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فى

العلوم الطبية البيطرية
الرقابة الصحية على اللحوم والأسماك ومنتجاتها
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**STUDIES ON MICROBIOLOGICAL QUALITY OF SOME MOLLUSCS
RETAILED IN ALEXANDRIA GOVERNORATE**

A Thesis Presented By

Kamal Hakim Aioub Gergis

(M.Vet. Sc. 2004)

For

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To

**Department of Food Hygiene
Faculty of Veterinary Medicine
Alexandria University**

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1. INTRODUCTION

The rapid increasing of world population and the growing demand for protein resources, follow on the demand of molluscs is also increased. To meet one's daily nutritional needs, man has relied on molluscs as they usually a readily available food source. Molluscs are rich in protein and trace minerals and have always filled a substantial portion of man's diet. (*Avril, 2005*).

Molluscs are soft bodied animals belonging to phylum Mollusca under a collective term "Shellfish" which include crustacean and molluscs. There are around 100.000 extant species within the phylum, which typically divided into ten taxonomic classes. (*Barnes et al., 2001*) Edible classes comprise; Cephalopoda as squid and Bivalvia as wedge shell *Donax Trunculus* and *Tapes de-Cussates*. The later two bivalves were locally named as Om Elkhouloul and Gandoufly, respectively.

Squid is a popular food in many parts of the world specially countries around the Mediterranean Sea, including Egypt, and often known by the name calamari. All parts of squid are edible except its beak and gladius, (*Xavier et al., 1999*.) It is usually eaten fried for less than 2 minutes after cutting into small pieces or sliced into rings, (*Marriam, 2003*). Gandoufly and Om El-Kholoul are traditional foods, soled by street vendors and fish markets in coastal areas of Egypt. They are economically important marine food species which found in estuarine and marine water near the shore (*Banwart, 1981*).

Raw and partially cooked molluscan shellfish (Clam, Oyster and Mussel) have along history as vectors of infection agents and marine biotoxins. Illnesses associated with these food sources originate principally from bacterial and viral pathogens and from toxins-producing dinoflagellates concentrated by shellfish during filter feeding process. Before 1950, the most common illness associated with the consumption of raw molluscan shellfish was typhoid fever (*Rippey, 1994*).

Public health concern related to consumption of molluscs is prompted by, 1- They are frequently consumed raw, lightly cooked or fried. 2- The whole animal is consumed (including all organs). 3- They lived near shore or at estuarine which exposed to many types of pollutants specially sewage pollutions, treated or untreated, because sewage treatment is designed to reduce organic or particulate matter and not to remove microorganisms (*wood, 1985*). 4- They lived on bottom over surface of mud or sand where sediments contain high concentrations of microorganisms due to sedimentation (*Lowman et al., 1971*). 5- Bivalve molluscs are filter feeders; can concentrate more than fourfold in their tissue particles present in the surrounding water. They feed and respire by passing water between the shells and over gill surfaces where particulate matter is strained off for ingestion, during this process they may ingest and get trapped faecal bacteria and viruses present in the water. So, they accumulate and concentrate enteric pathogens during their physiological activity (*Evison, 1985; Potasman et al, 2002 and Butt et al, 2004*). Therefore, the molluscs may contain higher levels of pathogens than that found in the water in which they grow to a level that may constitute a public health hazard.

Despite major advances and improvements in food and water quality, diagnostic methods, and surveillance systems; food-borne diseases remain a global public health

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problem (*Costantini et al 2006*). Enteric pathogens derived from human or animal sewage including salmonellae, *E. coli* and coliforms are of concern because molluscs are mostly eaten raw or lightly cooked (*ICMSF, 1980*). *E. coli* is responsible for colienteritis in children, traveler's diarrhea and gastrointestinal illness. It also produces enterotoxins and may cause colibacillosis in adults as well as peritonitis and cystitis (*Levine 1987*). Salmonella is one of the most important causes of human gastrointestinal disease world wide. It is not generally recognized as a part of normal bacterial flora in aquatic environment but presence of Salmonella indicates poor sanitation and hygiene or unhygienic post-harvest handling and processing contamination (*Nash et al., 1992*).

Vibrio parahaemolyticus is a halophilic organism found in coastal waters of virtually all temperate regions (*De Paola et al., 1990*). Its infection result in one of three clinical syndromes: gastroenteritis, wound infection and/or primary septicemia (*Hlady and Klontz, 1996*). Halophilic bacteria that are widely distributed in marine environment, in molluscs shellfish and sediment were recognized as common causes of food-poisoning following the ingestion of uncooked or partially cooked seafood. (*Frazier and Westhoff, 1988*).

Because of the high incidence of Staphylococcal food-poisoning which characterized by nausea, vomiting, diarrhea, general malaise and weakness, (*ICMSF, 1982*). The enumeration of *Staphylococcus aureus* should be routinely conducted in every regulatory food inspection.

The potential hazard associated with consumption of mould and yeast contaminated molluscs, their heterotrophic nature and their ability to adapt to a wide range of environmental condition, these fungi are frequently encountered as actively growing contaminants in and on various kinds of food. So studies should be prompted on determining the mould and yeast count. (*Mislivec 1981*).

Therefore, the objectives of this investigation were planned to throw light on the microbiological quality of some popular Egyptian molluscs (Gandoufly, Om Elkhouloul and squid) on the following criteria:

Part I: Microbiological examinations of molluscs

- a- Determination of total halophilic bacterial.
- b- Determination of total halophilic mould and yeast.
- c- Determination of total coliforms (MPN/g).
- d- Isolation and identification of some pathogens:
 - 1- Isolation and identification of *vibrio parahaemolyticus*.
 - 2- Enumeration and identification of *Staphylococcus aureus*.
 - 3- Detection of faecal coliform. (Eijkmann test)
 - 4- Detection and identification of *E. coli*.
 - 5- Detection and identification of salmonella.

Part II: Effect of some organic acids (citric acid and acetic acid 5%) on total halophilic and total coliform counts in Om-Elkhouloul

2. LITERATURE REVIEW

2.1 Characters of contaminants:

Sakazaki et al (1970); Bradshaw et al (1974) stated that *V. parahaemolyticus* grow well at pH ranging from 5-11 with an optimum pH 7.5-8.5 and at a temperature ranging from 22-42°C, but fails to grow at 2°C and killed by exposure to 100°C for 6 minutes.

Mitchell (1971) reported that the enteric bacteria may survive in sea water from a few hours to five days longer.

Paille et al (1987) examined the effect of seasonal variation in Faecal coliform population of Louisiana oysters. They found that *E. coli* was the principle faecal coliform when water temperature was <22°C. It was suggested that the safety indicator in the guideline for oyster meats should be changed from Faecal coliforms to *E. coli*.

El-Shenawy and Marht (1989), stated that the antimicrobial activity of organic acids is differed from one to other. Acetic and tartaric acids were the most effective while the lactic and citric acids were the least effective.

Depaola et al (1990) reported that *Vibrio Parahaemolyticus* is a halophilic estuarine organism found in coastal waters of virtually all temperate regions. In temperate regions, a seasonal occurrence in shellfish and in human infections has been reported, the majority in warmer months of the year. In subtropical regions such as Florida, illness can occur year a round.

Donn and Cameron (1991) mentioned that *Vibrio Parahaemolyticus* is a halophilic, motile, Gram negative rod widely distributed in marine and estuarine environments. The organism although is fairly sensitive to heat in shellfish, it is some what resistant to cold temperatures. Its destruction was done by heating at 100° for 1 minute. They also added that *Vibrio Parahaemolyticus* can survive processing and storage conditions.

Elliot et al (1995) found that *Vibrio Parahaemolyticus*, like many other gram negative bacteria, grow in the presence of relatively high level of bile salts. They are facultative anaerobic and grow well under alkaline conditions Thiosulfate Citrate Bile Sucrose (TCBS) agar is a medium commonly used for isolation of *Vibrio Parahaemolyticus*. This medium supports good growth of vibrio while inhibiting most non vibrio.

Muntada et al (1995) Indicated that high numbers of *Vibrio Parahaemolyticus* can be inactivated depending on the initial numbers and incubation temperature. It is possible to use this information to determine the storage time necessary to reduce *Vibrio Parahaemolyticus* hazards in fish and shellfish.

Cook (1997) stated that the sample of molluscan shellfish should be cooled at 7-10°C, immediately after collection, and analyzed as soon as possible. Direct contact of molluscan shellfish with ice should be avoided as it may influence the recovery rate of vibrios. Vibrios can be injured by rapid cooling, but grow rapidly in sea food at ambient temperatures.

Literature review

Haseqawa et al (2002) studied the survival of *Vibrio Parahaemolyticus* servovars in the presence of acetic, citric and hydrochloric acid. At pH 5.6, citric acid was more effective in reducing the number of viable cells of *Vibrio Parahaemolyticus* than acetic acid. However, at pH 4.5, acetic acid was more effective than citric acid. The number of viable cells decreased quickly in the presence of rice vinegar.

Gamal (2003) examined the effect of acetic and citric acids on the count of *Listeria monocytogenes* in artificially contaminated Gndoufly samples after dipping in both acids. It was found that reduction percentage in listerial count was directly proportional to the time of exposure to both acetic and citric acids.

Pfeiffer and Oliver (2003) suggest that TCBS agar is a superior medium for the isolation of *Vibrio* spp. from estuarine waters and sea foods. Because of the public health risk presented by *Vibrio Parahaemolyticus* and other vibrios (like *V. cholera* and *V. vulnificus*), the selection of the most appropriate medium for their isolation is extremely important.

Maruyama et al. (2005) examined the inhibition effect of table top dry ice coolers on *vibrio parahaemolyticus* in fillets of squid. They found that, table top coolers inhibited the growth of *Vibrio Parahaemolyticus* and no increase in viable count occurred in 3 to 4 hours.

De Abreu et al. (2007) reported that the combined UV light and chlorine treatments for purification or depuration system of molluscan shellfish resulted in total elimination of bacteria within 12 hours.

Kisla (2007) showed that both lemon juice and lemon dressing used as flavoring and acidifying agents for stuffed mussels caused slight decrease in *Salmonella typhimurium* as an immediate inhibitor and the effect was increased by time. The treatment of stuffed mussels with inhibitor need more than 5 minutes to prevent *Salmonella typhimurium* outbreaks related to stuffed mussels.

2.2 Sources of contamination to molluscs:

Tatini (1973) stated that, whether or not *Staphylococcus aureus* can grow and produce detectable amount of enterotoxins in a given food or food products depends on the nature of food composition, raw or semi processed, also an interactive influence the existent environmental conditions during various chains of handling, processing, packaging and storage.

Brown and Dorn (1977) stated that, fish can retain pathogens (e.g. *E. coli*, *Salmonella* spp., *Shigella* spp.) without becoming ill. Staphylococcal and streptococcal foodborne illnesses are usually due to contamination of fish from a fishing vessel or in processing plant.

Field (1979) mentioned the factors that complicated shellfish sanitation as: (i) during the process of feeding oysters, clams, and mussels filtered and retained harmful organisms and toxic substances present in the water in which they lived and high concentration of these substances might be present in shellfish meat, (ii) the environment in which shellfish grow was always subject to some degree of human, industrial or animal pollution, (iii)

Literature review

shellfish often were packed whole and alive and often were consumed either raw or partially cooked. So if the shellfish were exposed to polluted water with human and animal excrement, they become agents for disease.

ICMSF (1980) stated that, molluscs fed in polluted water, they would concentrate contaminating bacteria, including enteric pathogens and also viruses if they were present during feeding, molluscs filter microorganisms from the water. The volume of water pumped by an oyster could be as greater as 10 liters/ hour. Thus the ability to concentrate microorganisms was greater. Enteric pathogens derived from human or animal sewage, including salmonella, shigella, *Vibrio cholera* and *Escherichia coli* were concerned because molluscs might be eaten uncooked.

Banwart (1981) reported that, while bivalve molluscs feed, they filtered large quantities of water and concentrate bacteria that were normally found in water environment. Shellfish were normally found in water near the shore. This water was subjected to contamination of run-off water carrying soil microorganisms and sewage outfall. The accumulation and concentration of these microorganisms from the water is of particular concern when there were potential pathogens such as salmonellae.

Floccia and sanna (1981) studied the effect of pollution of seawater on molluscs culture. They found that, bivalve molluscs as well as the coastal microplankton. (e.g. Conyaulax, Gymnodinium) were easily contaminated and causing cholera, typhus or hepatitis in human especially when consumed raw. This problem occurred due to discharge of urban and industrial wastes into the sea and spread of pollution by natural processes (currents, tides).

Kampelmacher (1981) reported that microbiological contamination of worker's hands was studied, at 13 food and 3 non-food establishments. The pathogenic organisms most frequently isolated at various food establishments were Enterobacteriaceae (70-100% of workers) and faecal streptococci (7-100%) *Escherichia coli* (4-100%), *Staphylococcus aureus* (17-100), *Clostridium perfringens* (0-88%) and salmonella spp. He concluded that contamination of worker's hands by food pathogens especially that of animal origin, is of more importance than the consequence of toilet use.

APHA (1984) reported that bivalve molluscs such as oysters, mussels and clams were economically important marine food species found in abundance in estuarine and marine water. These organisms were filter feeders and commonly were eaten raw and whole in the living state, while feeding on plankton and other microflora. Shellfish might concentrate pathogenic bacteria if the water was contaminated with domestic pollution.

National Academy of Science (1985) stated that fish and shellfish are subjected to many risk of contamination from different sources starting from harvesting, marketing till reaching to consumers. The main sources of contamination are water, soil, sewage, working men and equipments. Such contamination may render the product resulting in significant economic losses and public health hazard to consumer.

West et al (1985) stated that, safe disposal of human and animal excrete was a major problem for communities. The common practice of discharging untreated or treated sewage into estuaries and rivers as well as the land disposal of sewage sludge frequently lead to faecal contamination of shellfish laying in estuarine and near-shore waters. All bivalve molluscs fed