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* (10^4-10^5CFU/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*

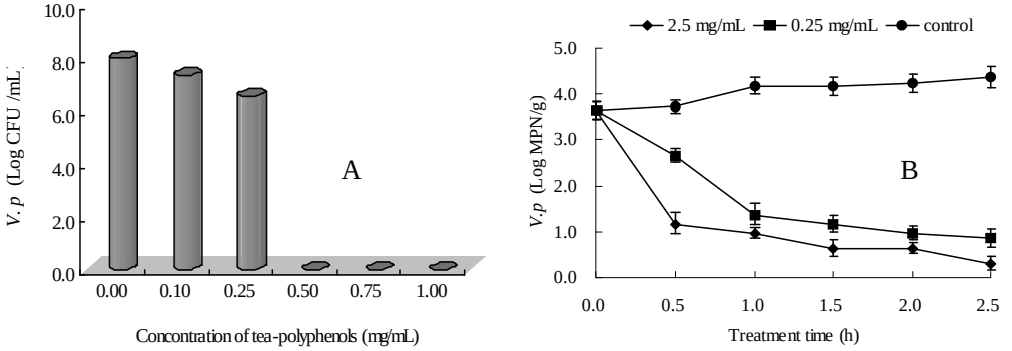

Figure 1 Inhibitory effect of tea-polyphenols against *V. parahaemolyticus* in culture broth (A) and shucked oysters (B)

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.

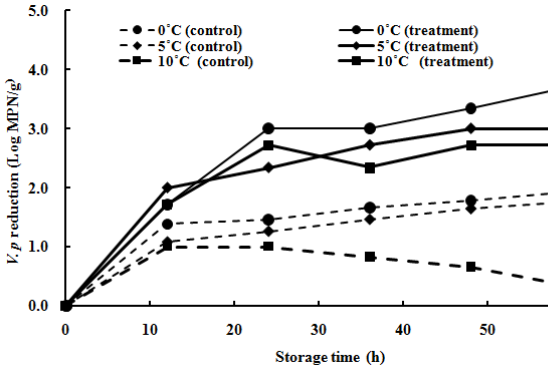

Figure 2 *V. parahaemolyticus* reduction in shucked oysters during storage with and without the treatment of tea-polyphenols

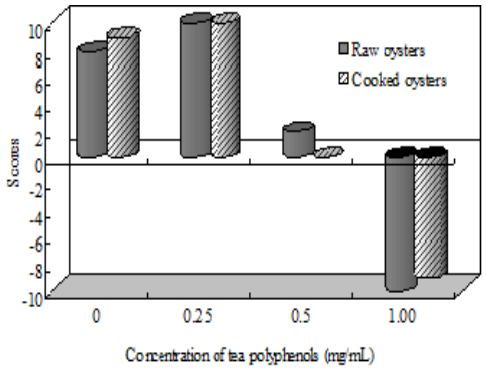

Figure 3 Effects of tea-polyphenols on the taste of raw and cooked oysters

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 like 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

An, B., Kwak, J., Son, J., Park, J., Lee, J., Jo, C., Byun, M. (2004) Biological and anti-microbial activity of irradiated green tea polyphenols. Food Chemistry. 88, 549-555.

Cook, D.W., Leary, P.O., Hunsucker, J.C., Sloan, E.M., Bowers, J.C., Blodgett, R.J., DePaola, A. (2002) *Vibrio vulnificus* and *Vibrio parahaemolyticus* in U.S. retail shell oysters. J. Food. Prot. 65, 79–87.

Paras, K., Deepak, K., Meera, S. (2006) Green tea polyphenol inhibits Mycobacterium tuberculosis survival within human macrophages. INT J BIOCHEM *CELL* B I 38, 600-609.

Pilar, M., Rosa, C,. Angel, J., Michael, H. (2008) Antioxidant and antimicrobial activities of tea infusions. Food Chemistry 108, 55-63.

Shen, X., Cai, Y., Liu, C., Liu, W., Hui, Y., Su, Y. C.(2009) Effect of temperature on uptake and survival of *Vibrio parahaemolyticus* in oysters (*Crassostrea plicatula*). Int J Food Microbiol 136, 129-132.

Si, W., Gong, J., Rong, T., Molosh, K., Raymond, Y., Yin, Y., 2006. Bioassay-guided purification and identification of antimicrobial components in Chinese tea extract. J Chrom 1125, 204-210.

Su, Y.C. and Liu, C., 2007. *Vibrio parahaemolyticus*: A concern of seafood safety. Food Microbiol. 24, 549–558.