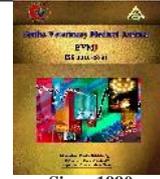




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Effect of Sodium Tri Poly Phosphate on the bacteriological quality of refrigerated Nile tilapia fillets

Hemmat M. Ibrahim¹, Mohamed A. Hassan¹, Reham A. Amin¹, Omayma M.A.L. Maghraby², Ahmed M. Mostafa²

¹ Food Hygiene and control Department, Faculty of Veterinary Medicine, Benha University

² Food Hygiene Dept., Animal Health Research Institute, Dokki.

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ABSTRACT

The effect of different concentrations of Sodium Tri Poly Phosphate (STPP) on the bacteriological quality of refrigerated Nile tilapia fillets was determined. There were significant differences ($P < 0.05$) among control with different concentrations of STPP treated samples on Total Aerobic plate count (APC) and the psychrotrophic bacteria counts. The control and 2% STPP treated samples exceeds the maximum permissible limit 6 log of APC after 6th and 9th day, respectively. While both 5% STPP and 10% STPP treated samples exceeds this limit after 12th day. Non-significant differences ($P > 0.05$) were detected among all examined samples at 0, 3rd, 6th or at 15th day on total coliform count. The highest reduction % of APC, total psychrotrophic count and total coliform count was (26.62% at the 3rd day, 32.99% at the 9th day and 20% at the 6th day) of 10% STPP treated samples, respectively. This study shows that, STPP have anti-microbial effect on APC and psychrophilic count but it has no effect on coliform bacteria. And it helps in extension of Nile tilapia fillets shelf life during refrigeration storage.

1. INTRODUCTION

Fish has highly nutritional value and digested easily. There are about 60% of people in a lot of developing countries depend on fish as a source of their animal protein supplies (El.Shehawey *et al.*, 2016).

After fish capture, some biological and chemical changes occurs in dead fish that lead to rejection for human consumption as a result of spoilage (FAO, 2013).

Preservation methods are needed for fish spoilage prevention and shelf life lengthening. Preservation methods made inhibition of the spoilage bacteria activity and decreasing the metabolic changes that lead to fish quality loss (Teklemariam, 2015).

The indirect antimicrobial effect of phosphates is due to metal ions chelation that is essential for metabolism of bacteria and integrity of cell (Goncalves *et al.*, 2008).

Treatment of fish fillets by Phosphate lead to pH increasing and moisture binding increasing (Young *et al.*, 1999). Treatment with STPP improves the fish and fish products shelf life (Kilinc *et al.*, 2009).

Using of phosphate by high amount will lead to formation of soapy taste, translucency, and slimy texture (Rattanasatheir *et al.*, 2008). and leave illegally high amount of phosphate residues in the final products (Wangtueai *et al.*, 2014).

The aim of the present study was to determine the effect of sodium tripolyphosphate on the bacteriological quality of refrigerated Nile tilapia fillets.

2. MATERIAL AND METHODS

2.1. Fish fillets preparation (Khalafalla *et al.*, 2015):

Perfectly three groups of Nile tilapia fish samples will be collected from different Egyptian fish markets located Cairo, El- Kalubia and Giza Governments at the harvesting day. Each group was separated into four subgroups. Furthermore, each fish was gutted, de-headed, cleaned and then filleted into 2 pieces of around 100g weights for each piece. Furthermore, the samples was wrapped in sterile bags from polyethylene and transferred directly to the laboratory in icebox in sterile condition within 2 h from samples purchasing.

2.2. Preparation of dipping solutions and fish fillets dipping (Moawad, *et al.*, 2017):

The food grade sodium tripolyphosphate was used for preparation of 0, 2, 5 and 10% solutions in previously chilled distilled water at 4°C. Furthermore, the samples was divided into four subgroups; 1st group was dipped into distilled water (control), 2nd group was dipped into 2% (w/v) solution /10 min, while 3rd was dipped into 5% (w/v) solution /10 min and 4th was dipped into 10% (w/v) solution /10 min in a refrigerator. After that, the dipping solution was removed. Finally, the samples will be leaved for draining about 1 min.

2.3. Packaging and storage of treated fish fillets samples

Treated samples of Nile tilapia fish fillets was labeled and packaged in sterile stomacher bags. After that, it was stored at 4 ± 1 °C in the refrigerator. The control and treated groups of samples were subjected to bacteriological examination at

* Corresponding author: dr_ahmed_gado64@yahoo.com

the zero days. And then, periodically every 3 days (0, 3rd, 6th, 9th, 12th, and 15th).

2.4. Bacteriological examination:

The prepared samples were subjected to the following examinations:

2.4.1. Determination of total aerobic plate count (ISO 4833-2, 2013).

2.4.2. Determination of total psychrotrophic count (APHA, 1992).

2.4.3. Determination of total coliform count (ISO 4832, 2006).

3. RESULTS

Regarding to data obtained in table (1), it is indicated that the initial TAPC were 4.71, 3.88, 3.81 and 3.73 (log cfu/g) in the control, 2% STPP, 5% STPP and 10% STPP fillet samples, respectively. While it was 7.95, 6.81, 6.48 and 6.50 (log cfu/g) at 15th day from refrigeration for the same examined samples respectively. A significant difference was recorded among control with STPP treated samples from each concentration in all days of examination.

The results reported in table (3) revealed that the initial total psychrotrophic bacterial counts were 2.94, 2.67, 2.20 and 2.36 (log cfu/g) in the control, 2% STPP, 5% STPP and 10% STPP fillet samples, respectively. While they were 7.47, 6.17, 5.64 and 5.45 (log cfu/g) at 15th day from refrigeration for the same examined samples respectively. There were significant differences among control with all STPP treated fish fillet samples in all days of examination.

The results recorded in table (5) indicated that the total coliform counts were 1, 1, 1 and 1 (log cfu/g) at the initial day for the control, 2% STPP, 5% STPP and 10% STPP fillet samples, respectively. And they were 2.12, 2.04, 2.03 and 2.08 (log cfu/g) at 15th day from refrigeration for the same examined samples respectively. There were non-significant differences between all examined samples at 0 day and at 15th day.

Table 1 Statistical analytical results of Total Aerobic plate count (log cfu/g) in the examined samples of tilapia fillet during refrigeration at 4°C (n=3).

Storage time (days)	APC (log cfu/g)			
	Control	2% STPP	5% STPP	10% STPP
Zero	4.71±0.08 ^A	3.88±0.11 ^B	3.81±0.10 ^B	3.73±0.06 ^B
3 rd	5.41±0.10 ^A	4.50±0.12 ^B	4.20±0.15 ^C	3.97±0.07 ^C
6 th	5.94±0.03 ^A	4.97±0.03 ^B	4.78±0.14 ^C	4.76±0.05 ^C
9 th	6.80±0.07 ^A	5.65±0.06 ^B	5.30±0.08 ^C	5.24±0.14 ^C
12 th	7.48±0.04 ^A	6.41±0.06 ^B	5.92±0.04 ^C	5.86±0.01 ^C
15 th	7.95±0.03 ^A	6.81±0.07 ^B	6.48±0.12 ^C	6.50±0.13 ^C

Mean values ± SD from the three replicates are presented. There are sig. diff. (P<0.05) between means having the different capital letters in the same row.

Table 2 Reduction % of the mean values of APC in the examined samples of refrigerated Nile tilapia fillet treated with different concentrations of STPP.

Storage time (days)	Reduction of APC (%)		
	2% STPP	5% STPP	10% STPP
Zero	17.62	19.11	20.81
3 rd	16.82	22.37	26.62
6 th	16.33	19.53	19.87
9 th	16.91	22.06	22.94
12 th	14.30	20.86	21.66
15 th	14.34	18.49	18.24

Table 3 Statistical analytical results of total Psychrotrophs count (log cfu/g) in the examined samples of tilapia fillet during refrigeration at 4°C (n=3).

Storage time (days)	Total Psychrotrophic count (log cfu/g)			
	Control	2% STPP	5% STPP	10% STPP
Zero	2.94±0.02 ^A	2.67±0.05 ^B	2.20±0.14 ^C	2.36±0.08 ^C
3 rd	3.56±0.10 ^A	3.30±0.16 ^B	2.87±0.05 ^C	2.80±0.05 ^C
6 th	4.96±0.03 ^A	3.98±0.02 ^B	3.73±0.07 ^C	3.33±0.11 ^D
9 th	5.88±0.05 ^A	4.85±0.04 ^B	4.22±0.15 ^C	3.94±0.05 ^D
12 th	6.77±0.02 ^A	5.78±0.05 ^B	4.92±0.03 ^C	4.79±0.04 ^D
15 th	7.47±0.06 ^A	6.17±0.10 ^B	5.64±0.05 ^C	5.45±0.09 ^D

Mean values ± SD from the three replicates are presented. There are sig. diff. (P<0.05) between means having the different capital letters in the same row.

Table 4 Reduction % of the mean values of Total Psychrotrophic count in the examined samples of refrigerated Nile tilapia fillet treated with different concentrations of STPP.

Storage time (days)	Reduction of Total Psychrotrophic count (%)		
	2% STPP	5% STPP	10% STPP
Zero	9.18	25.17	19.73
3 rd	7.30	19.38	21.35
6 th	19.76	24.80	32.86
9 th	17.52	28.23	32.99
12 th	14.62	27.33	29.25
15 th	17.40	24.50	27.04

Table 5 Statistical analytical results of total coliform count (log cfu/g) in the examined samples of tilapia fillet during refrigeration at 4°C (n=3).

Storage time (days)	Total coliform count (log cfu/g)			
	Control	2% STPP	5% STPP	10% STPP
Zero	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
3 rd	1.20±0.14	1.10±0.14	1.00±0.00	1.00±0.00
6 th	1.50±0.14	1.32±0.22	1.42±0.08	1.20±0.14
9 th	1.82±0.03 ^A	1.75±0.04	1.80±0.03 ^A	1.63±0.10 ^B
12 th	1.94±0.02 ^A	1.86±0.03	1.82±0.03 ^B	1.80±0.07 ^B
15 th	2.12±0.04	2.04±0.07	2.03±0.09	2.08±0.05

Mean values ± SD from the three replicates are presented. There are sig. diff. (P<0.05) between means having the different capital and small letters in the same row.

Table 6 Reduction % of the mean values of Total coliform count in the examined samples of refrigerated Nile tilapia fillet treated with different concentrations of STPP.

Storage time (days)	Reduction of Total coliform count (%)		
	2% STPP	5% STPP	10% STPP
Zero	0.00	0.00	0.00
3 rd	8.33	16.67	16.67
6 th	12.00	5.33	20.00
9 th	3.85	1.10	10.44
12 th	4.12	6.19	7.22
15 th	3.77	4.25	1.89

4. DISCUSSION

The reason of spoilage of several fresh and of most lightly preserved seafoods was the microbial activity (Lund et al., 2000).

In the present study there were difference between the values of total aerobic plate count (APC) of control and phosphate treated fish fillet samples during the storage at 4°C. At 0 day there was decreasing in APC of STPP treated samples than control one. Also, there a significant difference amongst control and all STPP treated samples. While there was no significant differences (P>0.05) amongst different concentrations of STPP treated groups. Cellular morphology changes were the cause of inhibition of bacterial growth that induced by phosphate treatment (Zaika et al., 1997). Chelation of metal ions that was essential for bacterial metabolism and cell integrity were the cause of indirect antimicrobial effects induced by phosphate treatment (Goncalves et al., 2008). Approving to, the maximum

permissible limit of APC is 6 log₁₀ CFU/g which was recommended by (Egyptian organization for standardization, 2005) in chilled fish, it was noticed that the control groups of samples exceeds this limit at 9th day of storage in refrigeration, and 2% STPP treated samples exceeds this limit at 12th day of storage. The same results reported by Moawad et al. (2017). While 5% STPP and 10% STPP treated samples exceeds this limit at 15th day of storage. The antimicrobial effects of STPP also determined by Moawad, et al. (2013), Ammar et al. (2014), Ghalati et al. (2017) and Moawad, et al. (2017).

In this study, the highest reduction % of APC was 26.62% at the 3rd day of 10% STPP treated samples. While, the lowest reduction % of APC was 14.30% at the 12th day of 2% STPP treated samples. The highest and the lowest reduction % of APC was 48.57 in STP treated Sea bass fillets and 29.03 in SMP treated saithe fillets, respectively, was reported by (Kilinc et al., 2007). According to Moawad, et al. (2017), the highest and the lowest reduction % of APC was 23.29 at the 15th day and 9.81 at the zero day of 2% STPP treated Nile tilapia fillets samples, respectively.

The reason of spoilage of aerobically stored chilled fish was the psychrotrophic organisms (Sallam, 2007). In the present work, the psychrotrophic bacteria counts of control and treated fish fillet samples raised by time raising of storage but, there was a decreasing in total psychrotrophic count of STPP treated samples comparing with control one. Also, there were significant differences between control with different samples of STPP treated in all days of examination. The psychrotrophic bacteria counts at 0 days of the Nile tilapia fillet samples ranges from 2.20 to 2.94 log₁₀ CFU/g, referring to the quality of the fish samples that was used in this study was good. There were significant differences between all examined groups of samples in the 15th day. The effect of STPP against psychrotrophic bacteria was also recorded by Abouel-Yazeed (2013), Moawad, et al. (2013), Ghalati, et al. (2017) and Moawad, et al. (2017).

In this study, the highest reduction % of Total psychrotrophic count was 32.99% at the 9th day of 10% STPP treated samples. While, the lowest reduction % of APC was 7.30% at the 3rd day of 2% STPP treated samples. The highest and the lowest reduction % of total psychrotrophic count was 46.92 in STP treated Sea bass fillets and 28.57 in SMP treated saithe fillets, respectively, as reported by Kilinc et al., (2007). According to Moawad, et al. (2017), the highest and the lowest reduction % of total psychrotrophic count was 15.30 at the 6th day and 6.53 at the 12th day of 2% STPP treated Nile tilapia fillets samples, respectively.

Presence of coliform bacteria was indication of unhygienic conditions that occurred during catching, handling and processing of fish and fishery products (The National Academy of Science, 1985). From the results presented in table (5) it was observed that the coliform bacteria had slow growth as storage time increased in the refrigerator. The same result described by Arannilewa et al. (2005). This may have occurred as coliforms are mesophilic so, they were inhibited by low storage temperatures (Mujica, 1988). Different result obtained by Al-Jasser and Al-Jasass (2014) who reported that the total viable bacterial and the total coliform were decreased by increasing time of storage in refrigerator. Non-significant differences among all examined samples at 0, 3rd, 6th or at 15th day were recorded. This may be due to gram-negative bacteria are less susceptible to inhibition by polyphosphate than gram-positive bacteria (Kim et al., 1995) and (Soultos and Genigeorgis, 1997). Referring to, the maximum permissible

limit of total coliform is 2 log₁₀ CFU/g which is recommended by Egyptian organization for standardization (EOS., 2005) in chilled fish, it could be observed that the control group and all treated groups exceeds this limit at 15th day of storage. So, there was no effect of STPP on total coliform counts.

In this work, the highest reduction % of total coliform count was 20% at the 6th day of 10% STPP treated samples. While, the lowest reduction % of APC was 0.00% at the zero days of 2%, 5% and 10% STPP treated samples. This results were partially agree with Kilinc et al., (2007), who reported that, the highest and the lowest reduction % of total coliform count was 46.90 in STP treated Sea bass fillets and 21.77 in SMP treated saithe fillets respectively.

5. CONCLUSIONS

STPP have anti-microbial effect on TAPC and psychrophilic count but it has no effect on coliform bacteria that helps in increasing of Nile tilapia fillets shelf life at refrigeration storage.

CONFLICT OF INTEREST

There is no conflict of interest.

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