

Bacteriological status of fish marketed in Beni-Suef City

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This study was carried out to evaluate the bacteriological status of fresh *Tilapia nilotica*, fresh *Clarias lazera*, frozen Mackerel and smoked Herring fish marketed in Beni-Suef City. The collected samples were examined for total bacterial count, coli form (MPN), faecal coli forms (MPN), *E. coli* (MPN), *Staphylococcus aureus* count, total proteolytic count, total lipolytic count, Aeromonas count and *Pseudomonas* count, as well as the isolation of *E. coli*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica* and *Listeria monocytogenes*. Most of the examined samples were within the permissible limits recommended by EOS and ICMSF, few samples of fresh *Clarias lazera* exceeded the recommended limits. The economic importance and public health significance of the isolated organisms were discussed.

Fish is an important protein source in many countries all over the world including Egypt. It is providing human by essential dietary protein of a high nutritive value, cardiovascular-protective omega-3 polyunsaturated fatty acids, essential minerals (phosphorus and calcium), vitamins (especially vitamin A), iodine, fluorine and appreciable amounts of cobalt, magnesium, iron, copper and very low quantities of cholesterol.

The microbial status of seafood after catch is closely related to the environmental conditions and microbiological quality of the water, these factors include water temperature, salt content, distance between the point of catch and polluted areas, natural occurrence of bacteria in the water, ingestion of food by fish, methods of catch and chilling, and post-harvest handling or processing conditions.

The rate of fish deterioration is highly temperature dependent and can be inhibited by the use of low storage temperature; therefore, it was advised by the need for careful attention to temperature control and the reduction of the time elapsing between fishing and consumption (Feldhusen, 2000 and Sivertsvik *et al.*, 2002). Freezing is one of the most appropriate preservation technologies of food used for fish. In addition to, the using of freezing and cold storage in fish preservation are more efficient than the other most traditional methods such as salting, smoking and drying (Jayasinghe *et al.*, 1996).

Fish can acquire pathogenic microorganisms or toxins from natural aquatic

environment, sewage, contaminated harvesting area as well as from workers, utensils and equipment during harvesting, processing, distribution and food preparation (National Academy of Science, 1985). Contaminated seafood has been implicated in food borne outbreaks in many countries; the majority of them have been caused by bacteria, viruses, histamine and marine biotoxins.

There is an increasing demand for fish and fish products around the world, although, there is substantial evidence that fish and seafood are high on the list of foods associated with outbreaks of food borne diseases. So, it became important to pay more attention to seafood safety and seafood-related diseases. Therefore, this work was carried out to evaluate the bacteriological status of the most popular consumed fishes that marketed in Beni-Suef City (fresh *Tilapia nilotica*, fresh *Clarias lazera*, frozen Mackerel and smoked Herring fish).

Materials and methods

Collection of the samples. A total of 120 fish samples were randomly collected from different fish markets at Beni-Seuf City; 30 each of fresh *Tilapia nilotica*, fresh *Clarias lazera*, frozen Mackerel and hot smoked Herring fish. Five samples were collected weekly. Organoleptic examination all of the collected samples were apparently healthy. The collected samples were identified and separately wrapped in sterile polyethylene bags and directly transferred in sterile icebox to the laboratory without delay to be examined within 6 hours after collection.

Preparation of the samples. Preparation of the muscle samples was carried out according to the muscle maceration technique recommended by (ICMSF, 1978).

Bacteriological techniques.

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Determination of aerobic plate count at 35 °C (APC). The pouring plate technique recommended by (AOAC, 1990) was applied.

Coli forms, faecal coli forms and *E. coli* (MPN). The applied technique was the three tubes method (MPN) recommended by (AOAC, 1990).

Isolation and identification of *E. coli* biotype I (true fecal type). The isolates were microscopically and biochemically identified according to the methods recommended by (AOAC, 1990).

Determination of *Staphylococcus aureus* count. The technique of *Staphylococcus aureus* count was performed according to (APHA, 1992).

Isolation and identification of coagulase positive *Staphylococcus aureus*. The isolates were identified according to the recommended technique by (APHA, 1992).

***Pseudomonas* / *Aeromonas* count.** The recommended technique by (Pireson *et al.*, 1970) was applied.

Isolation and identification of *Aeromonas hydrophila* and *Pseudomonas aeruginosa*. The isolates were identified microscopically and biochemically according to the recommended technique by (Koneman *et al.*, 1994).

Isolation of *Yersinia enterocolitica*. The applied technique was that recommended by (APHA, 1992).

Isolation and identification of *Listeria monocytogenes*. The applied technique was that recommended by FAO, (1992) using the tow-steps enrichment method.

Results and Discussion

Bacterial counts.

Fresh *Tilapia nilotica*. The illustrated data in Table (1) revealed that the mean values of APC at 35 °C, coli forms, faecal coli forms and *E. coli* (MPN), *Staph. aureus*, proteolytic, lipolytic, *Aeromonas* and *Pseudomonas* counts in muscle samples of *Tilapia nilotica* were 5.9×10^4 , 2.8×10^4 , 2.1×10^3 , 1.0×10^3 , 4.8×10^2 , 1.2×10^4 , 2.9×10^4 , 2.6×10^2 and 5.7×10^2 organisms/g muscle; respectively.

High figures were reported by (Abo El Alla, 2000; Abdel-Rahman *et al.*, 2004; Ali and Ibrahim 2004). While, low figures were obtained by (Mahmoud, 1999; Tzikas *et al.*, 2007). Nearly similar results were recorded by (Agnese *et al.*, 2001; Martins *et al.*, 2002),

The high data obtained in examined fresh fish could be attributed to high temperature of storage during marketing for long time, the using

of unhygienic utensils and equipment, as well as the current use of unhygienic water during marketing. In addition to, polluted water and mud from which the fish were caught play an important role in increasing the rate of contamination of fish flesh, this agrees with that reported by Fawzy *et al.*, (1981); Khalafalla, (1988).

Fresh *Clarias lazera*. The data in Table (1) showed that the mean values of APC at 35 °C, coli forms, faecal coli forms and *E. coli* (MPN), *Staph. aureus*, proteolytic, lipolytic, *Aeromonas* and *Pseudomonas* counts in muscle samples of *Clarias lazera* were 8.8×10^4 , 4.0×10^4 , 2.0×10^4 , 7.8×10^3 , 1.5×10^3 , 2.0×10^4 , 2.7×10^4 , 9.9×10^2 and 1.9×10^3 organisms/g muscle; respectively. High results were obtained by (Khalafalla, 1988; El-Atabany 1990). Low result was recorded by (Mousa and Mahmoud 1997; Mahmoud, 1999).

It was clear that the highest aerobic plate count was reported in fresh *Clarias lazera*, which could be attributed to the heavily polluted water inhabited by such fish. In addition to that, *Clarias lazera* are dirty feeders and the absence of scales gives more chance for surface contaminants to penetrate rapidly into fish muscles, this in accordance with that reported by (Khalafalla, 1988).

Frozen Mackerel. The recorded results in Table (1) revealed that the mean values of APC at 35 °C, coli forms, faecal coli forms and *E. coli* (MPN), *Staph. aureus*, proteolytic, lipolytic, *Aeromonas* and *Pseudomonas* counts in muscle samples of *frozen Mackerel* were 2.8×10^4 , 4.9, 1.8, 0.7, 2.4×10^2 , 2.3×10^3 , 2.9×10^3 , 9.0×10 and 3.3×10 organisms/g muscle; respectively. High figures were recorded by (Hwang *et al.*, 2004; Pacheco *et al.*, 2004; Geevarethnam *et al.*, 2008). While, nearly similar results were reported by (Pensrie *et al.*, 1991; El-Bakkly, 1997).

Low results were reported in frozen fishes as compared with fresh fish could be attributed to the inhibitory or the sub lethal effect of freezing and frozen storage on bacteria. This could be confirmed by that mentioned by (Magnusson and Martinsdottir, 1995) who stated that the frozen storage of fish at -25 °C leading to great reduction in the bacterial counts; 23 and 50 weeks of storage resulted in 80 % and 91 % bacterial counts, respectively.

Smoked Herring fish. The obtained data in Table (1) showed that the mean values of APC at 35 °C, coli forms, faecal coli forms and *E. coli*

(MPN), *Staph. aureus*, proteolytic, lipolytic, *Aeromonas* and *Pseudomonas* counts in muscle samples of *smoked Herring* were 3.9×10^3 , 0.6, <3, <3, 1.4×10^2 , 2.8×10^2 , 3.4×10^2 , 3.3 and 1.3×10 organisms/g muscle; respectively. High results were obtained by (Bastawrows *et al.*, 2000; Basti *et al.*, 2006). On the other hand, low counts were reported by (Gonzalez Rodriguez *et al.*, 2002).

The mean values in smoked Herring were low as compared with fresh fishes and this could be attributed to the inhibitory effect of salt in addition to the bacteriostatic and bactericidal effect of smoke compounds during the preparation, this held the view reported by El-Akeel, 1988).

Bacterial isolation.

Escherichia coli (biotype I). From the results represented in Table (2), it can be concluded that the incidence of *E. coli* in the examined muscle samples of *Tilapia nilotica* and *Clarias lazera* were 40 and 53.3 %, respectively. In addition, *E. coli* could not be recorded in the examined muscle samples of frozen Mackerel and smoked Herring.

High figure was recorded by (Silva *et al.*, 2002). While, low figures were recorded by (Youssef *et al.*, 1992; Agnese *et al.*, 2001). Similar figures were recorded by (Gonzalez Rodriguez *et al.*, 2002; Papadopoulou *et al.*, 2007).

In this respect, EOS (1-889/2005) recommended that frozen fishes should be free from *E. coli*. As well as EOS (288/2005) recommended that smoked fishes should be free from *E. coli*. So, all of the frozen Mackerel samples (100%) and all of the smoked Herring samples were within the Egyptian limits.

Staphylococcus aureus (coagulase positive). The results obtained in Table (2) revealed that the incidence of isolation of *Staphylococcus aureus* from the examined muscle samples of *Tilapia nilotica*, *Clarias lazera*, frozen Mackerel and smoked Herring were 26.6, 33.3, 6.6 and 10 %, respectively.

Nearly similar results of *Staphylococcus aureus* were obtained by (Ayulo *et al.*, 1994; Papadopoulou *et al.*, 2007). On the contrary, (Youssef *et al.*, 1992) could not isolate it from *Tilapia nilotica*. Low results were recorded by (Rodma *et al.*, 1991). EOS, (288/2005) stated that smoked fish should be free from Coagulase positive *Staphylococcus aureus*, so 10 % of the examined smoked Herring were not within the permissible limits recommended by EOS,

(288/2005). In this subject, sea foods are free from *Staphylococcus aureus* and contamination takes place upon handling (Bryan, 1980).

Humans are common carriers of *Staphylococcus aureus* in the nose, throat and in skin infections and can easily be transferred to food through handling, this held the view reported by (Pereira *et al.*, 1994). *Staphylococcus* food poisoning is characterized by nausea, vomiting, abdominal pain, and prostration, often with diarrhea but without fever, approximately one to 6 hours after ingestion of contaminated food. Although many patients usually completely recover after 24 hours without specific therapy and show a low overall case-fatality ratio, the symptoms are serious enough for up to 10 % of case to be admitted to hospital (Eley, 1992).

Aeromonas hydrophila. From the obtained results in Table (2), it could be concluded that the incidence of *Aeromonas hydrophila* in examined muscle samples of *Tilapia nilotica* and *Clarias lazera* were 20 and 16.6%, respectively.

High figures in fresh fish were recorded by (Abo El Alla, 2000; Davies *et al.*, 2001; Thayumanavan *et al.*, 2003; Papadopoulou *et al.*, 2007). On the contrary, low results were recorded by (Kumar *et al.*, 2000; Radu *et al.*, 2003). Nearly similar results were recorded by (Ali and Ibrahim, 2004).

On the contrary, *Aeromonas hydrophila* could not be isolated from the examined muscle samples of frozen Mackerel and smoked Herring; this was illustrated in Table (2).

Aeromonas spp. (principally *Aeromonas hydrophila*) are considered emerging food borne pathogens that are associated with septicemia, gastroenteritis, enterocolitis and wound infection in humans, this was reported by (Janda, 1991).

Pseudomonas aeruginosa. The results represented in Table (2) revealed that the incidence of *Pseudomonas aeruginosa* in examined muscle samples of *Tilapia nilotica* and *Clarias lazera* were 16.6 and 13.3 %, respectively. Low results were obtained by Mahmoud, (1990) and High figure was obtained by Ali and Ibrahim, (2004). In this respect, nearly similar result was reported by Abdel Rahman *et al.*, (2004). The same Table revealed that *Pseudomonas aeruginosa* could not be detected in frozen Mackerel and smoked Herring samples.

Pseudomonades are a good spoilage index in fishes and a marker for fish carcass deterioration, this held the view reported by Pacheco *et al.*,

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الحالة البكتريولوجية للأسماك المسوقة بمدينة بنى سويف

أجريت هذه الدراسة لتقييم الحالة الصحية لسمك البلطي الطازج، القرموط الطازج، الماكريل المجمد والرنجة المدخنة فى مدينة بنى سويف. الأسماك التى تم تجميعها قد فحصت ميكروبيولوجياً للعدد الكلى للبكتيريا الهوائية عند ٣٥° م والعدد الإجمالي لبكتيريا القولون و القولون البرازية وبكتيريا الإيشريشيا كولاى والعدد الكلى لبكتيريا المكور العنقودى الذهبى وللبكتيريا المحللة للبروتينات و للبكتيريا المحللة للدهون و أعداد السيدوموناس وأعداد الإيروموناس. بالإضافة إلى عزل كل من بكتريا الإيشريشيا كولاى و المكور العنقودى الذهبى والإيروموناس هيدروفيللا والسيدوموناس ايروجينوزا و اليرسينيا انتيروكوليتيكا و الليستريا مونوسيوجينز. وكما ناقشت الدراسة الأهمية الإقتصادية والصحية للميكروبات المعزولة.