

## The Inhibitory Effect of Lemon Juice (*Citrus limon*) on *Vibrio parahaemolyticus* in Raw Oyster (*Crassostrea virginica*)

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### ABSTRACT

**Introduction:** Serving raw oysters with lemon juice is a delicacy in many restaurants in Malaysia. Oysters (*Crassostrea virginica*) live in the seacoast and they share the same environment as *Vibrio parahaemolyticus*. Consumption of raw oysters contaminated with *V. parahaemolyticus* can lead to severe gastroenteritis. A study was performed to determine whether lemon (*Citrus limon*) juice is able to inhibit the growth of *V. parahaemolyticus* after being inoculated in raw oysters. **Methods:** Frozen oysters bought from a local supplier weighing 6 g each were minced and placed in two bottles using sterile technique. Approximately 1 ml of 10<sup>7</sup> CFU of *V. parahaemolyticus* (ATCC strain 17802) was added and mixed in both bottles. The mixture was treated with 1 ml of lemon juice in only one of the bottles and the other bottle served as a control. At every 30 s intervals for 2 min, 1 g of the sample was taken for enumeration of viable cells onto thiosulphate citrate bile salt sucrose (TCBS). **Results:** After 30 s of treatment with the lemon juice, it was observed that the number of colonies in the treated samples reduced from 7 Log to 3 Log. Subsequently, no viable *V. parahaemolyticus* was seen. It was also observed that there were 3 Log reductions of *V. parahaemolyticus* after 30 s in untreated samples, however the number of colonies remained stable until the end of the experiment. **Conclusion:** This study therefore shows that lemon juice has some antimicrobial effect on *V. parahaemolyticus* in raw oysters.

**KEYWORDS:** *Vibrio parahaemolyticus*, lemon juice, *Crassostrea virginica*

### INTRODUCTION

Oyster (*Crassostrea virginica*) is a type of bivalve mollusk and belongs to the family *Ostreidae*. The habitat of oyster is in briny water such as the sea coast or river banks. Oysters are filter feeders as they consume plankton and organic materials by filtering the water from the surroundings. Oyster's meat is rich in nutrient especially protein (16.8%) followed by vitamin A and B complex. The meat can be eaten raw, fried, or marinated.

*Vibrio parahaemolyticus* is a halophilic gram negative curved rod which needs brackish salt water to live. The clinical manifestations of infection with this organism are gastroenteritis such as watery diarrhoea, abdominal cramps, nausea and vomiting due to the toxin produced by the organism. Since vibrio shares the same environment as oysters, it has the potential to be present in oysters and hence there is a possibility of gastrointestinal infection in consumers. A recent report by Paydar *et al* (2013), 29% of raw seafood including

oysters in Malaysia was found to have *V. parahaemolyticus* [1].

In Malaysia and many other countries, eating raw oysters with squeezed lemon juice (*Citrus limon*) has been considered as an established delicacy. Not only rich in vitamin C, lemon juice also has the beneficial chemical content known as bioflavonoids. Miyake *et al.* (1998) reported that bioflavonoids in citrus have many properties such as antioxidant, anti-inflammatory and anticancer [2]. In addition, Tassou *et al.* (1996) found that bioflavonoids also play an important role as broad spectrum antimicrobial agents to many virulent organisms such as *Staphylococcus aureus* and *Salmonella enteritidis* [3]. In another study, Tomotake *et al.* (2006) applied lemon juice directly on *V. parahaemolyticus* and found that it inhibited the growth of the bacterium [4]. To date, there have not been any reports on whether lemon juice squeezed into raw oysters may kill *V. parahaemolyticus* in oysters. This study aims to determine the effect of lemon juice

on viable *V. parahaemolyticus* in raw oysters by spiking the oysters with infective dose of *V. parahaemolyticus*.

## METHODS

### Bacterial Strain

*Vibrio parahaemolyticus* ATCC strain 17802 was used in this study. A suspension of  $10^7$  colony forming unit per ml (CFU/ml) of overnight culture was prepared in phosphate buffer saline (PBS, pH 7.2) according to the standard method of the Clinical Laboratory Standard Institute [5].

### Preparation of Lemon Juice

A medium sized lemon was surface-sterilized with 70% alcohol before being aseptically cut and squeezed. The pH of the lemon juice was determined using litmus pH indicator strip (Merck, USA).

### Preparation of Oyster Samples

Frozen oysters of average weight of 6 g each, were bought from a seafood supplier in Batu Caves, Kuala Lumpur. They were transported in ice to our laboratory and kept frozen at  $-20^{\circ}\text{C}$  until used. Prior to the experiments, two oysters were thawed and checked for the presence of *Vibrio* spp. or other bacteria by culturing on thiosulphate citrate bile sucrose (TCBS) agar, blood agar and MacConkey agar. The oyster meat was minced by using scalpel in an aseptic technique for the following experiments. No growth of microorganisms was detected on the three types of culture media used.

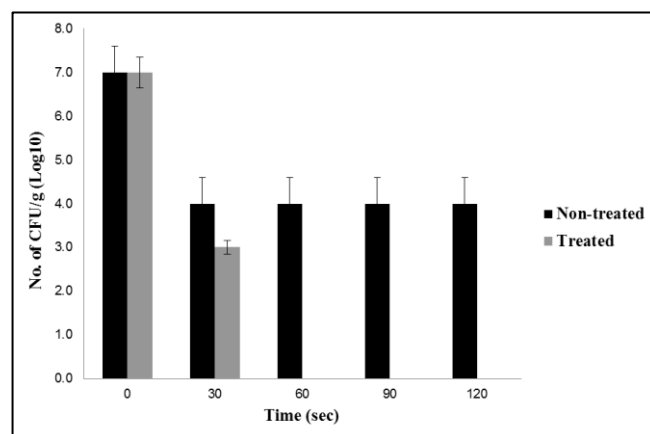
### Determining Inhibitory Effect of Lemon Juice on *Vibrio parahaemolyticus* in Raw Oysters

Two universal bottles containing 6 g of minced oyster's meat each were added with approximately  $10^7$  CFU of *V. parahaemolyticus*. Subsequently, 1 ml of lemon juice (average pH 2.1) was added to one of the bottles (treated sample) while the other bottle (control) was not treated with the lemon juice. Both the treated and control samples were left at room temperature for 2 min. At every interval of 30 s up to 2 min, a scoop of the meat was taken and weighed to get 1 g. It was then added to 100  $\mu\text{l}$  of PBS and serial dilutions were performed for enumeration of viable *V.*

*parahaemolyticus* on TCBS agar. Following 24 h incubation, the number of viable *V. parahaemolyticus* was counted using a colony counter. The whole experiment was repeated once and the viable count was averaged out.

## RESULTS

From this study, it was observed that after 30 s of treatment with lemon juice, the number of viable *V. parahaemolyticus* was reduced by 4 Log compared to 3 Log in the control. When the oysters were treated for 60 s, none of the organisms were detected by culture method in comparison to untreated samples in which the bacterium was found to be viable even at the end of the experiment. It was also observed that the number of vibrio in the untreated sample reduced to 3.5 Log CFU, 30 s after spiking the oysters with the bacterium (see Figure 1).



**Figure 1** Average number of viable *Vibrio parahaemolyticus* in oyster's meat after treatment with lemon juice. Each error bar represents standard error means

## DISCUSSION

The reduction in the viability of the bacterium seen this study can be attributed to the acidity of the lemon juice. A study by Tomotake *et al.* (2006) reported that *Vibrio* strains, particularly *V. parahemolyticus*, are very sensitive and dies readily at pH less than 4.5 but become tolerant to acidity and can grow at pH higher than 4.5 [4].

This finding supports the inhibitory effects of lemon juice on *V. parahemolyticus*. Being a halophilic and mesophilic organism, some of *V. parahaemolyticus* do not survive long because to survive, the bacterium requires brackish water which is

usually equivalent to 1-9% NaCl and temperature of 20°C for optimum growth [6]. We postulate that there are other factors which may have contributed to the inhibition of the growth of this organism such as the antimicrobial properties contained in oysters. Oysters contain defensive blood cells (haemocytes) which can kill *Vibrio* sp. in addition to histone H2B protein, which provides defense against gram-negative bacteria [7].

This experiment was conducted to simulate the condition where oysters are served in many restaurants in Malaysia. In most restaurants and hotels, oysters are usually served with the upper shell removed and the flesh in the lower shell placed on ice cubes or flakes. Slices of lemon are also served to complement the oysters. Not only the lemon juice adds flavor to the oyster, it also acts in a way to ‘cook’ the oyster. The inoculation with 10<sup>7</sup> CFU of *V. parahaemolyticus* into the oysters was to simulate the infective dose of this organism in causing gastroenteritis in food poisoning [8].

## CONCLUSION

This study showed that lemon juice has some inhibitory effect on *V. parahaemolyticus* in raw oysters. Since there was also some reduction of *V. parahaemolyticus* count in untreated samples, it is likely that other intrinsic properties of the oysters also contribute to the inhibition of *V. parahaemolyticus* in raw oysters.

## Conflict of Interest

Authors declare none.

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