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# EFFICACY OF WARM WATER, SODIUM HYPOCHLORITE AND TRISODIUM PHOSPHATE ON *E. Coli O157: H7* AND *Salmonella typhimurium* ARTIFICIALLY INOCULATED IN *Oreochromis niloticus* FILLETS

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***Abstract***

The Nile tilapia (*Oreochromis niloticus*) (*Tilapia nilotica*) is a native fish in Egypt and it is widely consumed as it represent a great protein source. The health of Egyptians is badly affected by some foodborne pathogens such as *E. coli O157: H7* and *Salmonella Typhimurium* which are being transmitted by eating the contaminated ***Oreochromis niloticus*** fillets. This experiment was carried out to decontaminate tilapia fillets samples by reference strains of *E. coli O157: H7* and *Salmonella Typhimurium*. This was done by dipping of samples in warm water (45°C) for 5 minutes, trisodium phosphate 8% for 20 minutes and sodium hypochlorite 50 ppm for 20 minutes. The count of examined *E. coli O157: H7* was decreased by 98.05%, 94.5% and 70.49% for previous treatments respectively while the count of *Salmonella Typhimurium* was decreased by 82.62%, 78.12% and 32.39% for previous treatments respectively. Some fish products with inferior quality were distributed in markets so the government must apply strict legislations on producers to ensure safe fish products for people, and must improve hygienic conditions during fishing, transportation, processing and storage of fish products.

**Key words:** Foodborne pathogens, *E.coli*, *Salmonella*, *Oreochromis niloticus*, warm water, decontaminators.

## INTRODUCTION

The production of *Oreochromis niloticus* in Egypt represents 53.67% of total fish production by 92034 ton (General Authority for Fish Resources Development, 2015). Although it is necessary to ensure food safety for the health of consumers and industry, *Salmonella* spp. and *Escherichia coli* commonly detected in many fishery products (EFSA, 2010). The incidence of food borne infections caused by bacterial pathogens continues to be a problem in industrialized nations and developing countries (Fang, 2005). These infections resulted in health and economic burdens in those countries and were especially severe in the immuno-compromised, old and young people (Bailey, 1998). Most of the reported outbreaks were caused by pathogenic bacteria especially *E. coli O157:H7* and *Salmonella* (Beuchat, 1996). *E. coli O157:H7* is one of the popular notorious foodborne pathogens, with an infectious dose of as low as a few hundred cells (Karmali, 2004). The most common food poisoning disease and widely distributed in the world is salmonellosis (Gomez *et al.,* 1997). Conventional culture-based methods, involving enrichment, isolation and confirmation steps have been used for over a century due to their sensitivity, low cost, ease of use and ability to monitor cell viability (Murakami, 2012). *E. coli O157:H7* and *Salmonella Typhimurium* are commonly found in a wide variety of raw meats, dairy products, vegetables, fish and water (Lang *et al.,* 2004; Rhee *et al.,* 2003; Strachan *et al.,* 2005; Vernozy-Rozand *et al.,* 2005; Wachtel and Charkowski, 2002). Contamination with *E. coli O157:H7* and *Salmonella* likely occurred on farms through the use of contaminated irrigation water and manure (Franz *et al.,* 2005; Johannessen *et al.,* 2005).

This study was carried out to estimate the effect of warm water (45 °C) for 5 minutes, trisodium phosphate 8% for 20 minutes and sodium hypochlorite 50 ppm for 20 minutes on *Tilapia* fillets experimentally contaminated by *E. coli O157: H7* and *Salmonella Typhimurium*.

## MATERIAL AND METHODS

**Strains:**

Reference strains (*E.coli O157: H7* and *Salmonella Typhimurium*) were collected from AHRI (Animal Health Research Institute) – Dokki – Giza – Egypt.

**Decontaminators:**

Warm water 45°C for 5 minutes dipping, trisodium phosphate 8% for 20 minutes dipping and Sodium hypochlorite 50 ppm for 20 minutes dipping were used as the decontaminators.

**Fish Samples:**

Thirty kilograms (30 kg) of *Oreochromis niloticus* were collected directly from markets in Cairo and Sharkia governorates and transferred immediately as possible in an ice-box to Laboratory of Animal Health Research Institute, Dokki, Giza, Egypt and immediately processed.

**Procedures:**

A loopful from specific strain was taken from soft agar to slope agar and then incubated for 24 hours at 37°C. Then a loopful was taken into 225 ml sterile peptone water and incubated for 24 hours at 37°C. The counts of each strain in inoculated peptone (X) (*E. coli* and *Salmonella Typhimurium*) were done.

Tilapia fillets were divided into nearly 100 gm cuts. Ten fish samples were dipped in an inoculated peptone for 15 minutes at room temperature of 25°C. Then, strain samples on sterile aluminum foil were divided into 2 groups. The first group (5 samples) was considered as inoculated samples (C) where 25 gm from each sample were added to 225 ml peptone water in stomacher bag and blended in a stomacher for 2 minutes at medium speed. The second group (5 samples 25 gm for each decontaminator) is experimentally decontaminated by decontaminators for specific dipping times and strained on sterile aluminum

foil. Then, 225 ml peptone water was added to each sample in stomacher bag and blend in a stomacher for 2 minutes at medium speed.

Bacteriological count and isolation of each sample were recorded to identify each strain by morphological characters and biochemical. Control samples were plain samples before dipping in decontaminators.

**Bacteriological Examination:**

***Escherichia coli* isolation:**

One ml of food homogenate was inoculated from each dilution into 5 tubes of 9 ml Lauryl sulphate tryptose broth (LST). LST tubes were incubated for 48 hours at 35°C and the tubes were examined for gas. From each positive tube, a loopful of suspension was transmitted to broth of Brilliant green lactose bile and incubated for 48 hours at 35°C and then tested for gas production for Colifom detection. From positive LST, a loopful was transferred from each positive LST to EC tube (*E. coli* broth). *E. coli* broth tubes were incubated in water bath for 48 hours at 45.5°C and tested for gas production for fecal Colifoms detection. Each positive EC broth was agitated gently and transferred a loopful of positive EC broth on L-EMB agar plate (Levine's eosin-methylene blue) and then incubated at 35°C for 18 to 24 hours. Plates were examined for suspected colonies of *E. coli* (violet flat colonies with a dark center with or without greenish metallic sheen) (FDA, 2011). The Biochemical tests were carried out using Indole, Methyl red, Voges Proskaur and Citrate utilization tests (Mac faddin, 2000).

***Salmonella typhimurium* isolation:**

Homogenized sample was incubated for 18 hours at 37°C (pre-enrichment). One ml of pre-enriched sample was transferred to 10 ml Rappaport-Vassiliadis (RV) and then incubated for 24 hours at 41.5°C. Loopfuls from enriched (RV) broth were separately streaked onto (XLD) Xylose Lysine Desoxycholate agar and incubated for 24 hours at 37°C. Typical colonies (red with dark center) were selected and streaked onto Triple sugar

iron (TSI) agar and incubated for 24 hours at 37°C (FDA, 2011). The Biochemical tests was carried out using Indole, Methyl red, Voges Proskaur, Citrate utilization, urea test and Hydrogen sulphide production tests (Quinn *et al.*, 2002; Mac Faddin, 2000).

**Calculation of results:**

Calculate count as follows:

$$\text{Count} = \frac{C}{v\,(n1 + 0.1n2)\,d^{\,x\ n3}}$$

**Where**: C = the sum of colonies on all plates counted, v is the volume applied to each plate, n1 = the number of plates counted at the first dilution, n2 = the number of plates counted at the second dilution, n3 = the original volume of neat suspension (i.e 10 for swab, 500 or 100 for other samples) and d = the dilution from which the first count was obtained e.g $10^{-2}$ is 0.01.

**Statistical Analysis:**

Results of microbiological analysis were reported as mean values ± standard error of mean (S.E). Microbial counts were converted to $\log^{10}$ CFU/g values. Statistical analysis of Data was done by using the statistical package for social sciences (SPSS Inc., Chicago, IL, USA) software. One way analysis of variance (ANOVA) at 95% level of confidence was done to determine significant differences in the microbial counts in samples. $P < 0.05$ was considered as significant.

## RESULTS AND DISCUSSION

To effectively inhibit growth of foodborne pathogens and spoilage bacteria in foods during storage, food antimicrobials have been added to food as a hurdle microbial growth, extending shelf life of various food products and also contribute positively to the sensory attributes. Among food antimicrobials, salts of organic acids (Knock *et al.*, 2006) and several chemical solutions (aqueous) treatments such as hypochlorite ($OCl^{-}$), trisodium phosphate

solution (TSP) were used to eliminate pathogens from seafood (Su and Morrissey, 2003).

Commonly used chlorine compounds are liquid chlorine solution (HOCl) and hypochlorite ($OCl^-$). More recently, chlorine dioxide ($ClO_2$) and electrolyzed oxidizing (EO) water have also been used for this purpose. Specifically, $ClO_2$ used as an alternative to hypochlorite and chlorine in US and Europe as it has a virucidal, bactericidal and fungicidal effect. In addition, EO water has also been shown to possess strong bactericidal activity against various foodborne pathogens (Kim *et al.*, 2000). (Rajkowski, 2012) showed the decrease in count of *E. coli* and *Salmonella* in Tilapia and catfish by heat inactivation.

**Table 1.** Effect of different decontaminants on *Oreochromis niloticus* fillet ($log^{10}$ CFU/g.) artificially inoculated with *E.coli*.

| **Parameters** | **Control** | **Warm water** | **Trisodium phosphate** | **Sodium. hypochlorite** |
|---|---|---|---|---|
| Minimum | 6.28 | 4.35 | 4.95 | 5.74 |
| Maximum | 6.48 | 4.95 | 5.30 | 5.95 |
| Mean ± SE | 6.36 ± 0.037[a] | 4.65 ± 0.099[d] | 5.1 ± 0.066[c] | 5.83 ± 0.039[b] |
| Reduction count (Reduction %) | | 1.71 (98.05 %) | 1.26 (94.5 %) | 0.53 (70.49 %) |

Reduction Count = Control - After treatment.
Reduction % = Control – After treated / Control.
[a-d]means carrying different superscript letters are significant ($P < 0.05$).

Results obtained (Table 1) showed that the mean value of *E. coli* count before dipping in different decontaminants was 6.36±0.037 log CFU/g (control), while after addition of decontaminants the mean values were 4.65±0.099, 5.1± .066 and 5.83±0.039 log CFU/g respectively for warm water (45°C) for 5 minutes, trisodium phosphate 8% for 20 minutes and sodium hypochlorite 50 ppm for 20 minutes respectively. After the addition of decontaminants, the lowest *E. coli* count was 4.35 log CFU/g obtained by

dipping in warm water for 5 minutes, while the highest *E. coli* count was 5.95 log CFU/g obtained by dipping in sodium hypochlorite 50 ppm for 20 minutes.

On the other hand, the most reduction count was obtained by dipping in warm water (45°C) for 5 minutes which represent 1.71 log CFU/g (98.05% reduction) followed by trisodium phosphate 8% for 20 minutes which represent 1.26 log CFU/g (94.5% reduction) then sodium hypochlorite 50 ppm for 20 minutes which represent 0.53 log CFU/g (70.49% reduction).

These results agreed with those reported by Northcutt *et al.* (2005), Sexton *et al.* (2007), Smith *et al.* (2007), NACMCF (2008), FDA (2009), Huang (2009), Kathleen (2012), Alonso-Hernando *et al.* (2012, 2013), Karuppasamy *et al.* (2015), while these results were lower than those obtained by Kim *et al*. (2004) (2.43 log CFU/g reduction count), Lee *et al.* (2009) (2.22log CFU/g), Xiao *et al.* (2011) (3.3 log CFU/g) in case of sodium hypochlorite and Purnell *et al.* (2014) (3.29 log CFU/g) in case of trisodium phosphate. However, these results were greater than that obtained by Purnell *et al.* (2014) (0.4 log CFU/g) in case of warm water. Most comparable studies applied on other food products as there were few researchers apply their studies on fish and its products.

Fecal coliform is an important group of coliform. *E. coli* are bacteria which are present naturally in the human intestinal tract. Its isolation indicates contamination of sea food samples by feces or sewage by using contaminated water, equipment and related persons (Pelczar *et al.*, 2005).

*Salmonellae* Spp. are important foodborne pathogen responsible for disease in humans. It has been the leading cause of many outbreaks and infections around the world and is considered as one of the major causes of human gastroenteritis worldwide (Rasschaert *et al.,* 2005). Seafood contaminated by *Salmonella* (through all the periods of production from farm to markets) is the main cause of bacterial outbreaks associated with seafood in the EU (EFSA, 2010), in the US (CSPI, 2009) and in other countries worldwide.

**Table 2.** Effect of different decontaminants on *Oreochromis niloticus* fillet ($log^{10}$ CFU/g.) artificially inoculated with *Salmonella Typhimurium.*

| Parameters | Control | Warm water | Trisodium phosphate | Sodium hypochlorite |
|---|---|---|---|---|
| **Minimum** | 4.95 | 4.20 | 4.41 | 4.90 |
| **Maximum** | 5.48 | 4.65 | 4.76 | 5.23 |
| **Mean ± SE** | 5.21 ± 0.103[a] | 4.45± 0.073[c] | 4.55± 0.061[bc] | 5.04 ± 0.062[a] |
| **Reduction count (Reduction %)** | | 0.76 (82.62%) | 0.66 (78.12 %) | 0.17 (32.39 %) |

Reduction Count = Control - After treatment.

Reduction % = Control – After treated / Control.

[a-c]means carrying different superscript letters are significant (P < 0.05).

From the results illustrated in (Table 2), the minimum, maximum and mean values of control samples were 4.95, 5.48 and 5.21±0.103 log CFU/g respectively. While by dipping in warm water for 5 minutes, these values were 4.20, 4.65, 4.45±0.073 log CFU/g. These data also recorded the minimum, maximum and mean values of *Salmonella Typhimurium* after dipping in trisodium phosphate 8% for 20 minutes (4.41, 4.76, 4.55 ± 0.061 log CFU/g). By using sodium hypochlorite 50 ppm for 20 minutes, the values of minimum, maximum and mean of *Salmonella Typhimurium* count were 4.90, 5.23 and 5.04±0.062 log CFU/g respectively.

The most reduction count was obtained by dipping fish samples in warm water for 5 minutes was 0.76 log CFU/g (82.62%), then 0.66 log CFU/g (78.12%) for trisodium phosphate 8% for 20 minutes, but the lowest reduction count was 0.17 log CFU/g (32.39%) for samples treated with sodium hypochlorite 50 ppm for 20 minutes. These results were in agreement with those obtained by Yuk *et al.* (2008), Juneja *et al.* (2010), Wenqian *et al.* (2012) and Karuppasamy *et al.* (2015), while these results were lower than the one obtained by Purnell *et al.* (2014) (2.1 and 3.29 log CFU/g reduction count) in case of using sodium hypochlorite and trisodium phosphate respectively.

However, these results were higher than the one obtained by Purnell *et al.* (2014) (0.4 log CFU/g reduction count) in case of using warm water.

## CONCLUSION

Overall, foodborne pathogens as *E. coli* and *Salmonella* in fish products could be greatly decreased by using some decontaminators as warm water (45°C), trisodium phosphate 8% and sodium hypochlorite 50 ppm. These food borne pathogens represent a public health hazard, so we need a strict governmental legislations on all food dealers to apply hygienic conditions and food safety programs to prevent or reduce food contamination. These results can be used to provide safe and hygienic food handling instructions to the consumer and producers.

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# تأثير الماء الدافئ وثلاثى فوسفات الصوديوم وهيبوكلوريت الصوديوم على الميكروب القولوني النموذجي والسالمونيلا فى شرائح أسماك البلطى الملوثة معمليا

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## الملخص العربى

فى هذه الدراسة تم فحص مدى تأثير بعض مزيلات التلوث (الماء الدافئ ٤٥°م لمدة ٥ دقائق – ثلاثى فوسفات الصوديوم ٨ % لمدة ٢٠ دقيقة – هيبوكلوريت الصوديوم ٥٠ جزء فى المليون لمدة ٢٠ دقيقة) على شرائح البلطى النيلى الملوث معمليا بميكربات التسمم الغذائى (الميكروب القولوني النموذجي – السالمونيلا).

أظهرت النتائج التأثير الايجابى لهذة المزيلات على ميكربات التسمم الغذائى تحت الدراسة، حيث انخفض العدد الكلى للميكروب القولوني النموذجي بنسبة ٩٨.٠٥ % ٩٤. ٥ % ٧٠و٤٩ % ، فى حين انخفض العدد الكلى لميكروب السالمونيلا بنسبة ٨٢.٦٢ % ٧٨.١٢ % ٣٢.٣٩ % على التوالى للمعاملات السابقة.

وفى ظل انتشار الأمراض الناتجة عن التلوث الغذائى وخاصة الأسماك ومنتجاتها فان السلطات الصحية يجب أن تهتم بصلاحية منتجات الأسماك في الأسواق مع منع تداول المنتجات مجهولة المصدر، مع الاهتمام بالأساليب الصحية في تداول المنتجات السمكية بداية من عملية الصيد وحتى التصنيع والتداول في الأسواق.