Analysis of bacterial communities of squid and shrimp skewers after immersion in a red wine vinegar-based solution

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Introduction

Seafood is a highly nutritious and essential component of a healthy diet.[1] However, due to its perishable nature, seafood products have short shelf lives, leading to high prices and reduced desirability. In order to address this issue, various innovative techniques, such as High Pressure Processing and Ozonation, have been explored to extend the shelf life of seafood. While efficiency of such techniques varies, the cost of their application is usually high, increasing final price or reducing profit margins. Therefore, easier and cheaper, methods such as the pulverization or immersion of the product in antibacterial solutions are gaining popularity.[2] To achieve the required bacterial inhibition and maintain an all-natural label, a seafood product, composed mainly of raw squid (*Loligo duvauceli*) and shrimp (*Parapenaeopsis*, *Penaeus* and *Metapenaeus* genus) was sprayed and immersed in a red wine vinegar-based solution. To perceive the efficiency of the treatment in the inhibition of unwanted spoilage bacteria, analysis of bacterial communities through sequencing of the 16S rRNA gene amplicons with NGS technologies (paired-end Illumina) was performed.

Methods

Immersion

•Complete submersion of the product in 550 ml of vinegar 50% (v/v) solution
•5 minutes
•Sampling

Pulverization

•Use a conventional dispenser to spray the solution on the product.
•3 to 4 sprays per face of the product.
•Sampling

In addition to the pulverization (PV) and immersion (IV) in the active solution, control samples were prepared for comparison. These control samples included untreated samples (Control), samples pulverized with deionized sterile water (PW), and samples immersed in deionized sterile water (IW). Samples were collected at different time points during the storage process under refrigeration: immediately after treatment (T0), 2 days (T2) and 5 days (T5), and total DNA was extracted following the Qiagen DNeasy® mericon® food kit protocol. This extraction was performed in triplicate and resulting DNA was pooled together to achieve a single sample. Amplification of the 16S rRNA gene, specifically the hypervariable V3/V4 region, was performed. PCR products were sequenced by the Illumina MiSeq® sequencer and obtained sequences were processed and analysed through EZBioCloud®. Results regarding the composition of samples were normalised and α-diversity and β-diversity of samples were determined by the Shannon-Wiener diversity index and Bray-Curtis dissimilarity analysis, respectively.

Results

Figure 1 – Taxonomic composition of samples. Family (A); Genus (B)

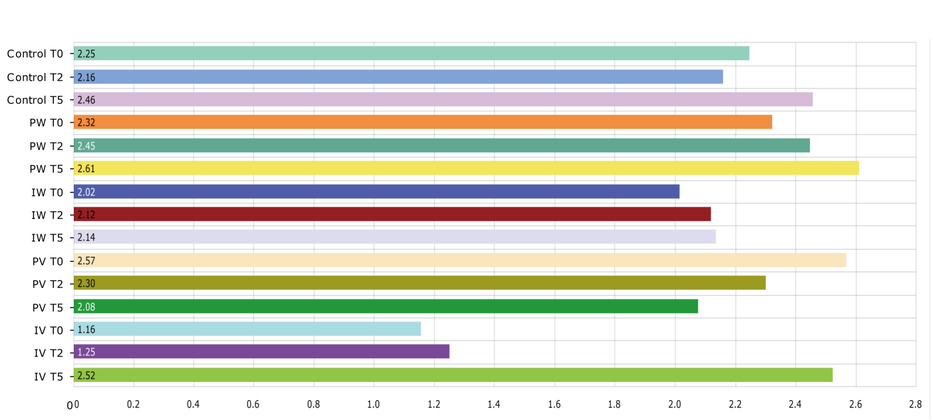

Figure 2 – Shannon–Wiener Diversity Index (A) of the squid and Shrimp samples under different treatments.

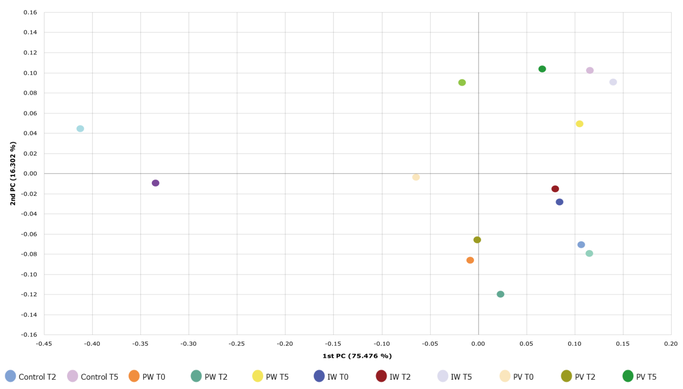

Figure 4 – PCoA (Principal Coordinate Analysis) based on a Bray-Curtis dissimilarity matrix calculated at Genus level.

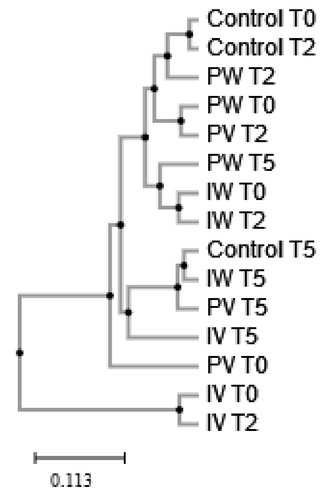

Figure 3 – β-diversity of squid and Shrimp samples using UPGMA clustering analysis.

- Dominance of *Leuconostoc* spp. and inhibition of *Pseudomonas* spp. in IV samples (Figure 1).
- Considerably lower diversity in IV samples (Figure 2).
- Slight impact immediately after pulverization with the vinegar-based solution (Figure 1).
- Antibacterial activity immediately after immersion in the vinegar-based solution and after 2 days of storage (Figures 1 and 2).
- IV samples, T0 and T2, are different from the controls, IA, PA and PV samples (Figure 4).
- After the 5 days of storage, the effect of the solution is lost, and the spoilage bacteria become dominant (Figures 1 and 3).

Conclusions

While not guaranteeing the complete inhibition of spoilage microorganisms, the immersion of this seafood product in a vinegar-based solution results in a significant reduction of Specific Spoilage Organisms. Alongside this, considerable reduction in bacterial diversity suggests susceptibility of a significant portion of colonizing bacterial species to vinegar. Although not a guarantee of extended shelf-life, this preliminary study showed that vinegar solutions could be an all-natural approach to preventing spoilage in such products.

References

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