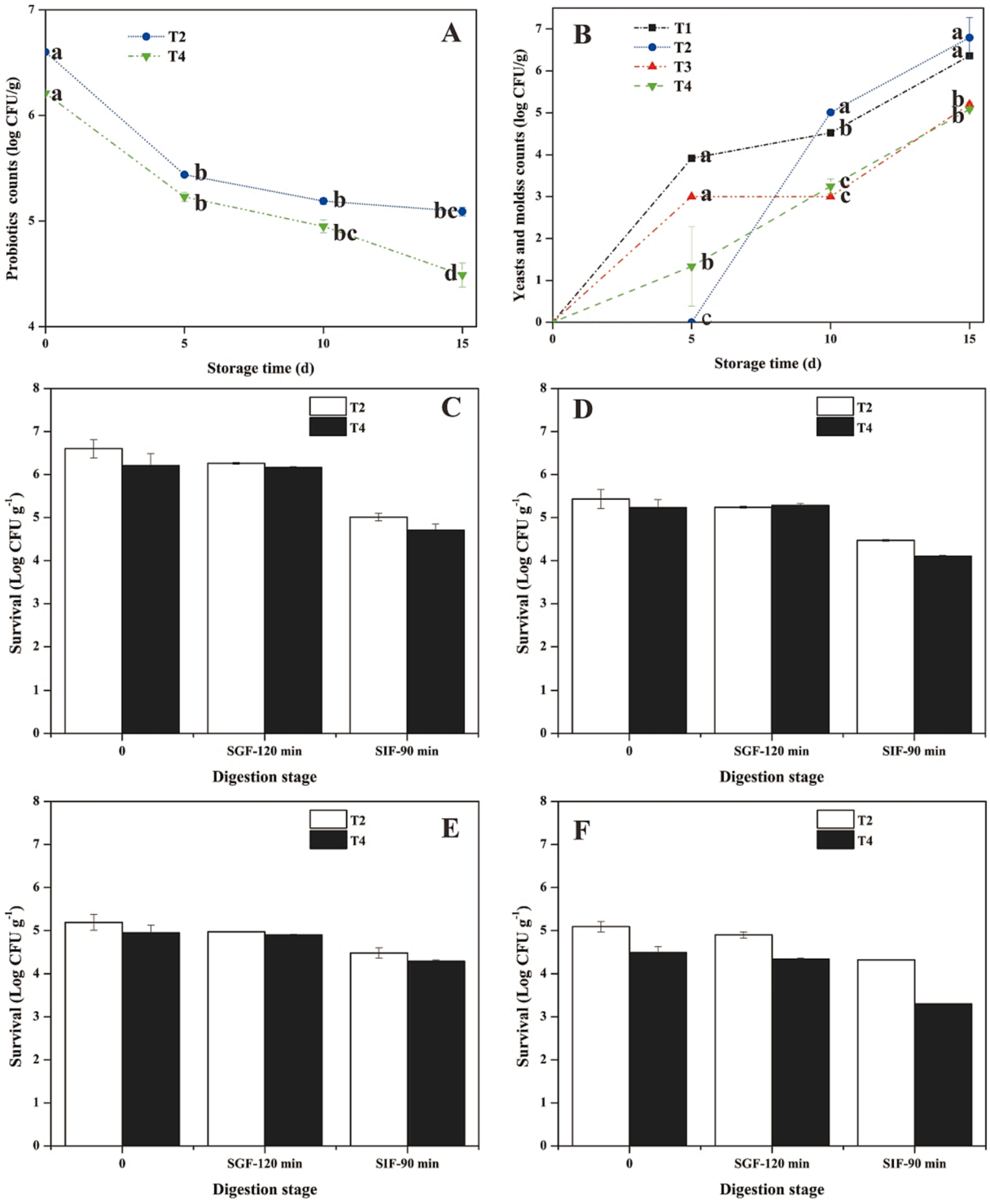

Fig. 4. Evolution of *L. plantarum* (A) and yeast and mold counts (B) on fresh-cut apples over 15 d of storage at 4 °C. Cell viability of encapsulated *L. plantarum* on fresh-cut apples under simulated gastrointestinal conditions on day 0 (C), 5 (D), 10 (E), and 15 (F). Different lowercase letters indicate significant differences at the same storage time ($P < 0.05$).

0.05) decreased the richness of microbiota on the surface of fresh-cut apples. In addition, the treatment of encapsulated *L. plantarum* and eugenol significantly ($P < 0.05$) decreased the diversity of the bacterial community.

Fig. 6A depict the β diversity of microbial communities. Structural changes in gut microbiota were analyzed by principal component analysis (PCA) to better assess the differences among of multiple samples. A significant separation between the microbiota was observed among the group containing and not containing *L. plantarum* ($P < 0.01$). In Fig. 6B, Relative abundance of the microbiota at the genus level in each group is shown. The addition of eugenol decreased the abundance of *Pseudomon* spp.and *Serratia* spp.and increased the abundance of *Pantoea* spp. Compared with the control group, the addition of *L. plantarum* decreased the abundance of *Pantoea* spp. In addition, the combination of eugenol and *L. plantarum* considerably decreased the relative abundance of *Pantoea* spp., compared to the *L. plantarum* treatment

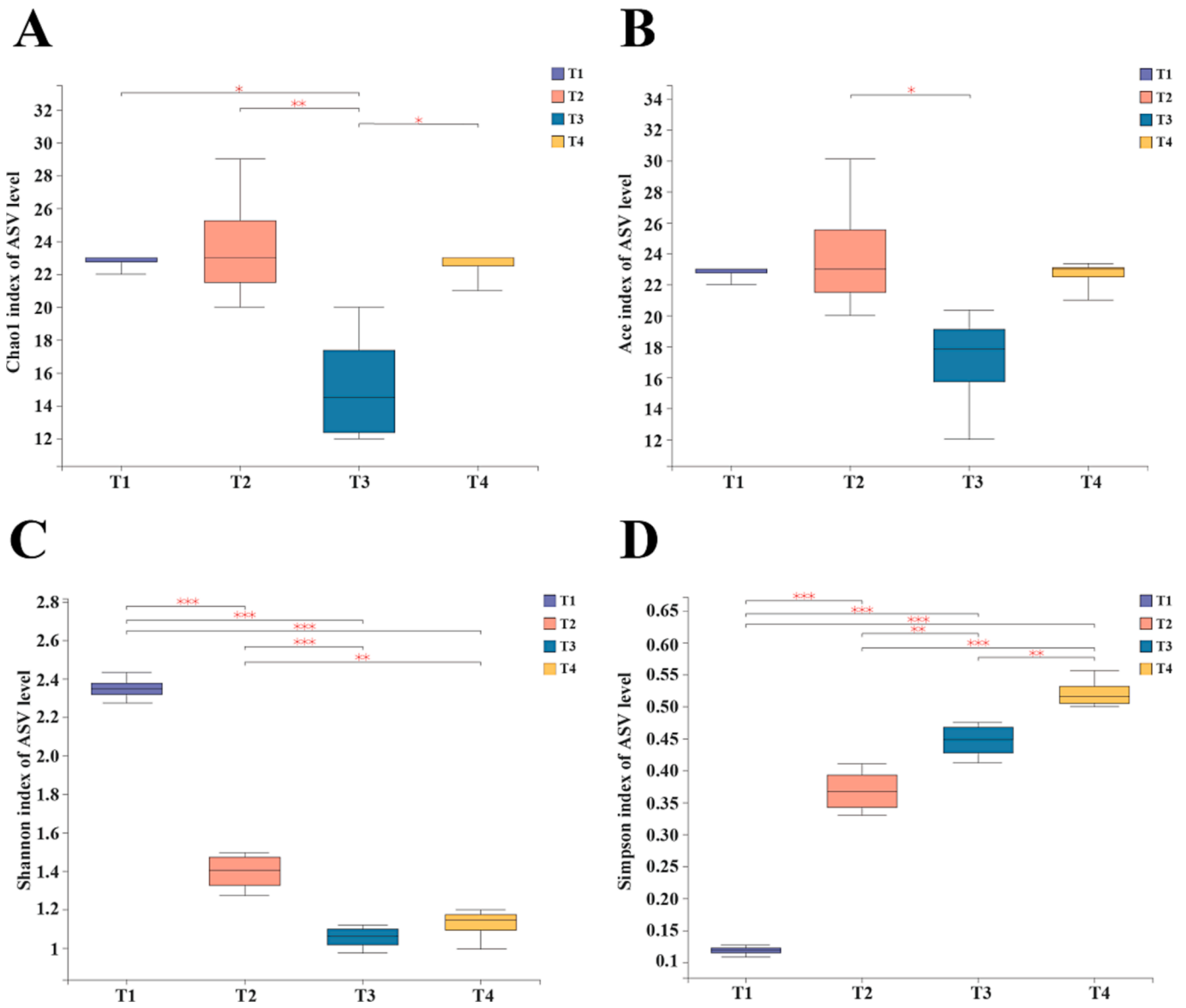

Fig. 5. Effects of encapsulated *L. plantarum* and eugenol treatment on the microbiome structure of fresh-cut apples. (A) Chao1, (B) Ace, (C) Shannon, and (D) Simpson indices in α-diversity analysis.

group. These results can be explained in terms of the differences in sensitivity to *L. plantarum* and eugenol treatments for different bacteria.

Pantoea was dominant bacteria in the eugenol group, while Pseudomonas was identified as dominant bacteria in the T4 group. Differences in microbial populations of fresh fruit and vegetables can result in different sensory characteristics (Siroli et al., 2015). Natural antibacterial agents, such as eugenol, have remarkably different effects on the microbial metabolism and volatile molecule profiles, which consequently affect the organoleptic characteristics of the product (Siroli et al., 2017). The differences in the abundances of *Lactobacillus* between two groups, based on the Kruskal-Wallis H test, are shown in Fig. 6C. The enrichments of *Lactobacillus* were significantly up-regulated by the addition of *L. plantarum*, but down-regulated upon simultaneous treatment with eugenol. As expected, the pyrosequencing analysis demonstrated that *L. plantarum* was only present in samples treated with probiotic solutions. The average relative abundance of *L. plantarum* in T2 samples (treated with *L. plantarum* alone) was about two to three times that of T4 samples (treated with *L. plantarum* and eugenol). In addition, the composition of the microbial population of T2 samples was similar to T4, but different relative abundances were observed. The addition of *L. plantarum*, as a biocontrol probiotic, resulted in positive outcomes for product color and flavor. The combination of encapsulated *L. plantarum* and eugenol, as well as other natural antibacterial and biocontrol agents, may be a suitable strategy to improve the safety and quality of minimally processed fruit.

Conclusion

The combined application of encapsulated *L. plantarum* and eugenol in fresh-cut apples presents clear advantages, such as reduced browning and microbial growth, which are important considerations when commercializing these products. The new bioactive agent enhanced CAT and SOD activities and induced scavenging of redundant ROS. Furthermore, the presence of *L. plantarum* and eugenol inhibited the growth of pathogens and spoilage microorganisms and resisted simulated gastrointestinal conditions. Thus, a high level of viability of *L. plantarum* was achieved, and the microbiological quality of apples were maintained. Therefore, the combination of encapsulated probiotic and natural antimicrobials may be a promising approach to enhance the safety and shelf-life of fresh-cut apples.

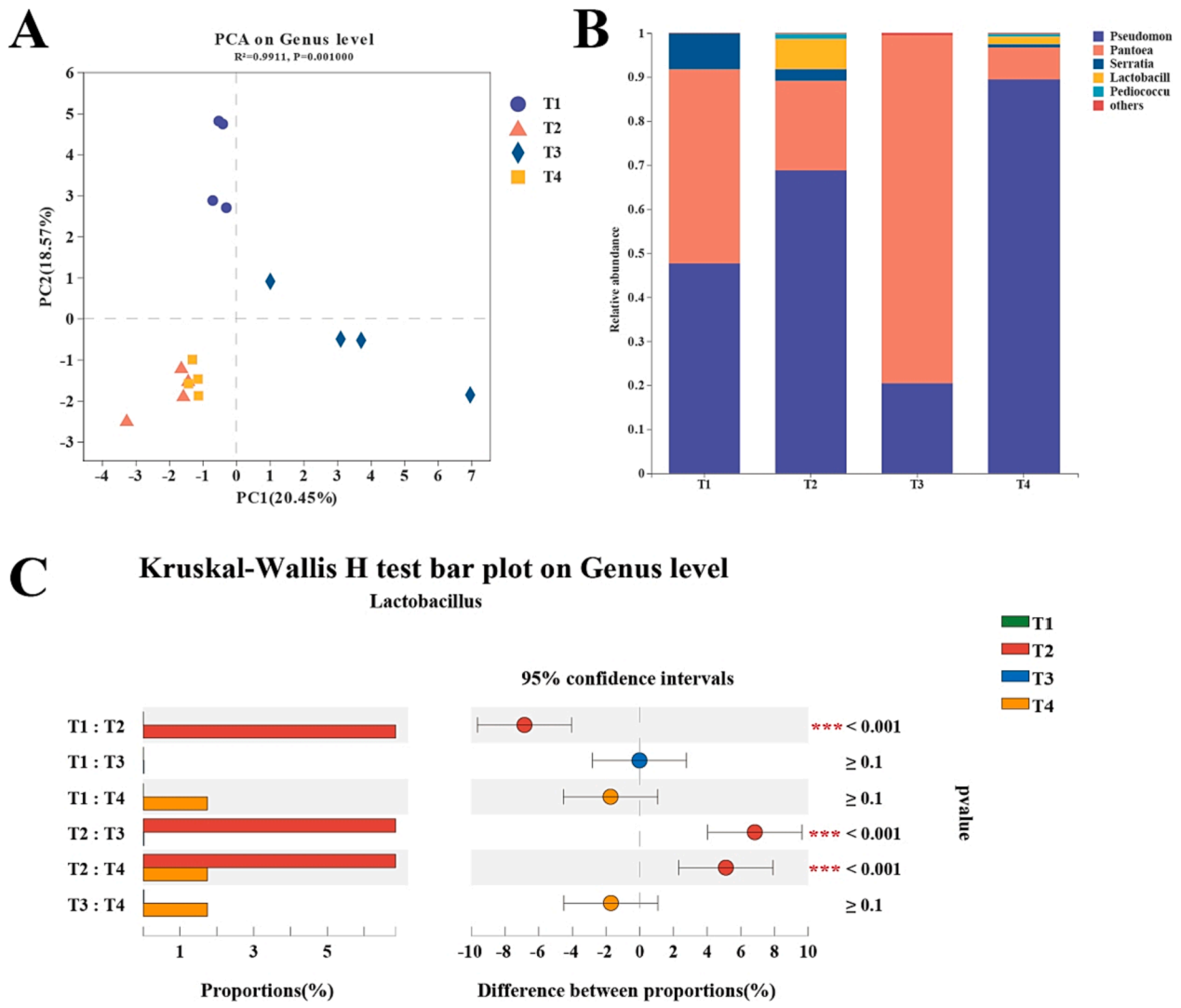

Fig. 6. (A) PCA analysis for bacterial communities in four groups. (B) Relative abundance of the bacteria in genus levels in each group. (C) Differences in *Lactobacillus* between two groups based on the Kruskal-Wallis H test. Data are presented as mean ± SD.

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.

Data availability

Data will be made available on request.

Acknowledgments

The research was supported by the national key Research and Development Program of the 13th Five-Year Plan (2019YFC1606703), and the agricultural Science and Technology Innovation Program of Shaanxi Province (NYKJ-2019-XA), China.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100563.

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