Supplementary Information

The effect of NaCl on the formation of key quality of tilapia fillet with fermentation of *Lactiplantibacillus plantarum* via multi-omics

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Bacteria community analysis

The bacterial diversity sequencing was measured according to the method of Wang et al. [1]. The 0.5 g of tilapia sample was minced in a 2 mL centrifuge tube for Extraction of DNA. E.Z.N.A™ Mag-Bind Soil DNA Kit was used to extract the total genomic DNA. Qubit 4.0 (Thermo, USA) was used to quantity and quality of extracted DNA. Amplification of the bacterial V3–V4 hypervariable region using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATC-3′). The quality of the amplification products was checked using the 2% agarose gel electrophoresis and NanoDrop 2000 UV–vis spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Illumina MiSeq system (Illumina MiSeq, USA) was used to sequence the PCR products.

Bacterial diversity and composition

The bacterial α-diversity analysis was shown in Fig. S1 and S2. The Chao index was used to assess the bacterial richness. The Shannon index were used to quantify the diversity [1]. The Chao index of untreated group was the highest, indicating that untreated tilapia had the highest bacterial abundance. Among the three groups of dry cured tilapias, the 8% group had the highest Chao index and the 6% group had the lowest Chao index. The Chao index of tilapia decreased after dry-curing fermentation, indicating that the bacterial abundance of the samples decreased during fermentation. With the increase of salt level, the bacterial abundance of tilapia first decreased and then increased. In addition, the untreated group had higher Shannon's index, indicating that the bacteria richness of untreated tilapia was the highest. This may be related to the environment of the fish. The 4% group had lowest Shannon's index, indicating that the bacterial richness of tilapia in 4% group was the lowest. This speculated that the inoculated foreign microorganisms become the dominant flora in tilapia and inhibit the growth of other bacteria. With the increase of salt content, the Shannon index of tilapia first increased and then decreased. It indicated that the bacterial richness first increased and then decreased. Higher salt inhibited the growth of exogenous *Lactiplantibacillus plantarum*, resulting the abundance of *Lactiplantibacillus plantarum* was decreased and the abundance of other bacteria was increased.

Fig. S3 shown the bacterial composition of dry cured tilapia. The most common bacteria in the untreated group was *Staphylococcus*. *Staphylococcus* mainly originates from the pre-fermentation

environment and raw materials. After curing and fermentation, *Lactiplantibacillus* gradually became the main microorganism in the 4% group, and the abundance of *Lactiplantibacillus* reached about 80%. Due to the addition of exogenous *Lactiplantibacillus plantarum*, we speculated that *Lactiplantibacillus plantarum* was the dominant microbe in 4% group tilapia. With the increase of salt content, the abundance of *Lactiplantibacillus* decreased and that of *Staphylococcus* increased. *Lactiplantibacillus* was inhibited at higher salt concentrations. *Staphylococcus* gradually became the main microbe in high-salt fermented tilapia, accompanied by a small number of other bacteria.

Sodium chloride content analysis

The steamed tilapia samples were taken out 5 g and added with 25 mL pure water. The mixture homogenized for 1 min and filter. The salinity analyze of the filtrate of the sample was measured by salinometer. The salinometer result was multiplied by the dilution of the sample to calculate the content of sodium chloride in the sample.

Sodium chloride content of different fermented tilapias

Fig. S4 shown the results of sodium chloride content. Untreated tilapia had a very low sodium chloride content. The contents of sodium chloride in 4% salt group, 6% salt group and 8% salt group were 3.32%, 5.08% and 6.76%, respectively. The variation trend of sodium chloride content in fish body was consistent with that of curing salt concentration.

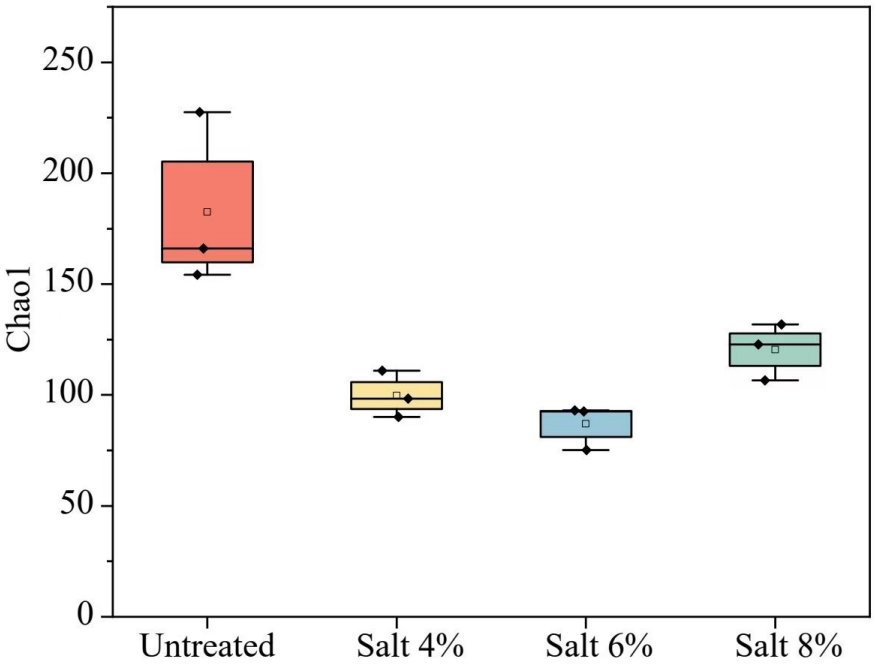

Figure S1. Chao index of the microbiota in fermented tilapia.

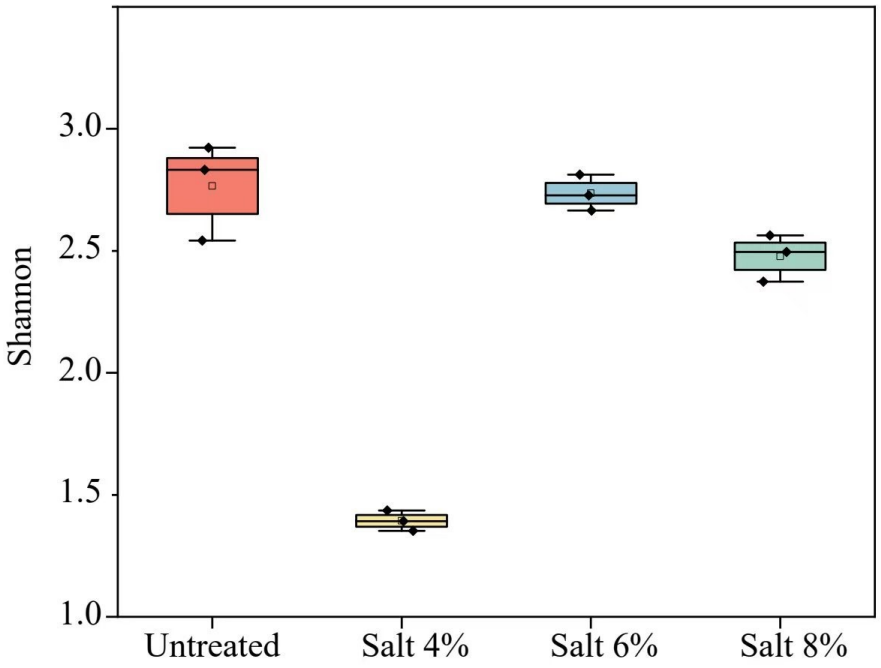

Figure S2. Shannon index of the microbiota in fermented tilapia.

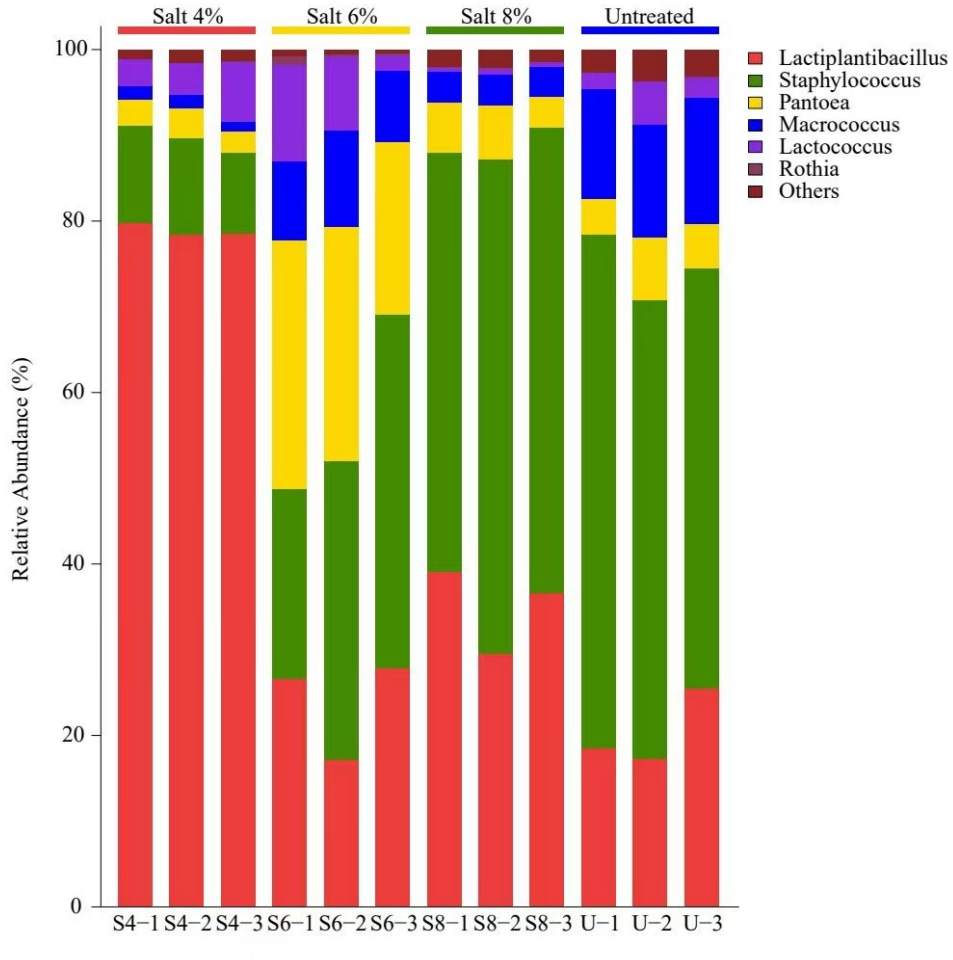

Figure S3. The relative abundance of bacteria at the genus in fermented tilapia.

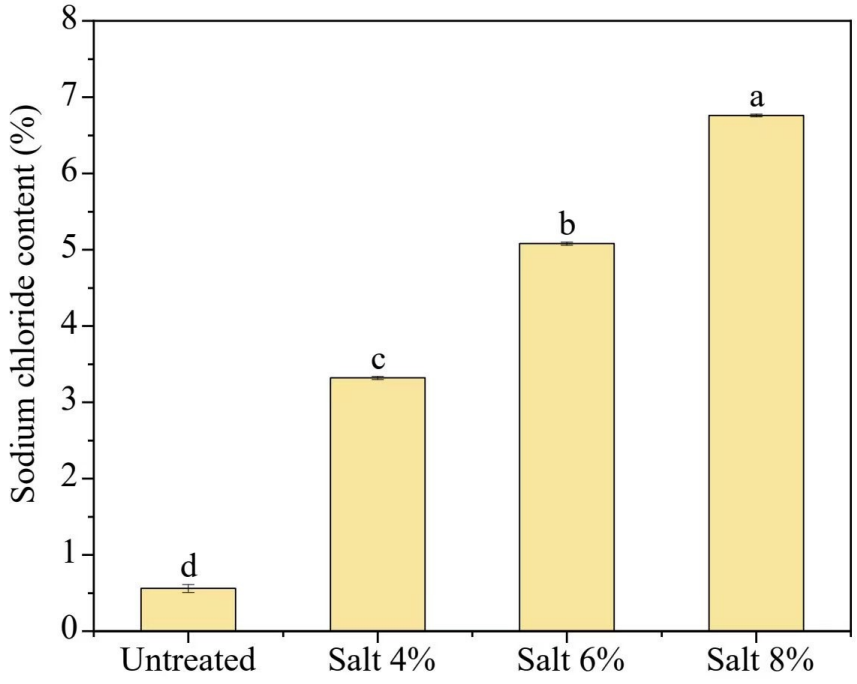

Figure S4. The sodium chloride content of fermented tilapia.

Reference

1. J. Wang, X.-S. Wang, Z. Zhang, D.-Y. Zhou, X.-H. Huang and L. Qin, Comprehensive insights into the organoleptic characteristics attributes of “HuangYuXiang”: Integration of volatilomics, sensomics, macrogenomics, lipidomics, and metabolomics, *Food Chemistry*, 2024, **460**, 140409.