Antimicrobial effects of tea-polyphenols on pathogenic *Vibrio parahaemolyticus* in shucked oysters (*Crassostrea plicatula*)

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**Introduction**

*Vibrio parahaemolyticus* is a Gram-negative human pathogen that is naturally present in inshore marine waters (Cook *et al.*, 2002) and recognized as the leading causes of foodborne gastroenteritis associated with seafood consumption throughout the world (Joseph *et al.*, 1982; Su and Liu, 2007). Tea-polyphenols are natural edible compounds extracted from tea leaves with excellent antimicrobial activity against food spoilage and foodborne pathogens including *Staphylococcus aureus, Escherichia coli, Streptococcus mutans, Mycobacterium tuberculosis, Bacillus cereus* and *Listeria monocytogenes* (An *et al.*, 2004; Paras *et al.*, 2006; Si *et al.*, 2006; Pilar *et al.*, 2008). However, there is no information about tea polyphenols against growth of pathogenic *V. parahaemolyticus*. This study investigated the inhibitory effects of tea-polyphenols against *V. parahaemolyticus* in both culture suspension and contaminated shucked oyster and provided useful information for improving safety of oysters for consumption.

**Materials and Methods**

**Bacteria strains and preparation of multi-strain cocktail suspension**

Two virulent strains *V. parahaemolyticus* possessing *tdh* gene (ATCC 33846 and ATCC 33847), and one non-virulent strain (VP 1602 isolated from oysters) were used and a multi-strain cocktail culture suspension was prepared according to Shen *et al.* (2009).

**Growth inhibitory test of tea-polyphenols against V. parahaemolyticus**

The minimal inhibitory concentration (MIC) of tea-polyphenols against *V. parahaemolyticus* was determined according to An *et al.* (2004).

**Inoculation of V. parahaemolyticus in oysters**

Live *C. plicatula* oysters were collected and inoculated with *V. parahaemolyticus* by holding oysters in artificial seawater containing *V. parahaemolyticus* (10⁴-10⁵CFU/mL) with circulating air at 10-12 °C for 4 h (Shen *et al.*, 2009)

**Effects of tea-polyphenols on V. parahaemolyticus in shucked oysters**
The inoculated oysters were shucked in a disinfected circumstance and then dipped in various tea-polyphenols solutions for 2.5 h. The populations of *V. parahaemolyticus* in shucked oysters were detected every 30 min. For storage experiment, the shucked oysters were treated with and without tea-polyphenols (0.5 mg/mL) and stored at 0°C, 5°C, and 10°C for 60 h respectively. The populations of *V. parahaemolyticus* in shucked oysters were determined every 12 h using the FDA’s Bacteriological Analytic Manual.

**Sensory test of shucked oysters with and without the treatment of tea-polyphenols**

The taste and flavor of raw and cooked oysters treated with and without tea-polyphenols were evaluated and scores were given to each sample (1: like; -1: unlike; 0: acceptable) by a test panel with 12 students from the lab.

**Results**

**Growth inhibitory test of tea polyphenols against *V. parahaemolyticus* in culture suspension and shucked oysters**

![Figure 1](image)

Figure 1: Inhibitory effect of tea-polyphenols against *V. parahaemolyticus* in culture broth (A) and shucked oysters (B).

Figure 1 (A) showed that the growth of *V. parahaemolyticus* in alkaline peptone water supplemented with 1.5% NaCl (APW-salt) at 37°C for 48 h was effectively inhibited by tea polyphenols. The MIC of tea-polyphenols against *V. parahaemolyticus* was 0.5 mg/mL. Figure 1 (B) demonstrated that the populations of *V. parahaemolyticus* in shucked oysters decreased greatly after the samples were treated with 0.25 and 2.5 µg/mL of tea polyphenols solutions. However, the population of *V. parahaemolyticus* in shucked oysters without treatment of tea-polyphenols was steadily increased. These results indicated the efficacy of tea-polyphenols to decontaminate *V. parahaemolyticus* in shucked oysters was correlated to both the treatment time and concentration of tea-polyphenols.

**Efficacy of tea polyphenols in decontamination of *V. parahaemolyticus* in shucked oysters during cold storage at different temperatures**

Figure 2: Cold storage, no matter the storage temperatures were at 0, 5 or 10°C could effectively reduce the *V. parahaemolyticus* count in shucked oysters (Figure 2). However, *V. parahaemolyticus* reduction was much greater when the oysters were treated with tea-polyphenols before storage, indicating that tea-polyphenols could enhance the inhibitory effects of cold storage against *V. parahaemolyticus* in shucked oysters.
Sensory acceptance of shucked oysters after the treatment of tea-polyphenols

Figure 3 shows that the acceptance of oysters was dependent on the concentration of tea-polyphenols. The panel liked the oysters treated by 0.25 mg/mL of tea-polyphenols, but did not like the oysters if the concentration of tea-polyphenols reached 1.00 mg/mL.

Conclusions

Tea polyphenols had bactericidal effects against *V. parahaemolyticus* in both culture suspension and shucked oysters. Its minimal inhibition concentration was 0.50 mg/mL. The optimal concentration of tea polyphenols to treat shucked oysters was 0.25-0.5 mg/mL based on both antimicrobial and sensory tests. Those results indicated that tea polyphenols might be applied to shucked oysters to reduce the contamination of *V. parahaemolyticus*.

Acknowledgements

This study was supported by Innovative Research Team Project of Universities and Leading Academic Discipline Project of Shanghai Municipal Education Commission (J50704).

References


