EFFECT OF COOKING PROCESS ON COLIFORMS AND *ESCHERICHIA COLI* IN SOME SEA FOODS AND THEIR PUBLIC HEALTH SIGNIFICANCE

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ABSTRACT

Received at: 25/3/2012

Accepted: 6/5/2012

Thirty samples of apparently healthy shellfish (30 each of baclawese "Tartufo di mare" and om EL-khloul "Donax trunculus anatinus" were randomly purchased from Port Said markets. The samples were examined for enumeration, isolation and identification of total coliforms, fecal coliforms and *Escherichia coli* before and after cooking technique. The incidence of positive fresh baclawese samples for total coliform, fecal coliform and *Escherichia coli* were 100% (30), 100% (30) and 20% (6), while that of fresh om EL-khloul samples were 100% (30), 100% (30) and 10% (3), respectively. Mean values of the total coliform counts were 2.4 X 10^6 and 1.8 X 10^5 MPN/100g of fresh baclawese and om EL-khloul respectively, while that of the cooked baclawese and om EL-khloul were 1.8 X 10^5, and 1.6 X 10^4 respectively. Meanwhile, the mean values of the *Escherichia coli* counts were 3.7 X 10^2, 2.0X 10^3 and 9.5 X 10^1 MPN/100g in fresh baclawese, fresh om EL-khloul and salted om EL-khloul respectively. The numbers of coliform isolates were 52, 47 for fresh baclawese and, om EL-khloul and that of cooked samples were 14, 8 respectively. While in case of salted om EL-khloul numbers of coliform isolates was 28. The number of *Escherichia coli* isolates were 6, 3 and 3 for fresh baclawese and, om EL-khloul and salted om EL-khloul samples respectively. The bacterial isolates in the examined samples were identified as *Escherichia coli, Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, Citrobacter koseri*, *Klebsiella pneumonia* and *Klebsiella Ozaenae*. The effect of cooking technique on coliforms and *Escherichia coli* in the examined samples were discussed.

تأثير الطهى بالحرارة علي الميكروبات القولونية وميكروب الايشيرشيا كولاى المتواجدة في بعض المأكولات البحرية وعلاقتها بالصحة العامة

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فـي دراسـة لتحديـد مـدي تـأثير الطهـى بـالحرارة علـي الميكروبـات القولونيـة وميكـروب الايـشيرشيا كـولاى المتواجـدة فـي بعـض المـأكولات البحريـة (البكلـويز والخلـول) تـم فحـص ثلاثـون عينـة صـالحة ظاهريـا والتـي تـم جمعهـا عـشوائيا مـن محـلات المـأكولات البحريـة بمدينـة بورسـعيد بهـدف عـد وعـزل الميكروبـات القولونيـة وميكـروب الايـشيرشيا كـولاى المتواجـدة فـي هـذه العينـات قبـل وبعـد الطهـى بـالحرارة بالاضـافة الـى تـاثير التمليـح علـى الخلـول حيـث انـه يمكـن تناولـه مملحـا بالاضـافة الـى مناقـشة اثرهـذه الميكروبـات علـي الـصحة العامـة. وأظهـرت النتـائج ان نـسبة العينـات الايجابيـة للميكروبـات القولونيـة الكليـة، الكـولي فـورم البرازيـة وميكـروب الاشريـشيا كـولاي كانـت ١٠٠% (٣٠)، ١٠٠ % (٣٠) و٢٠ % (٦) فـي البكلـويز الطـازج علـي التـوالي بينمـا كانـت ٣٣ ,٧٣% (٢٢) ، ٣٠ ,٥٣ % (١٦) وصـفر % (صـفر) فـي العينـات المطهيـة بـالحرارة بينمـا كانـت

نسبة العينات الايجابية للميكروبات القولونية الكلية ، الكولي فورم البرازية وميكروب الاشريشيا كولاي في الخلول الطازج ١٠٠% (٣٠)، ١٠٠ % (٣٠)، ١٠ % (٣) بينما كانت ١٦,٧% (٥)، ١٣,٣ % (٤) وصفر % (صفر) على التوالي في العينات المطهية بالحرارة. وكانت النسب فى الخلول المملح ٦٣,٣% (١٩) ،٦٠ % (١٨) و١٠ %(٣) على التوالى. كان متوسط العدد الكلي للميكروبات القولونية الكلية، الكولي فورم البرازية ١.٦X١٠ ٤ ، ٢.٩X١٠ ٢ خلية/ ١٠٠جرام بينما كان متوسط العدد الكلي لميكروب الاشريشيا كولاي ٣.٧X١٠ ٢، ٢X١٠ ٣ و ٥.٩X١٠ ١ خلية/١٠٠ جرام وذلك في عينات البكلويز والخلول الطازج والخلول المملح على التوالي. وتم عد وتصنيف العترات المعزولة فوجد أن عدد عترات الميكروبات القولونية ٥٢& ٤٧ في البكلويزو الخلول الطازج بينما كانت في البكلويزو الخلول المطهى ١٤& ٨ على التوالي وكان عدد عترات ميكروب الاشريشيا كولاي ٦ & ٣ & ٣ وذلك في عينات البكلويز الطازج والخلول الطازج والمملح على التوالي. وبتصنيف الانواع المعزولة من العينات موضع الدراسة وجد ان العترات المعزولة كانت تنتمى الى الاشريشيا كولاي ، انتيروبكتر كلواكا، انتيروبكتر ايروجينز، سترواكتر فروندراي، سترواكتر كوزيراي، كلبسيلا اوزينى فى كلا من البكلويزو الخلول ، بينما كلبسيلا نيمونيا عزلت فقط من البكلويز. وتم مناقشة تاثير المعاملات الحرارية على الميكروبات القولونية والاشريشيا كولاي المتواجدة في عينات المأكولات البحرية موضع الدراسة.

***Key words**: baclawese, om EL-khloul. Escherichia coli, Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, Citrobacter koseri, Klebsiella pneumonia and Klebsiella Ozaenae.*

INTRODUCTION

Shellfish is one of the most important and beneficial diets to consumer due to their highly palatability and digestibility, their poorness in fat and their rich in vitamins, mineral and omega 3 polyunsaturated fatty acid which is not produced within the body and reduce the risk of heart diseases and atherosclerosis Simopoulos (1997); Umemura *et al*. (2000); Smith (2005).

Bivalve shellfish use a filter feeding mechanism that filters water from their environment. If the water contains bacteria, viruses or other contaminates, these filter feeding organisms can accumulate toxins and concentrate bacteria within their bodies. The diseases found in the shellfish may be passed on to humans if the shellfish are consumed (Ghislaine *et al*., 2010).

Coliform is a group of bacteria inhabit the intestinal tract of warm blooded animals including man. Some species of them are opportunistic pathogens responsible for a wide range of infections while many other species of them are normally free living saprophytic. These bacteria are Gram-negative, aerobic and facultative anaerobic bacteria, non spore forming and rod shaped, motile by flagella except *Klebsiella* (non motile), capable of fermenting lactose with the production of acid and gas includes *Escherichia, Klebsiella, Enterobacter* and *Citrobacter* (Greenberg and Hunt, 1985; FAO, 1992; Hitchins *et al*., 1998; FDA, 2002)

The health problem of coliform group depends upon the production of different toxin and colonization factors. The enterotoxigenic strains of *Klebsiella*, *Escherichia*, *Enterobacter,* and *Citrobacter* have been isolated from infants and children with acute gastroenteritis. These toxins are heat labile and heat stable types and have other properties in common with *Escherichia coli* toxins. The endotoxins of most coliforms show a bacteraemia in human characterized by fever; vital organs hypo perfusion, depletion of complement, hypotension, irreversible shock and death. Coliforms rarely cause extra- intestinal diseases as bacteraemia and meningitis unless host defense is compromised (Guentzel, 1982; APHA, 1984).

The most virulent and major enteric pathogenic group particularly in developing countries was enterovirulent *Escherichia coli* group (EEC) which implicated in foodborne illness and includes entertoxigenic *Escherichia coli* (ETEC), enteropathogenic

Escherichia coli (EPEC), enterohemorrhagic *Escherichia coli* (EHEC) and enteroinvasive *Escherichia coli* (EIEC) (Hitchins *et al*., 1998; FDA, 2002). These groups have been incriminated in many cases of food borne disease outbreaks, travelers, diarrhea, infantile diarrhea and colibacillosis in adults.

Foodborne hazards are still of great concern for human health and in particular the risks connected with shellfish and seafood consumption continue to be important in developing and developed countries despite the advances in technology, changes in food processing, and packaging (Feldhusen, 2000; Egli *et al*., 2002).

The objectives are to evaluate the effect of cooking on coliforms count and *Escherichia coli* in baclawese "Tartufo di mare" and effect of cooking and salting om EL-khloul "Donax trunculus anatinus" besides the study of their public health significance in Port-Said city.

Om EL-khloul
"Donax trunculus anatinus"

Baclawese
"Tartufo di mare"

MATERIALS and METHODS

1: Samples collection:

A total of 60 apparently healthy samples of shellfish (30 each of baclawese "Tartufo di mare" and om EL-khloul "Donax trunculus anatinus" were randomly collected from Port Said markets. All specimens were prepared, treated and examined within 4 hours of collection. In case of baclawese the samples were divided into 2 parts; the 1st part directed to counting, isolation and identification of coliforms and *Escherichia coli*. The 2nd part was cooked before the bacteriological examination. While In case of om EL-khloul the samples were divided into 3 parts; the 1st part directed to counting, isolation and identification of coliforms and *Escherichia coli*. The 2nd and the 3rd part were cooked and salted before the bacteriological examination.

2: Bacteriological examination:

2-1: Preparation of the samples:

The meat and liquor of each sample was homogenized, and 10 g were drawn off into a sterile dilution cup and blended for 2 min in sterile phosphate buffer dilution water to yield 1:10 dilution of sample. Then serial dilution was prepared using sterile phosphate buffer dilution water according to (FDA, 2002).

2-2: Presumptive test for coliforms:

Five tubes each containing 10 ml of sterile lauryl tryptose broth (Difco) with Durham's tubes, were inoculated with 1ml from each of the original dilution. Inoculated tubes were thoroughly mixed before being incubated at 35°C for 48 hours. The inspection was done after 24 and 48 hours incubation for positive gas production (FDA, 2002).

2-3: Probable number of total coliforms:

Each positive LT tube was gently agitated then loopful of suspension was transferred to tube of brilliant green bile broth 2% (BGB) containing inverted Durham's tube. All tubes were incubated at 35°C for 48 ± 2 hr and examined for gas production. Enumeration of total coliforms was applied by using table of most probable number (MPN) based on combination of confirmed gassing LT tubes for 5 consecutive dilutions and the results were reported for 100 g of each sample.

2-4: Probable number of fecal coliforms:
Gentle agitation of each gassing LT tube was done and a loopful of the suspension was transferred to tube of EC medium containing inverted Durham's tube. All broth tubes were incubated at 44.5 ± 0.2°C for 48 ± 2 hr. The presence of gas in EC medium was considered to be confirmed evidence of the presence of fecal coliform bacteria in the shellfish sample. Enumeration of fecal coliforms was applied by using table of most probable number (FDA, 2002).

2-5: Enumeration of *Escherichia coli*:
A loopful of suspension from gassing EC medium tubes was streaked onto Levine's Eosin-Methylene Blue agar plate and iincubates at 35°C for 18-24 hr. Typical colonies with a dark center or a metallic sheen were selected, streaked for purity on nutrient agar, and incubated for 24 h at 35°C. Enumeration of *Escherichia coli* was applied by using table of MPN based on proportion of EC medium tubes in 5 consecutive dilutions which shown to contain *Escherichia coli* according to (FDA, 2002).

2-6: Biochemical identification of the isolates:
Pick three typical colonies from each agar plate and transfer to plate count agar (PCA) slants tube and incubate at 35°C for 18-24 hr. All PCA slants were directed to morphological and biochemical identification according to Holt *et al*. (1994) and Farmer (1995).

3: Statistical methods:
One-Way ANOVA test was performed on the parameter studied to describe data using Statistical Package for Social Scientists (SPSS) for windows 16.0 (SPSS Inc., Chicago, IL, and USA). Correlations between total coliform, fecal coliform and *Escherichia coli* counts based on the examined samples of baclawese "Tartufo di mare" and om EL-khloul "Donax trunculus anatinus" in relation to cooking and salting technique. Significant differences in parameters analyzed. P value was considered significant if less than 0.05 and 0.01 at 95% and 99% respectively (SPSS, 2007).

DISCUSSION

Marine bivalve like baclawese and om EL-khloul are globally important food resources. They are particularly important in developing countries; mostly because they are easily collected in shallow areas and have high nutritional value. These species are sedentary and filter-feeding which favor bioaccumulation of microorganisms, which make them frequently involved in outbreaks of gastroenteritis (Sockett *et al*., 1985; Potasman and Odeh, 2002).

Members of the coliform groups are referred as general indicator microorganisms to measure the potential presence of enteric pathogens (for example *Escherichia coli*) in foods, besides the measuring of fecal contamination of the food products and the sanitary condition in the food-processing environment (Greenberg and Hunt, 1985; APHA, 1992; FAO, 1992).

The results in Table 1 revealed that the incidences of positive fresh baclawese samples for total coliforms, fecal coliforms and *Escherichia coli* were 100% (30), 100% (30) and 20% (6) respectively. While that of om EL-khloul were 100% (30), 100% (30) and 10% (3) respectively (Table 2). The incidence of positive samples for coliforms was higher than the results recorded by Betty *et al*. (2008) but that of *Escherichia coli* was lower than those recorded by them. The high incidence of positive samples may be due to the water is highly contaminated by shipping and untreated sewage. Also may be attributed to the unsanitary conditions during the

collection and transportation of baclawese and om EL-khloul.

The mean values of the total coliforms, fecal coliforms and *Escherichia coli* counts in fresh baclawese were 2.4 X 10^6 , 2.3 X 10^5 and 3.7 X 10^2 (MPN/100g) while in case of om EL-khloul were 1.8 X 10^5 ,8 X 10^3 and 2 X 10^3 (MPN/100g) respectively (Tables 1 and 2). These results agreed with the results recorded by Carlos *et al*. (2007). On the other hand, our results were higher than that recorded by Hood *et al*. (1983); Humphey and Gawler (1986) and Colakoglu *et al*. 2010). Meanwhile the higher counts of fecal coliform and *Escherichia coli* may be attributed to recent fecal contamination (Caplenas and Kanarek, 1984; Greenberg and Hunt, 1985; APHA, 1992; FDA, 2002).

Regarding to the effect of cooking process on total coliforms, fecal coliforms and *Escherichia coli* presented in Table 1. It is evident that the incidences of positive cooked baclawese samples for total and fecal coliforms and *Escherichia coli* were 73.33% (22), 53.30% (16) and 0.00% (0.00) respectively. On the other hand the mean values of the total, fecal coliforms and *Escherichia coli* counts were 1.8X10^5, 5.6X10^3 and 0.00 for cooked baclawese respectively. While in case of the examined cooked om EL-khloul the incidence of positive samples decreased to 16.7% (5), 13.3% (4) and 0.00% (0.00) respectively (Table 2). The salting effect revealed that the percentage of the examined positive salted om EL-khloul samples were 63.3 % (19), 60 %(18) and 10 %(3) for total coliforms, fecal coliforms and *Escherichia coli* respectively. The mean values of the total, fecal coliforms and *Escherichia coli* counts were 3.5X10^4, 9.2X10^2 and 9.5X10^1 MPN/100g under effect of salting technique respectively results showed that the cooking process either in baclawese or om EL-khloul reduced the total and fecal coliforms and *Escherichia coli* counts to a different levels. This reduction effect agrees with the result recorded by Desmarchelier and Grau, (1997) and Yilmaz *et al*. (2005). This reduction was impossible to eliminate all forms of coliforms (Jay, 1978).

Statistically by using one way ANOVA test, a highly significant relationship between the counts of each of total and fecal coliforms and *Escherichia coli* recovered from fresh baclawese and om EL-khloul samples with that of the cooked samples. On the other hand, the relationship between cooking and salting technique in case of EL-khloul showed a significant relationship. This means that cooking technique was more effective and more efficient than salting technique in reduction effect (Yilmaz *et al*., 2002).

The obtained results in Table 3 showed that the number of coliforms isolates of fresh baclawese and om EL-khloul were 52, and 47 respectively. In case of cooked samples, the isolates were reduced to 14, and 8 for baclawese and om EL-khl oul repectively. The number of isolates in case of salted om EL-khloul was more than the number in the cooked om EL-khloul. This mean cooking technique was more effective and more efficient than salting technique. The coliforms organisms recovered from the examined baclawese samples were identified as *Escherichia coli*, *Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, Citrobacter koseri, Klebsiella pneumonia* and *Klebsiella Ozaenae*. While in om EL-khloul, the isolates were *Escherichia coli*, *Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, Citrobacter koseri* and *Klebsiella pneumonia*. Our results come in agreement with the result recorded by Gulsen *et al.* (2008). The main and important isolate found in fresh and treated samples was *Escherichia coli.* On the other hand *Citrobacter freundii* and *Citrobacter koseri* found only in total coliform and this may be attributed to that these group of microorganisms cannot tolerate high temperature but fecal coliform as *Enterobacter cloacae* and *Enterobacter aerogenes* can tolerate and considered thermotolerant organisms (Caplenas and Kanarek, 1984). The reduction in the number of isolates was more in cooking than in salting technique and this agrees with the

result recorded by Zahra *et al*. (1985) who showed that coliforms were easily killed and decreased markedly after heat treatment but heating process was not enough to ensure the safe eating quality (Bayhan *et al*., 1990) and impossible to eliminates all forms of coliforms (Jay, 1978). In conclusion, to increase the safety of Shellfish and decrease their coliforms loads and prevention the infections by these microorganisms to the consumers, a groups of measurement should be attempt such as removal the source of pollution as fecal contamination and sewage water and good sanitary condition during collection and transportation. Besides the equipment and utensils used for collection and transportation must be clean. sufficiently cooking of Shellfish and prevent the post cooking cross contamination and prohibiting people who are ill from working in food operations and strictly prevention the consumption of insufficient cooked Shellfish specially in compromised hosts reduce the consumers health problem.

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