

# Effects of Temperature on Growth and Survival of Pathogenic *Vibrio Parahaemolyticus*

Haiyan Zhang<sup>1</sup>, Cong Kong<sup>2</sup>, Yuan Wang<sup>3</sup>, Xiaosheng Shen<sup>\*4</sup>

<sup>1,2,3,4</sup> East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences  
No.300, Jungong Road, Shanghai City, China

<sup>1</sup> zhanghaiyan\_369@163.com; <sup>\*4</sup>foodsmc98@126.com

## Abstract

This study investigated the effects of temperature on the survival of *Vibrio parahaemolyticus* during cold storage and heating process. *V. parahaemolyticus* enriched in sterile alkaline peptone water (APW) supplemented with 1.5% NaCl (APW-salt broth) at 37°C overnight (12-16h) was subjected to cold storage (-30, -18, 0, 5, 10, 15 and 20°C) and heating treatment (50, 55, 60, 70, 80, and 90 °C) at various temperatures. The populations of *V. parahaemolyticus* were determined before and after the treatment. Results showed that *V. parahaemolyticus* in APW-salt broth increased rapidly when temperature was higher than 15 °C, while decreased gradually at 0 and 5 °C. Freezing treatment could greatly decrease the population of *V. parahaemolyticus* in APW-salt broth. Storage at -18 °C was more effective in inactivating *V. parahaemolyticus* than that at -30°C. Keeping *V. parahaemolyticus* frozen at -18 and -30 °C from 15 to 30 days could reduce the pathogen to non-detectable levels (<3 MPN/g). Heating at 60 °C for 5 min, 70 °C for 2 min, or 80 °C or higher for 1 min also reduced *V. parahaemolyticus* from 10,000 MPN/g to non-detectable level. In conclusion, *V. parahaemolyticus* did not survive well during frozen treatment or low temperature storage (<5 °C). In addition, heating treatment (at 60 °C) is also effective to inactivate *V. parahaemolyticus*. This study can be applied to reduce risks of infections associated with foodborne pathogen *V. parahaemolyticus*.

## Keywords

*Vibrio Parahaemolyticus*; Survival; Cold Storage; Heating Treatment

## Introduction

*Vibrio parahaemolyticus* is a Gram-negative, halophilic bacterium (Baumann and Schubert, 1984) and widely distributed in marine environments including water, sediment and seafood (Liston, 1990). This organism is the most prevalent foodborne pathogen in China (Chen *et al.*, 2007), which accounted for 31.1% of all

bacteria food poisoning events in thirteen provinces between 1992 and 2001 (Liu *et al.*, 2005). It has also been recognized as a leading cause of diarrhea associated with seafood consumption throughout the world including Japan, Canada and the United States (Su and Liu, 2007). Thermal treatment and cold storage (including refrigeration, icing and freezing) are the most effective means to destroy or limit the growth of foodborne pathogens including *V. parahaemolyticus*. This study investigated the fate of both virulent and non-virulent strains of *V. parahaemolyticus* in a multi-culture strain cocktail during chilled and frozen storage, and heating treatment at different temperatures.

## Materials and Methods

### Bacteria Cultures.

Two pathogenic strains of *V. parahaemolyticus* possessing *tdh* gene (VP33846 and VP 33847) were used in this study. The strains obtained from Microbiology Institute of Chinese Academy of Sciences, Beijing, China. Each culture was individually grown in sterile alkaline peptone water (APW) supplemented with 1.5% NaCl (APW-salt broth) at 37°C overnight (12-16h) and a two-strain cocktail culture suspension of *V. parahaemolyticus* was prepared according to our previous study (Shen *et al.*, 2009; Thompson and Thacker, 1973).

### Effects Of Temperatures On The Populations Of *V. Parahaemolyticus* During Chilled And Frozen Storage

*V. parahaemolyticus* in APW-salt broth was stored at the different temperatures (-30, -18, 0, 5, 10, 15 and 20 °C). Populations of *V. parahaemolyticus* in the broth were determined before and after storage using the pour-

plate method.

### Survival Of *V. Parahaemolyticus* During Heating Treatment

0.1 mL of fresh prepared *V. parahaemolyticus* cocktail suspension was added into a tube containing 9.9 mL of sterile APW-salt broth which was tempered at the set temperature (50, 55, 60, 70, 80, or 90 °C) in a water bath, and then continuously was incubated at the same temperature for a certain period of time (1, 2, 3, 5, 10 min). After the required contact time, the tubes were transferred to iced water to stop the action of the heat as quickly as possible. Populations of *V. parahaemolyticus* in the broth were determined before and after heating by using the pour-plate procedures.

### Microbiological Analysis

*V. parahaemolyticus* counting in culture suspension was detected by the pour-plate method using nutrient agar (Shanghai Reagent Providing and Research Center for Diarrheal Disease Control, Shanghai, China) supplemented with 1.5% NaCl.

### Results and Discussions

#### Effects Of Chilled Storage On Populations Of *V. Parahaemolyticus* At Different Temperatures

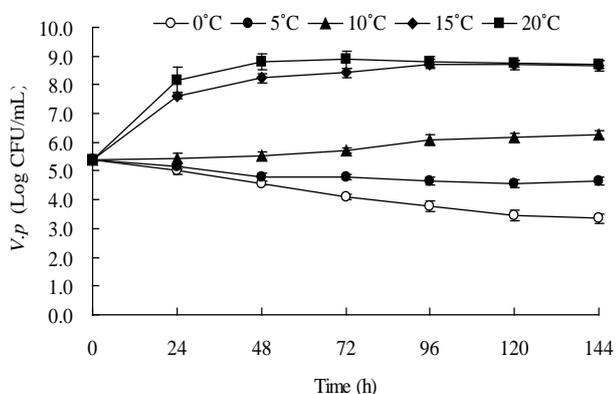


FIG.1 CHANGES IN POPULATION OF *V. PARAHAEMOLYTICUS* DURING STORAGE AT VARIOUS TEMPERATURES (0-20°C)

Figure 1 showed that population of *V. parahaemolyticus* in APS broth increased rapidly when temperature was higher than 15 °C, but decreased gradually at 0 and 5 °C. The initial number of *V. parahaemolyticus* in APS broth was 5.37 Log<sub>10</sub> CFU/mL, which increased by 3.28 and 3.34 Log<sub>10</sub>CFU/mL after storage for 144 hours at 15 and 20 °C, but decreased by 2.01 and 0.71 Log<sub>10</sub>CFU/mL after storage at 0 and 5 °C, respectively. At 10°C, *V. parahaemolyticus* multiplied slowly and its density increased by only 0.71 Log<sub>10</sub>CFU/mL after 144h storage that could be considered static for the

most time. These results were partly supported by previous studies. *V. parahaemolyticus* in oyster homogenates could decrease rapidly from the initial level (10<sup>3</sup> cells) to non-detectable level after storage at 0-4 °C; while multiplication was found to occur at 10-12 °C, but not at 8 °C (Thompson and Thacker, 1973). *V. parahaemolyticus* in crab meat decreased rapidly at 5 °C, with 5 log reduction observed after storage for 14 days (Ray, Hawkins and Hackney, 1978) and that on fish fillets could decline by 1-2 log<sub>10</sub> CFU/fillet after storage at 4-8 °C for 9 days (Vasudevan *et al.*, 2002). Therefore, keeping cold (< 10 °C) was an effective method to limit the growth of *V. parahaemolyticus* in foods. For reducing *V. parahaemolyticus* contamination in foods, the cold temperatures should be maintained from shipping, retail and home refrigeration.

#### Effects Of Freezing Treatment On The Survival Of *V. Parahaemolyticus*

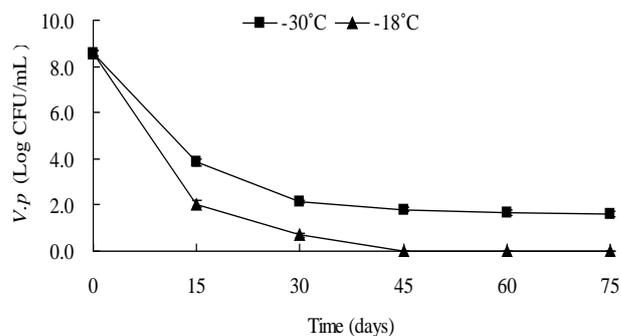


FIG.2 SURVIVAL LEVELS OF *V. PARAHAEMOLYTICUS* DURING FROZEN STORAGE AT DIFFERENT TEMPERATURES (-18,-30°C)

In addition to chilling, freezing is also a common method used to preserve food quality and enhance food safety. Figure 2 showed freezing treatment could decrease the population of *V. parahaemolyticus* in APS broth. Freezing the culture suspension of *V. parahaemolyticus* at -18 and -30 °C for 15 days could decrease the bacteria population from the initial level 8.59 to 2.04 and 3.84 Log<sub>10</sub>CFU/mL (with 6.55 and 4.75 log reductions), respectively, and continued to decline at a slower rate even to undetectable level during the storage. Storage at -18 °C was more effective in inactivating *V. parahaemolyticus* than that at -30°C. This might be explained by the fact that the bacterial cell damage caused by ice crystals at -18 °C was more serious than that at -30 °C since intracellular ice crystals formed in bacterial cells at a higher freezing temperature (-18 °C) were bigger than those at a lower temperature (-30 °C) (Jay, Loessner and Golden, 2005). Similar phenomena were also found in inoculated oysters (Liu and Su, 2009) and fish fillet (Vasudevan *et al.*, 2002). These results suggested that frozen storage

should also be an effective post-harvest treatment for reducing *V. parahaemolyticus* contamination in foods.

#### Effects Of Heating Treatment On Survival Of *V. Parahaemolyticus*

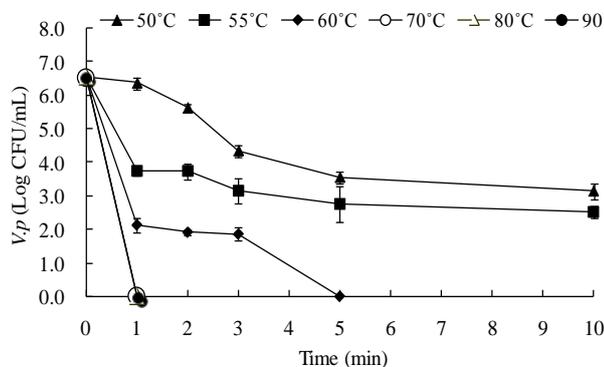


FIG.3 INACTIVATION OF *V. PARAHAEMOLYTICUS* DURING HEATING TREATMENT AT VARIOUS TEMPERATURES (50-90°C)

Figure 3 showed heating treatment was very effective to reduce *V. parahaemolyticus* level in APW-salt broth and the number of *V. parahaemolyticus* decreased rapidly upon heating at  $\geq 50$  °C. However, heating the culture broth at 50 or 55 °C for 10 min could not completely inactivate this organism, for  $10^{3-4}$  CFU/mL viable cells were detected after the 10 min heat treatment. Heating the culture cocktail at higher temperatures (60, 70, 80, 90 °C) could achieve greater than 6-log reduction, no viable cells were detected in the culture broth at 60 °C for 5 min and at 70 °C or higher for 1 min. Although Andrews *et al* (2000) (Andrews, Park and Chen, 2000) reported the 50 °C water heat treatment of inoculated shellstock oysters for 10 min could reduce the numbers of *V. parahaemolyticus* from  $10^5$  MPN/g to non-detectable level, the result of this study demonstrated that *V. parahaemolyticus* possessed high heat resistance. Heating *V. parahaemolyticus* in APW-salt broth at 50 °C for 10 min in our study could only achieve 3.41 log reduction. More than  $10^3$  viable cells of *V. parahaemolyticus* were still detected both in culture broth at 50 °C for 10 min. Vanderzant and Nickelson (1972) also reported that *V. parahaemolyticus* in shrimp homogenates showed higher heat resistance (Vanderzant and Nickelson, 1972) than that in shellstock oysters (Andrews, Park and Chen, 2000). Therefore, for full inactivation of *V. parahaemolyticus* in foods, cooking in hot water at 60 °C for 5 min, 70 °C for 2 min, 80 °C or higher for 1 min was recommended.

Although eating raw or cooked food is a matter of

personal preference, there are still a lot of people who like eating seafood raw or undercooked due to the special flavors of raw seafood. For example, in the United States today, there is a high demand for raw oysters on the half-shell (shooters) typically served at oyster bars. In China, clams are most often cooked before consumption, but undercooked is highly possible since the clams are just quickly boiled in hot water. These eating habits pose health risk because food borne pathogens including *V. parahaemolyticus* may survive in seafood. It is estimated that >60% of seafood-associated illness could be avoided if consumers would stop eating raw or undercooked molluscan shellfish (Liston, 1990). People at high risk groups, for example, young children, the elderly, pregnant women, and those in poor health conditions, should not eat raw or undercooked seafood including oysters and clams to reduce the potential risks of life-threatening disease caused by Vibrio infection.

#### Conclusions

In summary, cooling foods down to < 10 °C quickly after harvest and keeping them below 10 °C was effective to prevent *V. parahaemolyticus* from rapid multiplication. For reducing *V. parahaemolyticus* contamination in foods, cold storage at 0-5 °C was highly recommended. In addition, freezing was also an effective treatment to inactivate *V. parahaemolyticus*. Keeping foods at the range between -18 °C for 15-45 days could reduce *V. parahaemolyticus* contamination from 100 MPN/ml to non-detectable levels. Finally, cooking at 60 °C for 5 min, 70 °C for 2 min, 80 °C or higher for 1 min could reduce *V. parahaemolyticus* contamination from  $10^6$  MPN/ml to non-detectable level.

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