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EFFECT OF VARIOUS PROCESSING ON THE DIE-OFF OF PATHOGENIC BACTERIA INFECTING DONAX TRUNCULUS

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ABSTRACT

The effect of heat treatments, deep-frying, salting and addition of citric acid on the survival of Aeromonas hydrophila, Vibrio parahaemolyticus and Salmonella teshwinge on Donax trunculus (Om el kheloul) was studied. Both boiling and deep-frying destroyed the three organisms. Heating Om el kheloul for 5 min at 60°C did not destroy any of these bacteria. ***Vibrio parahaemolyticus*** *can survive at 2, 4, 6 and 8% salt concentration for 24 h. While* ***Salmonella*** *and* ***Aeromonas*** *endured can survive only at 2 and 4% NaCl. All tested bacteria were unable to survive at pH below 5.5.* ***Salmonella*** *was the only organism, which was reisolated after 24 h at pH 6.*

INTRODUCTION

Edible bivalves are often reported to be responsible for human diseases of bacterial origin, mainly due to pathogenic enteric bacteria. (Prieur ***et al.***, 1990, West 1989). The microflora of living fish and shellfish depends upon the microbial content of the water in which they live (West 1989 and El-Shenawy & El-Shenawy, 1995). Fish and crustaceans carry populations of predominant Gram negative psychrotrophic bacteria on their external surfaces, while the internal tissues and blood systems of healthy fish and crustaceans are usually sterile. (Morries ***et al.***, 1976 and Plusquellec, ***et al.***, 1983)

Aeromonas hydrophila considered to be the causative agent of a variety of systemic and localised diseases in both poikilothermic and homeothermic animals including humans (Davis ***et al.***, 1978 and Soliman, 1988 and El-Shamy ***et al.***, 1993).

Vibrio parahaemolyticus is an enteropathogenic. It has been implicated in food poisoning outbreaks associated with seafood in several countries. More than 70% of the cases of food poisoning in Japan are caused by ingestion of seafood, i.e., fish and shellfish, contaminated with ***Vibrio paraheamolyticus*** (Sakazaki ***et al.***, 1963 and Anand ***et al.***, 1981).

Furunculosis is a well-known bacterial disease of Salmonidae (Soliman 1988). Salmonellosis still constitutes a serious threat to human being and animals especially in the developing countries where the environmental sanitation is of low standard. Those diseases are prevalent and endemic in tropical and subtropical areas in which the agent is usually transmitted by food or water (Mc Caskey and Antony, 1979, Plusquellec, ***et al.***, 1990.)

The incidence rate of enteric fever in Egypt during the years 1978 to 1980 was over 122 per million which is a high figure in comparison to the more developed countries (WHO, 1983). The presence of large number of viable counts of Coliforms, Enterococci and ***Vibrio parahaemolyticus*** in examined fish and shellfish indicate that they carry infections when caught in polluted water of rivers, lakes or even sea which is now used for sewage disposal. So the presence of human enteric pathogens in fishes and shellfish may lead to human infection (Mousa, 1986).

In Egypt, Om el kheloul is one of the delicious edible bivalves, which consumed commonly either raw with a special sauce containing citric acid and salt (NaCl) or cooked. The present study aimed to obtain information about the effect of some traditional methods for processing Om el kheloul to kill the pathogenic gram-negative bacteria infecting it.

MATERIALS AND METHODS

Om el kheloul

About four and half kg of living Om el kheloul (***Donax trunculus***) were caught from Edko Lake. They were kept during the periods of acclimatisation (7 days) and through the experimental period (2 days) in three glass aquaria. Each aquarium contained about one and half kg of Om el Kheloul (about 50- 55 organisms). Volume of water in each aquarium was 20 litres of brackish water. The water was filtered and aerated at temperature of 25 ± 1°C.

Bacterial strains

Three pathogenic strains were used in this study. They were obtained from Fish Disease Laboratory, Dept. of Avian & Aquatic Animal Medicine, Fac. of Vet. Med. Alex. Univ.

a) Strain A 5 of ***Aeromonas hydrophila*** was isolated from ***Mugil capito*** liver and identified as described by Popoff and Veron (1976) and Soliman (1988).

b) Strain V115 of ***Vibrio parahaemolyiticus*** was isolated from bivalve and was identified according to Field, (1979).

c) ***Salmonella teshwinge*** was obtained from cloacal swabs from duck and identified according to Kuffman white scheme, described by Edwards and Ewing (1972).

Inoculation:

Bacterial suspensions containing 12 x 10^8 C.F.U./ml (colony forming units) were prepared for each of the studied strains in physiological saline. Eight ml of each was added to the three different Aquarium.

Processing technique:

During the bacterimia phase (which proved by reisolation of inoculated bacteria from Om el kheloul tissue after 48 hrs), the organisms were submitted to the following treatments: -

1- Blanching was in boiling water for 1, 2, 3, 4 and 5 minutes.
2- Heat treatment for 5 min. in hot water at different temp. namely 50, 60, 70, 80 and 90°C.
3- Deep frying in cottonseed oil at 150°C for 1, 2, 3, 4 and 5 minutes.
4- Salting immersed for 24 hrs in 0, 2, 4, 6 and 8% (w/v) brine NaCl solutions.
5- Citric acid treatment at various pH values (4.5, 5, 5.5, 6 and 6.5).

Reisolation technique:

Reisolation of the bacteria from the infected muscular tissue of Om el kheloul was done. About one gram from the tissue was ground in sterile mortar with one ml sterile saline. A loopful from the mixture was streaked on Rimer & Schotts media (RS) and nutrient agar containing 0/129 Vibrio static agent (0/129 medium). After 24 h incubation at 30°C, the plates of RS media were examined for the presence of yellow orange colonies (the specific colonies of Aeromonas stated by Rimer & Schotts, (1973). Plates of 0/129 medium were tested for growth (Aeromonas can grow while Vibrio cannot as stated by Schotts and Bullock, (1975).

Colonies grown on the 0/129 medium were tested for the presence/absence of oxidase and catalase activity.

In case of Salmonella, the pre-enrichment technique according to Harvey and Price (1980) was followed. Samples were streaked on MaConkey agar, plates and incubated at 37°C for 24h. Plates were examined for the presence of non-lactose fermenting colonies.

RESULTS AND DISCUSSION

Before examination: -

Om el kheloul and water in aquaria were previously checked for the presence of tested bacteria and proved to be negative. The results of reisolation of V. ***parahaemolyticus***, ***A. hydrophila*** and ***S. teshwinge*** from Om el kheloul tissues that were subjected to various treatments are presented in Table (1). The results show that deep-frying and blanching destroyed the three pathogenic organisms. It can be noticed that ***V. paraheamolyticus***, ***A. hydrophila*** and ***S. teshwinge*** were only isolated at 50 and 60°C after 5 min. heat treatment. These

may be explained by the ability of the microorganisms to survive at these heat treatments, or that the temperature is insufficient to destroy the bacteria inside Om el kheloul. El-Shenawy ***et al.***, (1993)& Rouf and Rigney, (1971) stated that, the mesophilic types of ***Aeromons*** have growth temperature range from 10 to 55°C. Temmyo (1966) reported that ***V. parahaemolyticus*** was destroyed after heating at 55°C for 10 min or at 60°C for 5 min. in peptonewater media.

Table (1): Results of reisolation of ***Vibrio-Parahaemolyticus, Aeromonas hydrophila*** and ***Salmonella teshwinge*** from muscular tissues of Om el kheloul after five types of processing.

Type of Treatment / Bacteria Strain	Frying (min.) 1-2-3-4-5	Temp.°c for 5 min. 50-60-70-80-90	Blanching (min.) 1-2-3-4-5	Salting (NaCl %) 0-2-4-6-8	Citric acid treatments (pH values) 4.5-5-5.5-6-6.5
Vibrio parahaemolyticus	- - - - -	+ - - - -	- - - - -	+ + - + +	- - - - - +
Aeromonas hydrophila	- - - - -	+ - - - -	- - - - -	+ + - - -	- - - - - +
Salmonella teshwinge	- - - - -	+ - - - -	- - - - -	+ + - - -	- - - + +

\+ = Positive.
\- = Negative.

In case of salting ***Vibrio*** was isolated after treatments with 2, 4, 6 and 8% NaCl. While ***Aeromonas*** and ***Salmonella*** were isolated only from 0, 2 and 4% NaCl. Kaneko and Gowell (1973) observed a considerable reduction in the propagation of ***V. parahaemolyticus*** when it was grown on nutrient medium containing 10% NaCl. Ro and Woodburn (1976) studied the survival of ***V. parahaemolyticus*** in oysters salted at three levels (3%, 6.8% and 10.6%) and observed that the highest survival rate was attained at the highest salt concentration. Graikaski (1973) isolated ***Salmonella*** from pickle-salted Mugil.

All three tested organisms were unable to survive at pH 4.5, 5 and 5.5. ***Salmonella*** was the only organism (out of the three organisms) which was reisolated after 24h incubation at pH 6 (room temp). The data agree with those obtained by Vanderzand and Nickelson (1972), who mentioned that ***V. parahaemolyticus*** was very sensitive to pH values below 6.0 in shrimp homogenates.

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