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Detection and Survival of *Vibrio* Species in Shrimp (*Penaeus indicus*) and Mussel (*Mytilus galloprovincialis*) at Landing and after Processing at Seafood Markets in Suez, Egypt

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ABSTRACT

This study was carried out to determine the safety of shrimp and mussels at landing and after post-harvest processing commonly carried out at markets in Suez. Fresh mussels and shrimp samples were collected from seafood Suez markets and were microbiologically evaluated raw or after have been subjected to one of the following treatments; steaming at 80°C for 5 min, chilling at 4°C for 24 hrs, or freezing at -5.0 ± 1.0°C for 24 hrs. Vibrio species including *Vibrio alginolyticus, V. fluvialis,* and *V. cholera* were identified in both samples. *Vibrio alginolyticus* was the most prevalent species in raw mussels (20%) and shrimps samples (20%). Post-harvest processing improved the safety of the final products. To estimate the efficacy of these processing treatments on *V. alginolyticus* in shrimp and mussels, the materials were inoculated with the bacteria at 7 Log CFU/g. Steaming eliminated the microbiological load of mussels to undetected level and reduced *V. alginolyticus* count in shrimps to < 1 Log CFU/g estimated. Chilling and freezing had a limited reduction of about 1 and 2 Log reductions, respectively, in the samples after 24 hrs.

INTRODUCTION

Raw and undercooked bivalve molluscs and shrimps are filter feeder; which represent an important vector of infectious agents due to their ability to concentrate pathogens. Vibrio species are microorganisms that are naturally and widely distributed in marine and estuarine environment and have been reported in seafood worldwide (Baffone *et al*., 2000). Most Vibrio species are halophilic and require 0.5–3% NaCl for optimal growth. Vibrio species are natural habitants of seawater, therefore majority (56.7%) of *Vibrio* food poisoning outbreaks are caused by the consumption of seafood (Farmer and Janda, 2004). Food and Environmental Hygiene Department, (2005) reported that crustaceans including shrimps, lobster, and others represented the highest percentage (28.7% of the 313 confirmed *Vibrio* foodborne outbreaks) of seafood outbreaks during 1999 to 2003 in Hong Kong.

V.parahaemolyticus, *V. vulnificus*, and *V. cholerae* are recognized as important food-borne pathogens, which can cause serious illness upon the consumption of raw or undercooked seafood (Cottingham *et al.*, 2003and Su and Liu, 2007). Several reports of foodborne or waterborne infections caused by other Vibrio species that cause severe diarrheal diseases such as *V. fluvialis* (Tacket *et al*., 1982 and Lu *et al*., 2014), *V. algenolyticus* (Gómez-León *et al*., 2005), and *V. mimicus* (Shandera *et al*., 1983).

V. alginolyticus has been categorized as a human pathogen since 1979 and an emerging foodborne pathogen of concern (Mustapha *et al*., 2013). Many surveys around the world have identified this species of *Vibrio* to be one of the most commonly isolated in shellfish and shrimps (Lhafi and Kühne, 2007; Su and Liu, 2007; Lafisca *et al*., 2008 and Adeleye *et al*., 2010) and has been shown to result in food poisoning (Li *et al*., 2009). In immunocompromised patients, infection with *V. alginolyticus* is fatal (Campanelli *et al*., 2008). In Korea, (Dong-Young *et al*. (2008) reported that *V. alginolyticus* caused septic shock in a cirrhotic patient. *V. alginolyticus* is related to *V. parahaemolyticus* as it possesses a thermostable direct hemolysin-related hemolysin (*trh*) gene previously reported only in *Vibrio parahaemolyticus* (González-Escalona *et al*., 2006). *V. alginolyticus* inhabits wide population of seafoods including fish, clams, crabs, oysters, mussels, and shrimp (Adeleye *et al*., 2010).

Harvesting and post-harvest handling of seafood should meet specified criteria to prevent multiplication of vibrios to potentially hazardous levels. Acceptable post-harvest handling processes for shellfish and shrimp include freezing, cold treatments, and mild-heat treatment or steaming (Cook and Ruple, 1992). *V. parahaemolyticus* and *V. vulnificus* counts in oysters subjected to mild heat treatment and freezing, were about 5 to 6 logs less than those in unprocessed raw oysters (DePaola *et al*., 2009). These post-harvest processes have been widely used in seafood markets by virtue of its overriding safety, prolonged quality, and economic benefits.

Mishandling and other uncontrolled factors could affect the quality and safety of seafood in markets, even with the application of post-harvest processes (Ye *et al*., 2013). Depending on methods of transportation and storage, seafood can be exposed to a variety of temperatures prior to any post-harvest process treatment (NSSP, 2015). The harvest water temperature can vary depending on the time and location of harvest. Shellfish and shrimp may be left on harvest vessels exposed to the sun without cooling for several hours or until the vessel docks. Moreover, they may also be held in cold seawater for up to 1 day in markets prior to selling (Zhang *et al*., 2014). Therefore, *Vibrio* survival and safety of shrimp and shellfish upon landing and after post-harvest processes such as chilling, freezing, steaming and peeling need to be evaluated from a safety point of view.

The objectives of this study were; i) to estimate the prevalence of Vibrio species in seafood harvested from Suez gulf and sold in Suez markets, ii) to investigate the safety of post-harvest processing of mussels and shrimp at retail markets in Suez that including refrigeration, freezing, and steaming as simple commercial methods used for peeling these seafood products and prepare them for sale, and iii) to determine the effect of steaming, chilling, and freezing processes on the inactivation of *V. alginolyticus* in inoculated mussels and shrimp samples.

MATERIALS AND METHODS

Materials

Shrimps and mussels (10 kg each) were collected from fresh market at Suez Government, Egypt. They were stored on ice that prepared in the lab from distilled

deionized water. Samples were then transported in sterilized cooler to the lab and processed immediately.

Methods

A total of 105 samples were carried out including 54 shellfish samples of mussels (*Mytilus galloprovincialis*), and 51 shrimp samples. Samples were randomly allocated to 4 treatment groups; Group 1 (raw): Mussels were scrubbed and washed under running water. Mussel bodies and intervalve water were aseptically removed chopped and a pooled sample (5 g) was prepared (n = 20). Shrimps were decapitated and a 25 g sample of pooled bodies was transferred into sterile bags (n = 15) under aseptic conditions and analyzed immediately. Group 2 (steamed): mussels (n = 16) and shrimps (n = 18) were steamed at 80°C for 5 min and treated as described above. Group 3 (chilled): mussels (n = 9) and shrimps (n = 9) were refrigerated at 4±1°C for 24 hrs as a mean to keep the quality of the product for short-time storage. At the end of the designated storage time, the samples were removed and treated as described for group 1. Group 4 (frozen): mussels (n = 9) and shrimps (n = 9) were frozen at -5±1 °C for 24 hrs. Samples then were taken out from the freezer and treated as described for group 1.

Cultural isolation and biochemical identification of Vibrio species

The mussel samples were transferred into a sterile bag with 45 ml of alkaline peptone water (APW, pH 8.6) (lab M, UK) containing 1% NaCl. The shrimp samples were transferred into a sterile bag with 225 ml of APW (lab M, UK) of pH 8.6, containing NaCl 1%. Samples were homogenized for 2 min using a stomacher (Stomacher® 400 Circulator, Seward UK) and incubated at 37°C for 24 hrs.

Plating of enrichment culture onto thiosulphate-citrate-bile salts-sucrose (TCBS) (Lab M, UK) agar plates with NaCl 1% was carried out after incubation at 37°C for 24 hrs. The green / blue-green, and yellow colonies on TCBS agar plates, presumptively selected as Vibrio colonies, were transferred to Trypticase Soy Agar (TSA) plates (Lab M, UK) containing 2% NaCl. After incubation at 37°C for 24 hrs, the isolates were subjected to biochemical identification (FDA, 1998; Angela *et al*., 2008).

Gram staining and microscopic examination were performed to recognize gram-negative, pleomorphic organisms exhibiting curved or straight rods with polar flagella. Motility test was performed by stabbing the column of the motility test medium (lab M, UK) to a depth of approximately 5 cm, after incubation overnight at 36°C. A circular outgrowth from the line of stab represented a positive test.

Biochemical tests were used for species identification including, oxidase reaction, motility, salt tolerance, and API 20E diagnostic strips and Arginine glucose slant (AGS, lab M, UK). AGS was incubated with loose cap overnight at 35° ±2°C. Alkaline (purple) slant indicative for sugar fermentation and an acid (yellow) butt indicated as arginine is not hydrolyzed. The ability of the isolated bacteria for growth at different salt concentration was tested at 0, 3, 6, 8, and 10 % (W/V) NaCl and the c the mix was incubated at 35° ±2°C for 24 hrs. Oxidase reaction was performed by transfer the incubated culture growth to a filter paper saturated with the oxidase reagent, dark purple color within 10 sec was considered as a positive test. Species were identification according to FDA bacteriological analytical manual (BAM, 2004). Further identification was done using API 20E diagnostic strips (Biomérieu, France) where culture suspensions in 2% NaCl solution were used.

Identification of Vibrio species by PCR and 16S rRNA gene sequencing

Randomly selected samples were used for this technique either for confirmation of API results or for identification of unknown samples. The technique was performed according to Azwai1 *et al*. (2016). DNA extraction was done using bacterial DNA preparation kit (Jena Bioscience, Thuringia, Germany). Partial 16S rDNA was amplified using the universal oligonucleotides primers forward 5′-GAGTTTGATCCTGGCTTAG-3′ and reverse 5′-GGTTACCTTGTTACGACTT-3′. Briefly, 2 µl DNA templates (20 ng/ µl) was added to 12.5 µl Master Mix (Qiagen, Hilden, Germany) and 10.5 µl deionized H_2O for a total volume of 25 µl. The mixture was then amplified in a DNA Thermal Cycler (Techne Progene, Marshall Scientific, Hampton, NH) using the following program: one denaturation step at 94 °C for 5 min; 37 cycles (30s at 94ºC, 30s at 51 ºC, and 30s at 72 º C); and a final extension for 5 min at 72 ºC. Agarose gel (1.5%) with Tris-acetate-EDTA (1X, TAE) buffer was used for gel electrophoresis.

DNA Sequencing

Purification of the PCR products was performed using QIAquick Kit (Qiagen, Hilden, Germany). Second PCR was performed using BigDye Terminator v3.1 cycle sequencing kit. Each reaction (a total of 20 µL) contained a terminator ready reaction mix (8 µL), primer (3.2 pmol), DNA template quantized according to the PCR product size, and deionized water. Thermal profile for Cycle Sequencing PCR was 1 min at 96ºC; 25ºC cycles as follows: 10 s at 96ºC, 5 s at 50ºC, and 4 min at 60ºC. After an additional step of purification with CENTRI-SEP Columns (Princeton Separations, Freehold, NJ), DNA sequencing was carried out by 3500 Genetic Analyzer (Applied Biosystems, Massachusetts, USA). The obtained consensus sequences were subjected to BLAST search through the Mega program (7.0.20).

***V. alginolyticus* culture preparation**

The strain of *V. alginolyticus* used in this study was isolated from the shrimp, identified, and confirmed by PCR and sequencing of 16S rDNA. The bacteria were grown in 10 mL of trypticase soy broth containing 1.5% NaCl (TSB with salt) overnight at 37 °C. The enriched culture was streaked on TCBS plates and incubated at 37 °C for 24 hrs. A single colony formed on the TCBS plate was picked and enriched in 10 mL of TSB with salt at 37 °C for 24 hrs.

Inoculation of shellfish and shrimp with *V. alginolyticus* under hygiene measures

V. alginolyticus culture was inoculated to sterilized shellfish homogenates and shrimp homogenates to obtain a level of approximately 107–8 CFU/g. To prepare shellfish homogenates, mussels obtained from a local market shucked, and their bodies and fluid were homogenized by sterile stainless steel laboratory blender. The shrimp samples were treated in the same manner after been decapitated. Homogenates of mussels and shrimps were then sterilized at 121 °C for 15 min to eliminate naturally occurring bacteria. The sterilized homogenates (10 g) were transferred to sterile bags and inoculated with *V. alginolyticus* culture and were left at room temperature for 2 hrs. The inoculated samples

were treated as control samples (group 1). Samples were steamed at 80°C for 5 min (group 2), refrigerated at 4°C for 24 hrs (group 3), and frozen at 0°C for 24 hrs (group 4). All samples and processing techniques were carried out under aseptic conditions.

Bacterial count *of V. alginolyticus*

The viable count of *V. alginolyticus* in treated and untreated samples was carried out as described by Phuvasate and Su (2015) using the spread plate method with TCBS agar supplemented with 1% NaCl. The samples were serially (10-fold) diluted with sterile phosphate buffered saline, and 1 ml was plated on TCBS-salt plates and incubated at 35–37 °C for 24 hrs. Colonies were counted and results were reported as logs of colony forming units (CFU/g), and 3 replicate measurements were conducted for each treatment. The results are means ± standard deviations.

RESULTS AND DISCUSSION

Prevalence of Vibrio species and other pathogens

The yellow, and green / blue–green colonies on TCBS agar plates were isolated. Biochemical identification as shown in Table 1 with API 20E confirmation test identified. The prevalence for Vibrio species in the mussels and shrimp samples is shown in Table 2. *V. alginolyticus* was the most prevalent species in raw mussels (20%) and shrimp (20%) samples, followed by *V. fluvialis* (10% and 6.7% in mussels and shrimp, respectively), and *V. cholera* (5% and 6.7% in mussels and shrimp, respectively) with no further pathogenicity investigations. The prevalence of *V. alginolyticus* in our study was similar to the findings of several studies. Adeleye *et al* (2010) had reported that *V. alginolyticus* was the most predominant vibrio species (31.8% of 25 samples) in shrimps and crabs in Lagos, Nigeria. The incidence of *V. alginolyticus* in fresh seafood products were found to be 81.48% with greater isolation frequency was found for mussels (18.9%) (Baffone *et al*., 2000). Lafisca *et al*. (2008) reported *Vibrio alginolyticus* strains as the most predominant strain (32.4%) isolated from bivalves harvested in Italy (Venice Lagoon area), and Brazil (Guanabara Bay). *V. alginolyticus* was the species most frequently detected (51.2%) mong Vibrio isolated from mussel samples taken in seven shellfish-growing areas of the German Wadden Sea (Lhafi and Kühne, 2007). Neither *V. parahaemolyticus* nor *V. vulnificus* were found in this study.

Table 1. Biochemical characteristics of *Vibrio* species isolated from mussels and shrimp samples.

TCBS agar		*V. alginolyticus* Yellow	*V. cholerae* yellow	*V. fluvialis* yellow
oxidase		+	+	+
AGS		K/A	K/A	K/K
motility		+	+	+
Growth in (w/v):	0% NaCl	-	+	-
	3% NaCl	+	+	+
	6% NaCl	+	-	+
	8% NaCl	+	-	-
	10% NaCl	+	-	-
Api 20 E biochemical tests:				
ONPG		-	+	+
Arginine dihydrolase		-	-	+
Lysine decarboxylase		+	+	-
Ornithine decarboxylase		+	+	-
Citrate		-	+	+
H_2S		-	-	-
Urease		-	-	-
TDA		-	-	-
Indole		+	+	-
Voges-Proskauer		+	v	-
Gelatinase		+	+	+
Acid from:	glucose	+	+	+
	Mannitol	+	+	+
	Inositol	-	-	-
	Sorbitol	-	-	-
	Rhamnose	-	-	-
	sucrose	+	+	+
	Melibiose	-	-	-
	Amylose	-	-	-
	arabinose	-	-	+

KK = Slant alkaline / Butt alkaline, KA = Slant alkaline /Butt acidic.

Table 2. Prevalence of *Vibrio* species and other species identified in raw, steamed, chilled, and frozen mussels and shrimps samples collected from retail market in Suez, Egypt.

Vibrio species	Prevalence %							
	Raw		Steamed		Chilled		Frozen	
	mussel	shrimp	mussel	shrimp	mussel	shrimp	mussel	Shrimp
V. alginolyticus	20% (4/20)	20% (3/15)	18.8% (3/16)	16.7% (3/18)	22.2% (2/9)	11.1% (1/9)	11.1% (1/9)	22.2% (2/9)
V. fluvialis	10% (2/20)	6.7% (1/15)	6.3% (1/16)	5.6% (1/18)	11.1% (1/9)	11.1% (1/9)	ND (0/9)	ND (0/9)
V. cholera	5% (1/20)	6.7% (1/15)	ND (0/16)	ND (0/18)	ND (0/9)	ND (0/9)	ND (0/9)	ND (0/9)
Others								
Aeromonas hydrophila	-	6.7% (1/15)	-	-	-	22.2% (2/9)	-	11.1% (1/9)
Shewanella putrifaciens	25% (5/20)	6.7% (1/15)	-	5.6% (1/18)	-	-	-	-
Pseudomonas fluroroscens	5% (1/20)	6.7% (1/15)	-	-	-	-	-	-
Proteus mirabilis	-	6.7% (1/15)	-	-	-	-	-	-

values in parentheses indicate the number of samples that contained respective species out of the number of total samples analyzed in each group. ND: not detected

Other bacterial species with public health importance have been identified including; *Aeromonas hydrophila* and *Shewanella putrifaciens. Aeromonas hydrophila* was found in raw shrimp (6.7%). *Aeromonas hydrophila* is psychrotrophic that is associated with gastroenteritis and can cause traveler's diarrhea (Vila *et al*., 2003). *Shewanella putrifaciens* has been identified in raw mussels (25%) and shrimp (6.7%) samples. This bacterium has been associated with food poisoning and causes human infections such as cellulitis, abscesses, and bacteremia (Sharma and Kalawat, 2010).

Application of post-harvest processes

With the application of post-harvest processes in mussels, *V. alginolyticus* was most predominant in chilled (22.2%), followed by raw (20%), then steamed (18.8%), and frozen (11.1%) mussels samples, respectively. A similar order was found with *V. fluvialis*, it was most common in chilled (11.1%), and then raw mussels (10%), and steamed

(6.3%). In addition, in shrimp samples, *V. alginolyticus* was most prevalent in frozen (22.2%), followed by raw samples (20%), steamed (16.7%), and chilled (11.1%) samples. Although reports indicated Vibrio species are sensitive to cold, seafood can be protective for Vibrio species at refrigeration temperature. Vibrio species could survive low temperature for different storage times. The initial number of *V. parahaemolyticus* in alkaline peptone water supplemented with 1.5% NaCl broth was 5.37 Log10 CFU/mL, decreased by 2.01 and 0.71 Log10CFU/mL after storage at 0 and 5 ˚C for 144 hrs, respectively (Zhang *et al*., 2014).

Several Vibrio species such as *V. mimicus, V. fluvialis* strains, their survival in tryptic soy broth at 4°C and -30°C were tested. *Vibrio Fluvialis* decreased by about 2 log CFU/ml (from 6.69 ± 0.13 to 4.14 ± 0.18) when stored at 4°C and decreased to not detected levels -30°C after 5 days of test period. While, *V. mimicus* decreased from 7.69 ± 0.05 to 6.93 ± 0.07 at 4°C and to not detected level at -30 °C at the end of 5 days test time. (Wong *et al*., 1994). Chilling is capable of achieving reductions of Vibrio species in oysters, Thompson and Vanderzant (1976) reported that *V. parahaemolyticus* count in shucked oysters decreased after 7 days of storage at 3 °C from 11,000 to 0.36 MPN/g. Prolonged freezing could decrease *V. vulnificus* (from approximately 105 CFU/g to approximately 101 CFU/g) when inoculated in oysters at −20°C for 30 days (Parker *et al*., 1994). It is worth noting that *V. alginolyticus* grow well on culture media at 10 °C and 40 °C (mesophilic and psychrophilic environment) (Mustapha *et al*., 2013).

Steaming times, temperatures, hygiene measures are key factors for the safety of seafood. In this study, Vibrio species were recovered from shrimp and mussels steamed at 80°C for 5 min (table 1). Survival of Vibrio species even after steaming might be due to several factors such as high population of bacterial contamination. *V.parahaemolyticus* cells survived heating in shrimps at 60°C and even 80°C for 15 min with high population of 2x105 CFU/g, (Vanderzant and Nickelson, 1972). Time of processing exposure, in this study shrimps and mussels were exposed to steaming for 5 min. Prolonged time of application was associated with better results; West (1989) reported shellfish should be heated at least 10-15 min. to an internal temperature of 60°C to eliminate pathogenic Vibrio species. Uneven steam application might decrease the internal temperature of shrimps and mussels to less than 60°C, in this case it requires longer time of application. Ye *et al*. (2012) reported when oysters heated at low temperature (40, 45°C) for longer time (20 min) reduced *V. parahaemolyticus* and *V. vulnificus* by 0.7- 2.5 log MPN/g while at higher temperature (50°C) for only 15 min more than 7 log MPN/g of bacterial count were reduced. Because of previous factors Venugopal (1993) suggested combining other processing techniques accompanied with steaming for peeling shrimp such as dipping in 10% brine containing 5% sodium tri-polyphosphate followed by steaming the salted product for 10 min. The final products resist bacterial contamination for 25 d at 3±0.5°C. In addition, hygienic practices needed to be followed, avoiding contamination of product before and after steaming (NACMCF, 2008).

In the market, several poor hygiene practices were observed where mussels and shrimps were not completely covered with ice with some uneven distributions of ice. This practice might expose mussels and shrimps to summer weather temperature in Suez (35±2). Vibrio species can multiply rapidly between 20 and 40°C. Growth at 37°C can be very rapid with a short generation times of 9 to 10 minutes have been reported (ICMSF, 1996). Utensils where mussels and shrimps were contained in the market were not cleaned enough, as dusts and dark colored edges were observed. In confirmed *Vibrio* seafood poisoning outbreaks (313 cases) during 1999 to 2003, contaminated raw food accounted for 4.5% of the cases followed by contamination by utensil 2.2%, and improper holding temperature 1.9% (FEHD, 2005). Proper steaming, chilling and freezing are critical control measures to prevent growth of these microorganisms.

Application of low temperature such as chilling or freezing or high temperature such as steaming as the post-harvest process was intended to facilitate shucking and decapitating process in the market. Therefore, mussels and shrimps can be sold in easier and faster ways. For these purposes, post-harvest processes had short time applications. Time of application is considered as a very important factor, most of the reports indicated that time duration of at least 3 days was required to control the growth of Vibrio species in sea food. Su and Liu (2007) have shown that reducing the amount of Vibrio species in seafood can be achieved by cold storage (3°C) for 7 days. *V. parahaemolyticus* count in shellfish, and shucked oysters required at least 96 h storage at 5 ºC to be declined by 1.42 and 2.0 log MPN/g, respectively, and same time (96 h) storage at 0°C to decline 2.11 and 2.38 log MPN/g, respectively (Shen *et al*., 2009).

Handling or poor hygiene and sanitation during food production might give a chance for further multiplication of bacteria during and after application in shucking and decapitating action, due to human activity (WHO, 2015). In addition, sanitation levels and food handlers' hygiene in the market were not controlled. Therefore, contamination can quickly reach the infective dose evaluated 105 bacteria per gram, a few hours if kept in poor conditions (CDC, 1998). Yeung and Boor (2004) reported that the number of bacteria is 50 times higher in shellfish after 10 hrs storage at 26 °C and 760 times higher after 24 hrs at the same temperature. To maintain safety of seafood products some recommendations needed to be followed such as establish a clean-up and disinfection program to clean and sterilize equipment and utensils. Food-handlers should wash their hands with soap and clean water thoroughly before and after preparing food and every interruption in food preparation (FEHD, 2005).

Survival of *V. alginolyticus* in the model system

V. alginolyticus isolated in this study was identified by PCR and 16S rDNA gene sequencing. The nucleotide sequence of *V. alginolyticus* OM2 has been submitted to the GenBank with accession number KY212749, and represented in a phylogenic tree in Fig.1.

Post harvesting processes; steaming at 80°C for 5 min, chilling at 4±1°C for 24 hrs, or freezing at -5.0 ± 1.0°C for 24 hrs were performed in this part of the study with samples inoculated with *V. alginolyticus* OM2. Samples were sterilized and inoculated with *V. alginolyticus* OM2. Bacterial count in the mussels and shrimp samples after inoculation were 7.9± 0.9, 7.7± 0.9 logs CFU/g, (Fig. 2). When exposed to similar post-harvest processing, steaming

was the most effective treatment and resulted in *V. alginolyticus* OM2 count reduction to <1 log estimated. Freezing reduced the bacterial count by 1.7 and 1.8 log reductions in mussels and shrimp, respectively, to 6.2 ± 0.9, 5.9 ± 0.1. Chilling reduced the *V. alginolyticus* by 1.1 log reduction in both materials to 6.8 ± 0.9, 6.6 ± 0.1, respectively. Steaming was effective in inactivating *V. alginolyticus* and this heating regime is in agreement with those recommended by food safety agencies. The U.S Food and drug administration (FDA) recommends steaming shellstock oysters, clams, and mussels for 4-9 min, frying shucked oysters for 10 min at 375°C or baking oysters for 10 min at 450°C (NSSP, 2015). On the other hand, any defects in steaming application might affect the safety of seafood. Cases of *Vibrio* food poisoning (60%) were due to the consumption of inadequately cooked food from 1999-2003 (FEHD, 2005). Cooking and to avoid cross contamination are the most important key factors in successful prevention of *Vibrio* food poisoning.

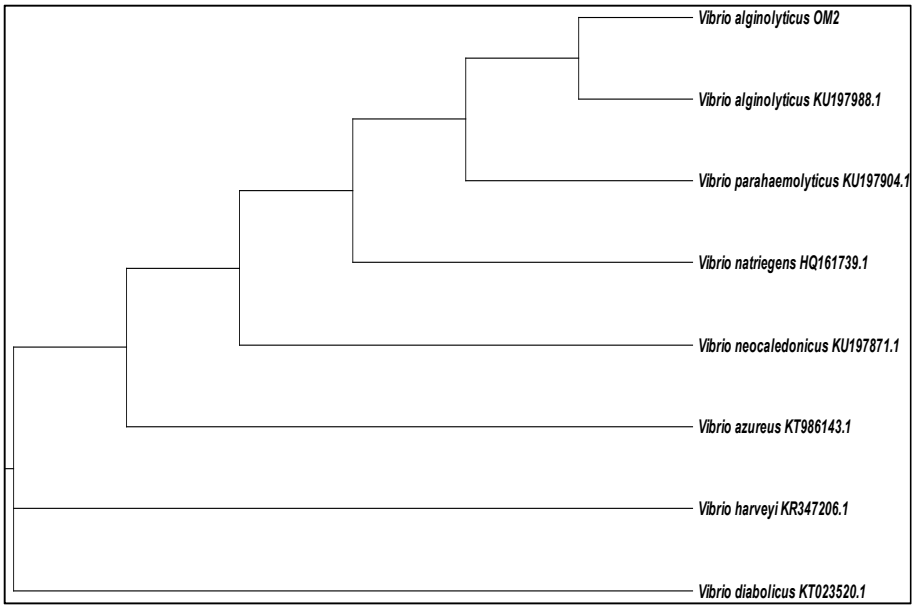

Fig. 1. Phylogenic tree represented sequenced *V.alginolyticus* OM2.

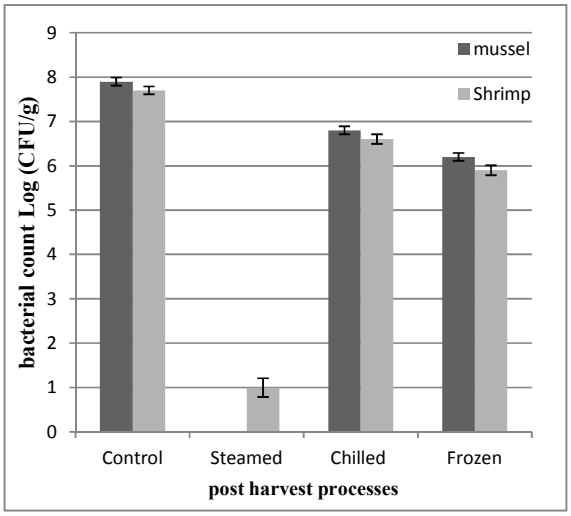

Fig. 2. Total bacterial count means (Log CFU/g) of *V. alginolyticus* exposed to different post-harvest processes.

Chilling and freezing had a limited reduction of about 1 and 2 logs reductions, respectively, in the samples after 24 hrs. This result agreed with the Guide for the Control of Molluscan Shellfish, it recommended after harvesting of raw shellfish, it required to be cooled down to less than 10 °C within 10 h (NSSP, 2015). Holding chilled ingredients and finished products at above 4°C for more than 2 hrs should be avoided (FEHD, 2005). Temperature and duration control of chilling and freezing as a post-harvest process should be monitored. A Risk Profile on *Vibrio parahaemolyticus* in Bivalve Molluscan Shellfish has identified the potential to survive and multiply after harvesting, depending on the ambient temperature as follow (ESR, 2016). Vibrio will multiply in shellfish stock stored at 20°C or above. The concentration can increase by as much as 1 log per gram in one day at 20°C, and more at higher temperatures. Growth to stationary phase occurs within 1-2 days. Vibrio will multiply in shellfish at 15°C, increasing by approximately 2 logs over two days of storage. Vibrio will not grow in shellfish stored unfrozen at 4°C or lower. The concentration has been observed to remain stable or decrease at these cool temperatures. Vibrio dies under frozen storage but can survive for up to six months. The data suggests that death is more rapid at -10°C or -18°C compared with -30°C. This has been attributed to the formation of larger intracellular ice crystals at the higher temperatures, causing greater cell damage (Shen *et al*., 2009).

CONCLUSION

In summary, steaming at 80°C for 5 min in model system completely eliminated *V. alginolyticus* to < 1 log estimated, in mussels and shrimps. Chilling at 4±1°C for 24 hrs and freezing at -5±1°C for 24 hrs maintained the microbial shelf life with minimal reduction (<2 logs reduction). In commercial samples, prevalence of *V. alginolyticus* in chilled (22.2%) and steamed mussels

(18.8%) was high even when compared to raw (20%). Same results for shrimp samples, where Vibrio species in frozen handled samples were more prevalent (22.2%) than the raw one (20%). Sanitation and hygienic measures, time-duration of freezing and chilling application, and temperature penetration to internal bodies are key factors that needed to be addressed to control Vibrio species in mussels and shrimps.

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حصر أنواع Vibrio species في الجمبري (Penaeus indicus) وبلح البحر (Mytilus galloprovincialis) بعد معاملات ما بعد الحصاد في أسواق المأكولات البحرية في السويس ، مصر

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اجريت هذه الدراسة لتحديد سلامة الروبيان وبلح البحر فى الأسواق وبعد تطبيق معاملات ما بعد الحصادالشائعة في أسواق السويس ، تم جمع بلح البحر الطازج وعينات الجمبري من أسواق المأكولات البحرية في السويس وتم تقييمها ميكروبيولوجيًا خامًا أو بعد إخضاعها إلى أحد المعاملات التالية ؛ تبخير عند 80 درجة مئوية لمدة 5 دقائق ، تبريد عند 4 درجة مئوية لمدة 24 ساعة ، أو تجمد عند -5.0 ± 1.0 درجة مئوية لمدة 24 ساعة. تم التعرف على الأنواع Vibrio بما في ذلك Vibrio alginolyticus و V. fluvialis و V. cholera في كلتا العينات. كانت بكتريا Vibrio alginolyticus أكثر الأنواع انتشارًا في بلح البحر الخام (20٪) وعينات الجمبري (20٪). أدت معاملات ما بعد الحصاد إلى تحسين سلامة المنتجات النهائية. لتقدير فعالية معاملات المعالجة هذه على V. alginolyticus في الجمبري وبلح البحر ، تم تلقيح المواد مع البكتريا في 7 Log CFU / g. وأدت عملية التبخير إلى القضاء على الحمل الميكروبيولوجي من بلح البحر إلى مستوى لم يتم حصره وتقليل تعداد الالجينوليتيكوس في الروبيان إلى <1 لوغ. وفى التبريد والتجمد تم انخفاض محدود من 1 إلى 2 لوج، على التوالي ، في العينات بعد 24 ساعة.