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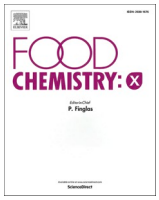

Effect of plasma-activated lactic acid on microbiota composition and quality of puffer fish (*Takifugu obscurus*) fillets during chilled storage

Xiaowei Sheng [a], Longfei Yan [a,b], Lanqing Peng [c], Luling Zhao [a], Fanwei Dai [b], Feiping Chen [b], Ling Wang [b], Yulong Chen [b], Mingqiang Ye [b,*], Jin Wang [d,*], Jianhao Zhang [a,*], Vijaya Raghavan [e]

[a] *National Center of Meat Quality and Safety Control, Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China*
[b] *Sericulture & Agri-Food Research Institute Guangdong Academy of Agriculture Science, Key Laboratory of Functional Foods, Ministry of Agriculture and Rural Affairs, Guangdong Key Laboratory of Agricultural Products Processing, Guangzhou 510610, China*
[c] *Guangdong Supply and Marketing Green Agricultural Products Production and Supply Base Operation Co., Ltd, Huizhou 516100, China*
[d] *Key Laboratory of Environmental Medicine and Engineering, Ministry of Education, and Department of Nutrition and Food Hygiene, School of Public Health, Southeast University, Nanjing 210009, China*
[e] *Department of Bioresource Engineering, Faculty of Agricultural and Environmental Sciences, McGill University, Sainte-Anne-de-Bellevue, QC, H9X3V9, Canada*

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ABSTRACT

Fresh puffer fish (*Takifugu obscurus*) are susceptible to microbial contamination and have a very short shelf-life of chilled storage. Hence, this study aimed to evaluate the effects of plasma-activated lactic acid (PALA) on microbiota composition and quality attributes of puffer fish fillets during chilled storage. The results showed that PALA treatment effectively reduced the growth of bacteria and attenuated changes in physicochemical indicators (total volatile basic nitrogen, pH value, K value, and biogenic amines) of puffer fish fillets. Additionally, insignificant changes were observed in lipid oxidation during the first 8 days ($p > 0.05$). Illumina-MiSeq high-throughput sequencing revealed that PALA effectively inhibited the growth of *Pseudomonas* in puffer fish fillets and maintained the diverse characteristics of the microbial community. In combination with sensory analysis, PALA extended the shelf life of puffer fish fillets for 4 days, suggesting that PALA could be considered a potential fish fillet preservation method.

1. Introduction

The obscure puffer fish (*Takifugu obscurus*) is a food fish cultivated in both seawater and freshwater with a long history (Zhou, Liu, Xie, & Wang, 2011). Renowned for its robust nutrition and exquisite taste, this fish has garnered considerable popularity among discerning consumers (Zhang, Liu, Zhou, Wang, Fan, & Liu, 2022). However, the susceptibility of fresh fish meat to microbial spoilage and chemical deterioration leads to short shelf life, significantly limiting the development of the fish industry (Cao et al., 2020; Zhuang, Li, Hong, Liu, Shu, & Luo, 2020). Low-temperature storage technologies such as chilling, super-chilling, and freezing are currently primarily employed to extend the shelf life of puffer fish and ensure its quality (Yang et al., 2019). Although lower temperatures can slow down the activity of enzymes and bacteria in fish meat, they cannot effectively inhibit the growth of spoilage bacteria, thus the shelf life of chilling storage is limited (Zheng et al., 2022). Furthermore, during the frozen storage conditions, there may be adverse effects on fish meat quality, including undesirable sensory changes, crystallization, lipid oxidation, and protein loss (Li et al., 2020; Yang et al., 2019). To improve the shelf life of puffer fish, it is crucial to design a safe and effective antibacterial treatment.

Plasma-activated water (PAW) has been demonstrated its efficacy as a potent disinfectant agent capable of inactivating foodborne pathogens and food spoilage microorganisms (Chanioti et al., 2023; Qian et al., 2022; Shanker et al., 2023). Importantly, this is achieved while preserving the inherent quality characteristics of food products and extending

* Corresponding authors at: National Center of Meat Quality and Safety Control, Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China (J. Zhang); Department of Nutrition and Food Hygiene, School of Public Health, Southeast University, Nanjing 210009, China (J. Wang).
E-mail addresses: yemingqiang@gdaas.cn (M. Ye), jin.wang6@mail.mcgill.ca (J. Wang), nau_zjh@njau.edu.cn (J. Zhang).

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their shelf life. PAW is a solution enriched with reactive species, charged particles, and ions, generated through the treatment of water with non-thermal plasma (Qian, Wang, Zhuang, Nasiru, Zhang, & Yan, 2021; Qian et al., 2022). The disinfection potential of PAW is attributed to the presence of reactive oxygen and nitrogen species (RONS), encompassing hydrogen peroxide, peroxynitrite, nitrate and nitrite ions, as well as its low pH (Chanioti et al., 2023; Liao et al., 2018). These RONS can target a variety of biological macromolecules in bacterial cells, damaging bacterial morphology, intracellular oxidative levels, and antioxidant capacity. For instance, RONS can react with unsaturated fatty acids present in cell membranes, facilitating lipid peroxides formation, subsequently leading to bacterial cell death (Qian, Wang, Zhuang, Zhang, & Yan, 2020).

To enhance the inhibitory effect of PAW on microorganisms, various solvents (such as 6 % hydrogen peroxide (Fan, Vinyard, & Song, 2022), 10 % phosphate buffer (Zhao, Ojha, Burgess, Sun, & Tiwari, 2020), and 0.9 % saline solution (Yang et al., 2021) were added into the water before the plasma treatments. Our research team has demonstrated that adding lactic acid solution to water can prepare plasma-activated lactic acid (PALA) solution, which has a better antibacterial effect than PAW solution (Qian et al., 2021; Qian et al., 2020; Qian, Zhuang, Nasiru, Muhammad, Zhang, & Yan, 2019; Wang et al., 2023). It is worth noting that lactic acid is widely recognized as a safe additive in producing and processing of aquatic, livestock, and poultry products (Jaspal et al., 2021; Li et al., 2022; Wang et al., 2023). Previous studies have also indicated that PALA used as a cleaning agent for livestock and poultry products, can effectively reduce microbial growth rates in beef and chicken while simultaneously enhancing their quality characteristics (Qian et al., 2021; Qian et al., 2019). However, in terms of fish, relevant research has not been reported.

This study aims to use PALA as a decontamination agent to increase the shelf life of puffer fish fillets. The effect of PALA on the shelf life of the puffer fish fillets was assessed by monitoring variations in quality parameters, encompassing total volatile basic nitrogen (TVB-N), pH value, 2-thiobarbituric acid reactive substances (TBARS), K value, biogenic amines, and sensory evaluation. Furthermore, microbial enumeration and high-throughput sequencing techniques were employed to assess the changes in the total viable count, psychrophilic bacteria count, and microbial composition in puffer fish fillets induced by PALA treatment during storage.

2. Material and methods

2.1. The preparation of plasma-activated lactic acid solution

Based on our team's previous research, a plasma jet, with specific operating parameters (voltage: 19 kV, frequency: 20 kHz, current: 0.024 mA), was positioned 10 mm below the liquid surface to treat a 300 mL lactic acid solution with a volume fraction of 0.15 % for a duration of 120 s, resulting in the preparation of PALA (Fig. 1a) (Qian et al., 2019). Subsequently, the physical and chemical properties of PALA were subjected to various measurements. The pH, temperature, and

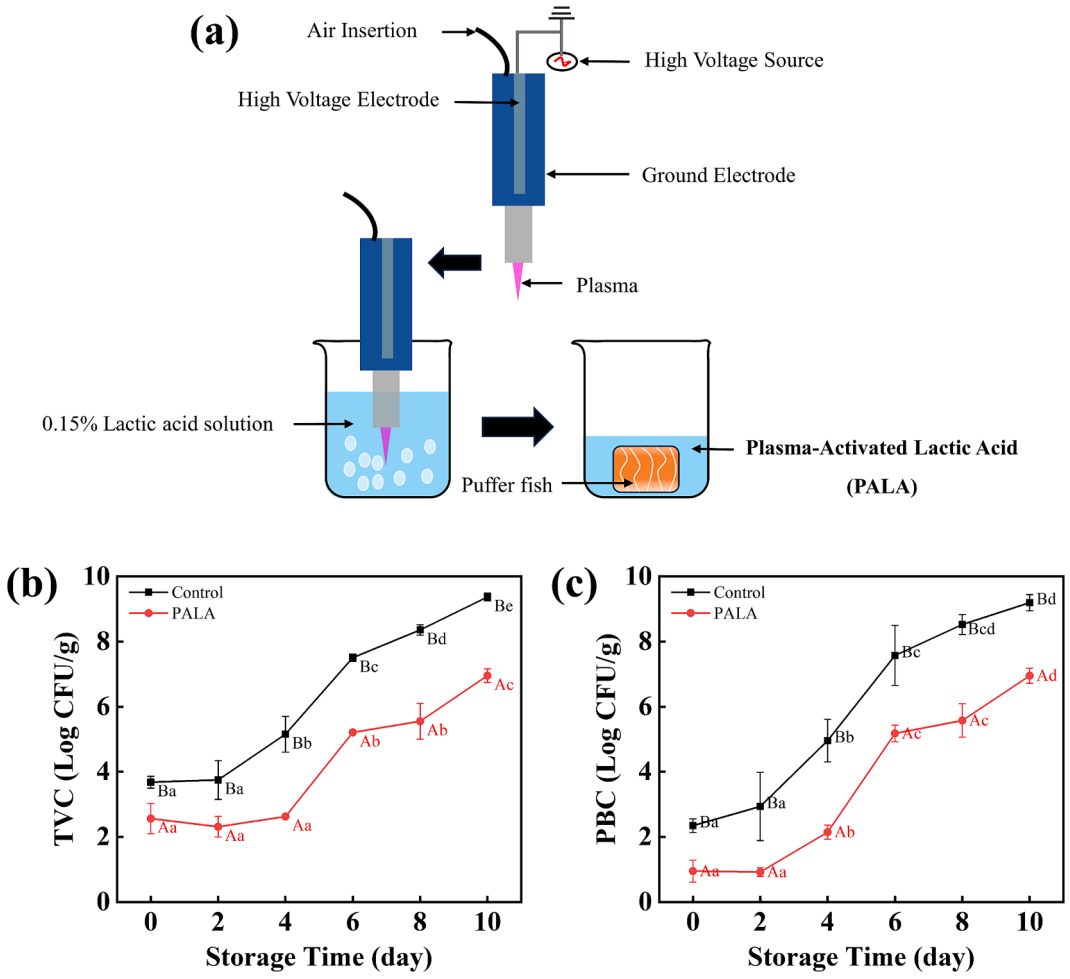

Fig. 1. **(a)** The schematic diagram of the application of PALA on puffer fish fillets. Changes in **(b)** total viable count (TVC) and **(c)** psychrophilic bacteria count (PBC) of puffer fish fillets in the control and PALA groups during storage at 4 °C. Notes: Control: samples immersed in deionized water; PALA: samples treated with Plasma-activated Lactic Acid solution.

oxidation–reduction potential (ORP) of PALA were measured using a pH meter (Sartorius, PB-10, Germany), a thermometer (CEM, DT-880, China) and an ORP meter (Bell Analytical Instrument Co., Ltd., BPP-920, China), respectively. Additionally, the concentrations of nitrite anions (NO_2^-) and nitrate anions (NO_3^-) in PALA were evaluated through spectrophotometric methods (Qian et al., 2021). The relevant parameters of PALA were as follows: pH value (2.61), temperature (25.8 °C), ORP value (590 mV), and the concentrations of NO_2^- and NO_3^- (1889 μM and 510 μM, respectively).

2.2. Sample pretreatment

Live puffer fish (*Takifugu obscurus*), without any external injuries, were purchased from Guangdong Jinyang Aquaculture Co., Ltd. The pufferfish were bled, and after removing the eyes, skin, and viscera, they were thoroughly washed in sterile water. Under sterile laboratory conditions, the fish meat was dissected along the dorsal spine to remove the bones and subsequently sliced horizontally into approximately 2 cm × 3 cm × 1 cm fish fillet samples, each weighing an average of 10 ± 0.1 g. The samples were randomly divided into two groups, with 90 samples in each category. The fish fillet samples were soaked in the PALA solution that had been prepared for 5 min, with a ratio of 1 part sample to 5 parts solution (w:v). After removing the samples from the solution, excess moisture was drained, and the samples were packed in sterile sealed bags and stored at a refrigerated temperature of 4 °C. Samples immersed in deionized water were recognized as the control group. Microbiological (Microbial enumeration and Illumina-Miseq high throughput sequencing), chemical (TVB-N, pH value, TBARS, K value, and Biogenic amines), and sensory analysis were conducted on the samples at 0, 2, 4, 6, 8, and 10 days, ensuring consistent sampling locations for different groups.

2.3. Microbial enumeration

The 10 g puffer fish fillet was transferred to a sterile homogenization bag with 90 mL of sterile 0.9 % NaCl solution. After homogenization for 2 min at a low speed using a homogenizer (Nanjing Ningkai Instrument Co., Ltd., XC07-II, China), the microbial suspension was serially diluted in a 10-fold manner using sterile NaCl solution. Each dilution (100 μL) was plated onto plate count agar (Qingdao Haibo Biotechnology Co., Ltd., China) plates and incubated at 30 °C for 3 d and at 4 °C for 10 d. The resulting colonies were counted as total viable counts and psychrophilic bacteria counts, respectively. The results were presented as a logarithm of colony-forming units per gram (lg CFU/g).

2.4. Illumina-MiSeq high-throughput sequencing

The Illumina-MiSeq high-throughput sequencing was conducted following the previous method (Zhang, Li, Yang, Liu, Hong, & Luo, 2021). The 10 g puffer fish fillet was combined with 20 mL of sterile 0.9 % NaCl solution and agitated on a rotary shaker (100 rpm) for 15 min. The blend was then subjected to centrifugation at 200 g for 5 min to eliminate fish particles. The resulting supernatant underwent further centrifugation at 10,000 g for 10 min to collect bacterial cell pellets and the bacterial DNA was extracted from the pellets using a bacterial genomic DNA extraction kit (QIAamp, Germany). The quality of the isolated total DNA was assessed by electrophoresis on 1 % agarose gel. The TransStart FastPfu DNA Polymerase kit (Beijing full-style Golden Biology Co., Ltd., China) was used following the manufacturer's instructions to amplify the V3-V4 hypervariable region of the 16S rDNA gene. The primers used for amplification were 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Denaturation was initially placed at 94 °C for 3 min, then there were 25 cycles of denaturation for 45 s, annealing for 60 s, and extension for 90 s, with a final extension for 10 min at 72 °C. For high-throughput sequencing on the Illumina Miseq platform, a library was made using magnetic beads to filter the amplified products.

2.5. Determination of TVB-N and pH

According to the previous method, the TVB-N content was determined by the semi-micro nitrogen determination method (Jia, Liu, et al., 2019). The TVB-N concentration was given as mg N/100 g fish. A piece of 5 g of minced puffer fish fillet was homogenized in 50 mL of deionized water and swirled for 30 min. The mixture was centrifuged at 2000 g for 3 min, and the supernatant was obtained using Kjeldahl equipment (FOSS, Kjeltec8400, Denmark) for TVB-N analysis. The pH of the supernatant was concurrently measured using a digital pH meter.

2.6. Determination of lipid oxidation

Furthermore, lipid oxidation was determined following the previous method (Qian et al., 2021). The value of 2-thiobarbituric acid reactive substances (TBARS) was represented as mg MDA/kg fish.

2.7. Determination of k-value

The K value was obtained using High-Performance Liquid Chromatography (HPLC), following the previous method (Li et al., 2020). Adenosine triphosphate (ATP) and its breakdown products, including adenosine monophosphate (AMP), adenosine diphosphate (ADP), hypoxanthine riboside (HxR), inosine monophosphate (IMP), and hypoxanthine (HX), are quantified as part of this analytical technique. Specifically, 2 mL of a cold 10 % perchloric acid solution was used to homogenize 1 g of fish flesh. The resulting homogenate underwent centrifugation at 8,000 rpm for 10 min at 4 °C, enabling the supernatant collection. The pellet was then subjected to two washes with 2 mL of a cold 5 % perchloric acid solution, and the supernatants were combined. Subsequently, the pH of the combined supernatant was carefully adjusted to a range of 6.0–6.5 using 1 M and 10 M sodium hydroxide solutions. Following this, the mixture underwent centrifugation at 7,000 rpm for 15 min at 4 °C, and the resultant solution was passed through a membrane with a pore size of 0.22 μm. The filtered samples were stored at −20 °C until further analysis. The calculation of the K value follows the formula: $K\ value\ (\%) = [(HxR + Hx) / (ATP + ADP + AMP + IMP + HxR + Hx)] \times 100$.

2.8. Determination of biogenic amines

The extraction procedure was conducted following the previous method (Huang, Liu, Jia, & Luo, 2017). Briefly, 5 g fish flesh was combined with 20 mL of 0.4 M perchloric acid solution and homogenized at 8000 rpm for 2 min. Subsequently, the homogenate underwent centrifugation at 12000 rpm for 10 min, and the resulting supernatant was collected. This homogenization and centrifugation process was repeated, and the combined supernatants were adjusted to a final volume of 50 mL using perchloric acid. For the derivatization step, 1 mL of the extracted solution was combined with 200 μL of a 0.2 M NaOH solution, followed by the addition of 300 μL of a saturated $NaHCO_3$ solution and a 10 mg/mL dansyl chloride solution. The reaction was conducted in the dark at 40 °C for 40 min and terminated by adding 100 μL of a 25 % (v/v) ammonia solution. The resulting mixture was adjusted to a final volume of 5 mL using acetonitrile and filtered through a 0.22 μm membrane to analyze the organic phase. HPLC analysis was performed following the previous method (Li & Zhang, 2023).

2.9. Sensory evaluation

Sensory evaluation was determined following the previous method (Zhang et al., 2021). The sensory evaluation panel comprised 10 trained and experienced food experts, including 5 males and 5 females. They conducted sensory evaluations on puffer fish fillets, focusing on the

attributes of color, odor, texture, and overall acceptability. Each quality parameter was assessed on a scale of 0 to 10, with a score of 10 representing the highest quality. Samples receiving a score below 5 were considered unacceptable. Sensory scores: 7–10 represented good quality, 5–7 represented acceptable quality, 3–5 represented unacceptable quality, and 1–3 represented intense dislike.

2.10. Statistical analysis

ANOVA was used for the statistical analysis in this work with the SPSS 20.0 package (SPSS Inc., Chicago, IL, USA) to assess the significance of the test data. The chosen significance level was set at $p < 0.05$. Additionally, the *t*-test was employed to evaluate the differences between the two groups. Graphical representations were generated using Origin software (version 2021, OriginLab, USA). All treatments and measurements were conducted in triplicate, and the data are expressed as mean values with accompanying standard deviations.

3. Results and discussion

3.1. Microbiological analysis

3.1.1. Microbial enumeration

The initial total viable counts (TVC) were 3.68 ± 0.18 and 2.56. ± 0.47 lg CFU/g for control and PALA-treated samples, respectively (Fig. 1b). This indicated that some bacteria in puffer fish fillets were eliminated immediately after immersing in the PALA solution for 5 min. Previous studies have reported that the growth rate of microorganisms in fresh fish fillets was relatively slow during the early stages of storage, a phase often referred to as the lag phase (Verheyen, Bolivar, Perez-Rodriguez, Baka, Skara, & Van Impe, 2018). The TVC results demonstrated that the lag phases for the control and PALA-treated groups were determined to be 0–2 and 0–4 days, respectively. Previous studies have confirmed that PALA shows antibacterial effects mainly by the generation of reactive species (such as NO_2^-, NO_3^-, H_2O_2, and $ONOO^-$), which were considered a dominant species for bacterial inactivation due to their high permeability. Upon through the cell membrane, these reactive species permeate the interior of bacterial cells, inciting oxidative damage to intracellular lipids, DNA, and proteins(Qian et al., 2019). This cascade of events ultimately culminates in the demise of surface bacteria on the fish fillets (Chanioti et al., 2023). This suggests that the application of PALA treatment offers an advantage in extending the lag phase duration for microbes in puffer fish fillets. These findings align with the results reported in studies involving the application of electrolyzed water-chitosan on puffer fish (Zhou et al., 2011), and the utilization of fish gelatin-grape seed extract on seabass fillets (Zhao, Chen, Wongmaneepratip, He, Zhao, & Yang, 2021). The TVC in the control group developed quickly during storage following the lag phase and reached 7.51 ± 0.11 lg CFU/g on day 6, above the maximum edible limit (6.0 lg CFU/g) for chilled seafood products (International Commission on Microbiological Specifications for Foods., 1988). However, the TVC in the PALA group finally increased to 6.95 ± 0.21 lg CFU/g on day 10, exceeding the edible limit 4 days later than the control group. These findings suggested the antimicrobial efficacy of PALA, which was consistent with previous studies (Qian et al., 2019; Wang et al., 2023).

As depicted in Fig. 1c, the initial psychrophilic bacteria count (PBC) of the PALA-treated group was 0.95 ± 0.34 lg CFU/g, which was significantly lower than the control group ($p < 0.05$). During chilled storage, the antimicrobial effects of PALA on psychrophilic bacteria exhibited a trend similar to the results of TVC. After storage for up to 6 days, the PBC value of fish fillets in each group was close to its TVC value and exhibited no substantial difference ($p > 0.05$). These findings demonstrated that psychrophilic bacteria were identified as the primary spoilage microorganisms in the later storage stages of puffer fish fillets, which was consistent with earlier observations (Silbande et al., 2018). Similar studies have also discovered that psychrophilic bacteria flora exhibited greater adaptability to growth and metabolic processes in low-temperature environments compared to other bacteria flora (Dehghani, Hosseini, Golmakani, Majdinasab, & Esteghlal, 2018; Zhuang, Hong, Zhang, & Luo, 2021). Hence, the regulation of psychrophilic bacterial growth plays a pivotal role in the preservation and freshness maintenance of the product.

3.1.2. Microbiota composition

To attain a deeper understanding of microbial variations in puffer fish fillets during storage at 4 °C, the succession of microbial flora was further analyzed. A total of 615,862 gene sequences were acquired after quality filtering through the Illumina sequencing of the bacterial 16S rRNA gene (Table 1). The Goods coverage values exceeded 0.997 in all groups, suggesting that the vast majority of bacterial phylotypes were detected and identified with a sufficiently high sequencing depth (Zhao et al., 2021). Studies have reported that the Shannon and Simpson indices represent sample richness and species evenness, while the Chao 1 and ACE indices are influenced by the number of bacterial species in the community (Cao et al., 2020). These four indices collectively offer an objective reflection of species diversity. Overall, the diversity indices exhibited a gradual decrease during storage, suggesting a decline in microbial diversity with the prolonged storage time. Similar studies have reported a decrease in the bacterial diversity of largemouth bass fillets with an increase in storage time (Zhuang et al., 2020). However, during the 4th day of storage, the PALA-treated group exhibited Shannon, Simpson, Chao 1, and ACE values of 5.50, 0.95, 146.02, and 150, respectively, which were significantly higher than those of the control group. These results suggested that PALA treatment retained the characteristics of microbial community diversity during the initial state of fish fillets.

As illustrated in Fig. 2a, *Proteobacteria* and *Firmicutes* were the predominant bacterial phyla in fresh puffer fish fillets, which was consistent with previous research (Zhang, Li, Liu, Lei, Regenstein, & Luo, 2019). As storage time increased, the relative abundance of *Firmicutes* in control samples decreased, while the relative abundance of *Proteobacteria* increased rapidly from 64.39 % (day 0) to 95.93 % (day 4), and finally to 99.97 % on day 8. However, a slight alteration in the relative abundance of *Proteobacteria* and *Firmicutes* was observed in the PALA-treated group compared to the control group on day 0. Similar studies have reported that the bacteria in *Firmicutes* and *Proteobacteria* exhibited different growth capacities under the modified atmospheric conditions, with significant changes in their relative abundance (Zhang et al., 2021). Furthermore, the relative abundance of bacterial phyla in the PALA-treated samples showed minimal changes from day 0 to day 4. These findings indicated that PALA treatment exhibited a noticeable impact on the changes of the predominant bacterial phyla in puffer fish fillet samples.

The composition and relative abundance of different genera are shown in Fig. 2b. The initial microbiota composition of puffer fish fillets was quite diverse. *Ralstonia* (47.1 %), *Selenomonas* (9.4 %), *Megasphaera* (7.5 %), *Pseudomonas* (7.0 %), and *Dialister* (6.2 %) were the top five genera of the initial microbiota. This suggests that *Ralstonia* had a relatively high relative abundance in fresh fish fillets, which was possibly due to the presence of residual intestinal microbiota on the fish surface after slaughtering. Previous studies have reported that *Ralstonia* was not a dominant genus among spoilage bacteria in fish fillets, but rather a dominant genus in the intestinal microbiota of fish (Carda-Dieguez, Mira, & Fouz, 2014). Compared to the control group, the initial relative abundance of *Ralstonia* in the PALA-treated samples had completely diminished, while the relative abundance of *Pseudomonas* rapidly increased to 69.92 %. This result suggested that other bacterial genera in puffer fish fillets were more easily inactivated by PALA, compared to *pseudomonas*. On day 4, the control sample neared spoilage (TVC: 5.15 lg CFU/g), with the microbiota genus predominantly composed of *Pseudomonas* (95.77 %) and *Macrococcus* (3.87 %). Complete decay was observed on day 8, with *Pseudomonas* accounting for a

Table 1
Alpha diversity indices of microbiota of puffer fish fillets in the control and PALA groups during storage at 4 °C.

Storage time (days)	Treatment	Total tags	OTUs	Shannon	Simpson	Chao1	ACE	Goods coverage
0	Control	118,587	100	4.01	0.80	115.69	119	0.9986
	PALA	123,217	119	4.18	0.89	134.77	139	0.9985
4	Control	138,316	25	2.22	0.70	58.21	62	0.9999
	PALA	102,143	146	5.50	0.95	146.02	150	0.9977
8	Control	67,598	18	1.96	0.62	23.48	27	0.9998
	PALA	66,000	16	1.48	0.45	23.50	24	0.9998

Notes: Control: samples immersed in deionized water; PALA: samples treated with Plasma-activated Lactic Acid solution. OTUs: operational taxonomic units; Shannon: the Shannon index of community diversity; Simpson: the Simpson index of community diversity; Chao 1: the Chao1 estimator of community richness; ACE: the ACE estimator of community richness; Coverage: the good's community coverage.

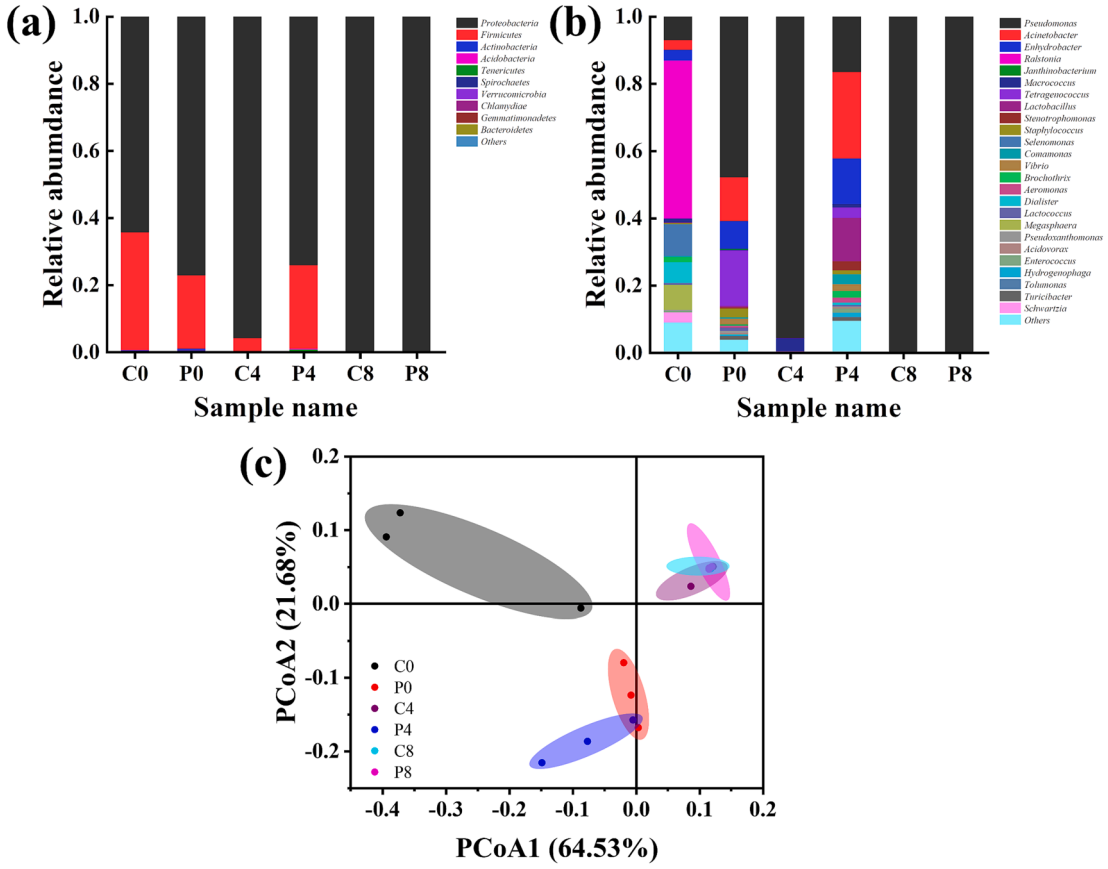

Fig. 2. Relative abundance of the bacteria at **(a)** the phylum level and **(b)** the genus level, **(c)** and principal coordinates analysis of microbiota in the control and PALA groups during storage at 4 °C. Notes: C0, C4, and C8: Control samples on days 0, 4, and 8; P0, P4, and P8: PALA-treated samples on days 0, 4, and 8.

relative abundance of 99.91 %. Similar studies have reported a rapid increase in the relative abundance of *Pseudomonas* on seabass fillets to 88.01 % on day 12, consistent with the findings of our study (Silbande et al., 2018). The results indicated that *Pseudomonas* exhibited significantly higher spoilage activity compared to other spoilage bacteria (Zhuang, Li, Jia, Hong, Liu, & Luo, 2019). Therefore, the dramatic increase in *Pseudomonas* in the control group on the 4th day of storage indicates a faster spoilage process compared to the PALA-treated group. Moreover, it is worth noticing that PALA treatment led to a decreasing trend in the relative abundance of *Pseudomonas*, from day 0 (47.85 %) to day 4 (16.55 %). This can be explained by the fact that PALA can effectively inhibit the growth of *Pseudomonas* in puffer fish fillets during storage, thus delaying the succession process of spoilage bacteria. Principal Coordinate Analysis (PCoA) based on β-diversity analysis was employed to evaluate the differences in microbial community composition of samples under various storage durations. As shown in Fig. 2c, significant differences were observed between samples in C0 and P0, indicating that PALA altered the microbiota composition. However, no significant difference occurred in the microbial community composition between day 0 and day 4 in the PALA-treated group. This observation further supports the proposition that PALA treatment can prolong the shelf life of puffer fish fillets.

3.2. Chemical analysis

3.2.1. TVB-N and pH value

During the first four days of storage, the TVB-N concentration of both the control and PALA-treated samples displayed a constant range of 5–9 mg N/100 g (Fig. 3a). Then, starting on day 4, the TVB-N level in control samples exhibited a significant rise, reaching 26.91 mg N/100 g on day 8, above the allowable limit for fish products (20 mg N/100 g) (Li et al., 2020). The TVB-N level of the treated samples changed very little due to PALA's antibacterial activity, only rising to 9.96 mg N/100 g on day 10. Consequently, even after storage had ended, the TVB-N concentration of

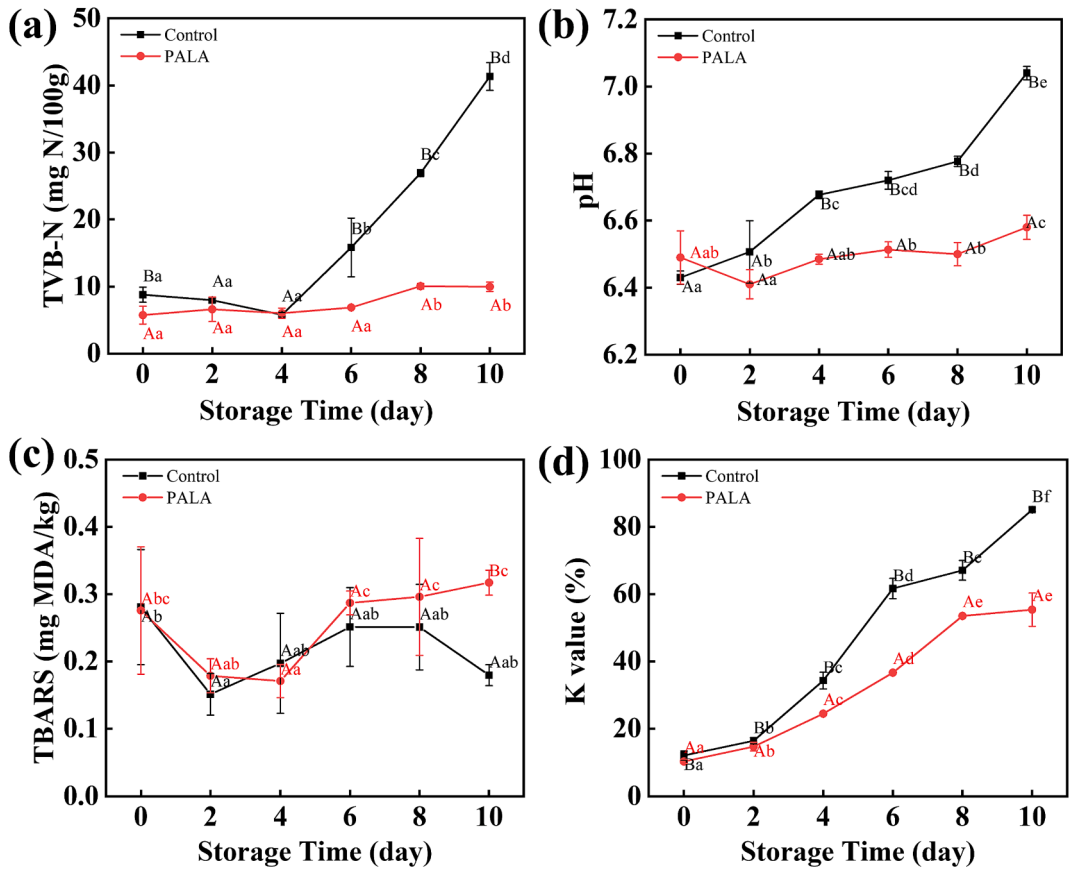

Fig. 3. Changes in **(a)** TVB-N, **(b)** pH value, **(c)**TBARS, **(d)** and K value of puffer fish fillets in the control and PALA groups during storage at 4 °C. Notes: Control: samples immersed in deionized water; PALA: samples treated with Plasma-activated Lactic Acid solution.

samples that had received PALA treatment did not exceed the maximum limit. Previous studies have established that during the process of fish decay, TVB-N compounds predominantly originated from the microbial metabolism of nitrogen-containing compounds, such as free amino acids and peptides (Parlapani et al., 2018). Furthermore, earlier research has documented that *Pseudomonas* plays a significant role as a TVB-N producer in fish flesh (Huang et al., 2022; Li, et al., 2019). Thus, the reduced TVB-N values in the treated samples may be ascribed to the antibacterial effect of PALA on spoilage bacteria, which was consistent with the findings of the high-throughput sequencing results.

Fig. 3b displays the variations in the pH values of the two groups. The initial pH values of the control and PALA-treated samples were 6.43 and 6.49, respectively, which was consistent with the pH value (6.4) of fresh puffer fish fillets as reported in a previous study (Dehghani et al., 2018). As the treatment time increased, the control group exhibited a pronounced upward trend in pH values, ultimately reaching a value of 7 on day 10. In contrast, the pH of the PALA-treated group remained relatively stable during storage, significantly lower than that of the control group from day 4 to day 10. Previous research has demonstrated a close correlation between microbial metabolism and pH alterations in aquatic products (Y. Li, Jia, et al., 2020). Microorganisms tend to accelerate protein degradation, leading to the accumulation of basic nitrogen-containing compounds such as ammonia and biogenic amines, subsequently elevating the pH of the sample (Zhou et al., 2011). Therefore, the results showed that PALA could effectively inhibit the increase in the pH value of fish fillets by reducing the growth of spoilage bacteria.

3.2.2. *Lipid oxidation and K value*

To evaluate the effect of the reactive species on fish meat, the lipid oxidation of fish fillets was tested, as depicted in Fig. 3c. During the first 8 days, no significant differences were observed between the control and the PALA-treated samples, and the TBARS values for all samples remained at relatively low levels, approximately ranging from 0.1 to 0.3 mg MDA/kg. Previous research has established that when the TBARS value of meat is below 0.6 mg MDA/kg, it will not adversely affect the sensory quality and flavor of meat (Qian et al., 2021). Similar studies have reported that the TBARS value of untreated bighead carp fluctuated slightly and remained low with the prolongation of storage time (Hong, Luo, Zhou, Bao, Lu, & Shen, 2013). Furthermore, Liao et al. (2018) found that the impact of reactive species in PAW on the lipid oxidation of shrimps was slight and could be ignored, which is consistent with the findings of our research. The non-significant increase in TBARS values of fish meat after PALA treatment could be attributed to the effect of $NO^{\bullet}$. The $NO^{\bullet}$ produced by PALA may bind to the iron center of myoglobin, avoiding the lipid oxidation induced by free iron (Qian et al., 2021). It is noteworthy that, based on microbial results, both the control and the PALA-treated samples had reached the spoilage stage on day 10. At this point, the TBARS values of the PALA-treated group were significantly higher than those of the control group, possibly due to varying degrees of spoilage, leading to distinct levels of lipid oxidation among the samples.

The K value serves as a crucial parameter for assessing the freshness of seafood products. In fish flesh, ATP undergoes degradation through enzymatic and bacterial processes following the ATP → ADP → AMP → IMP → HxR → Hx pathway (Zhuang et al., 2020). This degradation process leads to a progressive increase in the K value as ATP depletes, indicating the deterioration in sample freshness (Jia, Liu, et al., 2019). The initial K values of puffer fish were 12.03 % and 10.24 % for the control and PALA-treated group (Fig. 3d). These values exhibited a gradual increase with the progression of storage time, ultimately reaching 85.07 % and 55.38 % at the end of storage, respectively. During the entire storage period, the K value of the PALA group was lower than

that of the control group. However, the difference in K values between the two groups was more significant during the mid-storage and final-storage periods compared to the initial storage period (0–2 days). Prior research has reported that fish meat primarily experiences cell autolysis in the initial 1–2 days of storage, leading to the conversion of ATP to IMP. Subsequently, this IMP is further metabolized into HxR and Hx by bacterial enzymes, resulting in an escalation of the K value (Cao et al., 2020). Therefore, the slow increase in K values for the PALA-treated group may be attributed to the inhibition of microbial proliferation during storage.

3.2.3. Biogenic amines

The accumulation of biogenic amines is a consequence of proteolysis and amino acid decarboxylation by microorganisms (Li et al., 2020). The changes in the content of three biogenic amines in puffer fish fillets during storage were illustrated in Table 2. The content of putrescine (PUT), cadaverine (CAD), and tyramine (TYR) remained low during the initial storage period, falling below the detection limit. However, towards the end of storage, a significant increase in the content of these biogenic amines was observed. Similar studies reported that the PUT and CAD contents in fish meat were low at the initial stage of storage. Among them, PUT and CAD were produced by the physiological activities of Aeromonas, Pseudomonas, and Acinetobacter (Cao et al., 2020). Studies have also shown that Aeromonas was a powerful producer of PUT and CAD in fish fillets, and inhibiting the growth of Aeromonas helps delay the deterioration of fish fillets (Li et al., 2020). Therefore, PUT and CAD contents are highly correlated with food safety and quality deterioration, often employed to evaluate the freshness and microbial spoilage of fish (Zhuang et al., 2019). Based on the high-throughput sequencing results (Fig. 2), PUT and CAD in puffer fish fillets may be produced by the physiological activities of *pseudomonas* (Cao et al., 2020). In our study, PUT and CAD contents were detected on day 6 and day 4, respectively, and exhibited an increase as the storage duration extended. Throughout the entire storage period, the PALA group consistently displayed lower concentrations of PUT and CAD in comparison to the control group. By the 10th day of storage, the contents of PUT and CAD in the control group were 21.22 and 16.88 mg/kg, while the contents of the PALA group were only 1.07 and 3.15 mg/kg, respectively. The results showed that PALA could effectively inhibit the production of PUT and CAD during storage, which might be due to the inhibitory effect of PALA on spoilage bacteria (pseudomonas).

3.3. Sensory analysis

According to Fig. 4, the initial sensory evaluations of puffer fish fillets' color, odor, texture, and overall acceptability were 9.20 ± 0.45, 8.82 ± 0.84, 9.41 ± 0.55, and 8.84 ± 0.84, respectively. Compared with the control group, PALA treatment exhibited no significant difference in sensory evaluations on day 0. Similar studies also reported that the quality parameters of beef and chicken subjected to PALA treatment showed a relatively stable state in the initial stages of storage (Qian et al., 2021; Qian et al., 2019). Overall, each group displayed a decreasing trend in the sensory properties during storage, suggesting a progressive deterioration in the quality of puffer fish fillets (Chanioti et al., 2023; Zhang et al., 2021). However, the sensory scores of the PALA-treated were consistently better than the control. The main factor might be attributed to the reactive species generated by PALA, which inhibit the growth of spoilage microorganisms in the fish fillets, thus retarding the deterioration of sensory quality (Kulawik & Tiwari, 2019; Rahman et al., 2022). After 6 days of refrigerated storage, the color, odor, texture, and overall acceptability scores in the control group reduced to 6.20 ± 0.84, 4.70 ± 0.71, 4.90 ± 0.36, 5.40 ± 0.55, respectively. Notably, both odor and texture were below the minimum acceptance level (score = 5). However, PALA-treated samples began to become organoleptically unacceptable (odor scores lower than 5) on day 10. Therefore, the application of PALA significantly enhanced the sensory attributes of the fish fillets, resulting in a prolongation of the shelf life by approximately 4 days.

4. Conclusion

In this study, PALA demonstrated its potential as a novel sanitizer to extend the shelf life of puffer fish fillets. PALA treatment effectively reduced the growth of bacteria (TVC and PBC) and attenuated changes in chemical indicators (TVB-N, pH value, K value, and biogenic amines) of puffer fish fillets. Additionally, insignificant changes were observed in lipid oxidation during the first 8 days ($p > 0.05$). Illumina-MiSeq high-throughput sequencing revealed that PALA effectively inhibited the growth of *Pseudomonas* in puffer fish fillets during storage, maintaining the diverse characteristics of the microbial community, thus delaying the succession process of spoilage bacteria. In combination with sensory analysis, PALA extended the shelf life of puffer fish fillets for 4 days. Therefore, PALA is suggested as a novel sanitizer for puffer fish fillets stored at 4 °C.

CRediT authorship contribution statement

Xiaowei Sheng: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Writing – original draft, Writing – review & editing. **Longfei Yan:** Data curation, Formal analysis, Investigation, Methodology. **Lanqing Peng:** Data curation, Investigation, Methodology. **Luling Zhao:** Data curation, Investigation, Methodology. **Fanwei Dai:** Data curation, Formal analysis, Methodology. **Feiping Chen:** Data curation, Investigation, Methodology. **Ling Wang:** Data curation, Investigation, Methodology. **Yulong Chen:** Data curation, Investigation, Methodology. **Mingqiang Ye:** Funding acquisition, Supervision. **Jin Wang:** Supervision, Writing – review & editing. **Jianhao Zhang:** Funding acquisition, Supervision, Writing – review & editing. **Vijaya Raghavan:** Investigation, Methodology.

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.

Table 2
Changes in biogenic amine content of puffer fish fillets in the control and PALA groups during storage at 4 °C.

Biogenic amines	Treatment	Storage time (days)					
(mg/Kg)		0	2	4	6	8	10
Putrescine	Control	ND	ND	ND	2.88 ± 0.23^{a}	14.41 ± 0.65^{Bb}	21.22 ± 3.53^{Bb}
	PALA	ND	ND	ND	ND	0.33 ± 0.26^{Aa}	1.07 ± 0.58^{Aa}
Cadaverine	Control	ND	ND	0.38 ± 0.10^{Ba}	1.72 ± 1.60^{Bb}	9.26 ± 1.52^{Bc}	16.88 ± 1.16^{Bd}
	PALA	ND	ND	0.02 ± 0.09^{Aa}	0.08 ± 0.08^{Aa}	0.26 ± 0.21^{Aa}	3.15 ± 0.49^{Ab}
Tyramine	Control	ND	ND	0.27 ± 0.02^{a}	1.59 ± 0.11^{Bb}	6.78 ± 0.52^{Bc}	13.22 ± 2.52^{Bd}
	PALA	ND	ND	ND	0.36 ± 0.13^{Aa}	0.25 ± 0.03^{Aa}	1.16 ± 0.24^{Ab}

Notes: Control: samples immersed in deionized water; PALA: samples treated with Plasma-activated Lactic Acid solution. ND: Not detected.

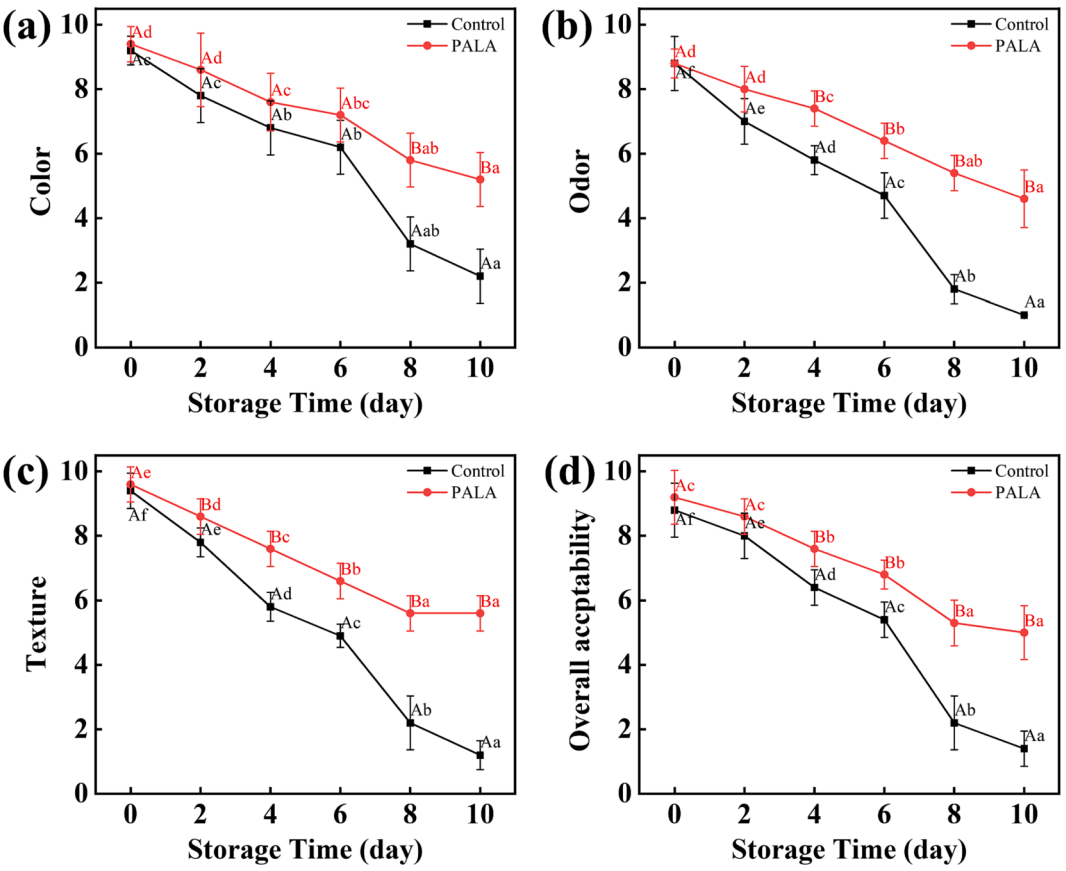

Fig. 4. Sensory evaluation of puffer fish fillets in the control and PALA groups during storage at 4 °C. **(a)** color, **(b)** odor, **(c)** texture, **(d)** overall acceptability. Notes: Control: samples immersed in deionized water; PALA: samples treated with Plasma-activated Lactic Acid solution. Sensory scores: 7–10 = good quality, 5–7 = acceptable quality, 3–5 = unacceptable quality, 1–3 = intense dislike.

Data availability

Data will be made available on request.

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