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EFFICIENCY OF BIFIDOBACTERIUM WITH/OR SALTS OF SORBIC ACID ON THE QUALITY OF CHILLED AND FROZEN FISH FILLET

(With 6 Tables and 12 Figures)

By

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تأثير بكتيريا البفيدو وأملاح حمض السوربيك على جودة الأسماك المحفوظة بالتبريد والتجميد

نادية عبد الفتاح أبوسريع

تم حفظ شرائح السمك البلطى مقسماً إلى مجموعتين رئيسيتين، الأولى كمجموعة ضابطة وأما المجموعة الثانية فقد قسمت إلى ثلاثة مجموعات فرعية معالجة (الأولى عولجت بالبيفيدوبكتيريا والثانية بمادة الصوديوم سوربات ١,٥٪ والثالثة عولجت بخليط من البيفيدوبكتيريا ومادة الصوديوم سوربات ١,٥٪) وحفظت فى درجة حرارة ٤° م لمدة ١٤ يوم والأخرى فى الفريزر المنزلى عند درجة حرارة – ١٨° م لمدة ستة أشهر فى وجود مجموعة ضابطة مع كل منهما. وتم تعيين فترة الصلاحية للمجموعات الثلاثة بالتقييم الحسى والكيميائى والبكتيرى وقد تبين أن معالجة شرائح السمك بخليط من البيفيدوبكتيريا ومادة الصوديوم سوربات ١,٥٪ كانت من أفضل المعاملات التى تحافظ على جودة شرائح سمك البلطى طوال فترة التخزين فى كل من درجات الحرارة السابقة. ولذلك يمكن الإشارة إلى أن إستخدام مخلوط من البيفيدوبكتيريا ومادة الصوديوم سوربات ١,٥٪ مناسباً للحفاظ على جودة شرائح سمك البلطى طوال فترة التخزين سواءً بالتبريد عند درجة حرارة ٤° م أو التجميد عند درجة حرارة – ١٨° م.

SUMMARY

Bolti fish (*Tilapia nilotica*) fillets were divided into two groups, 1st group considered as a control one and the 2nd group was subdivided into three subgroups (1st group was treated with Bifidobacterial culture, 2nd group was treated with potassium sorbate 1.5% and the 3rd group was treated with a combination of Bifidobacterial culture and potassium sorbate 1.5%). These treated groups were stored with a control group at 4°C for 14 days and the other was stored at -18°C for 6 months. Some of physiochemical, microbiological and organoleptic characteristics of

Bolti fish fillets were studied and evaluated. The results indicated that using of mixture of potassium sorbate and Bifidobacterial culture maintained fish fillets in a good condition up to 24 weeks (storage period) for samples kept at -18°C and improved the physiochemical, microbiological and organoleptic characteristics for samples stored at 4°C for 12 days. The use of combination of Bifidobacterial culture and potassium sorbate 1.5% for keeping stored Tilapia fillets stored at both 4°C or at -18°C was recommended.

Key words: *Fish, bifidobacterium, sorbic acid.*

INTRODUCTION

Consumption of seafood is increasing and most consumers prefer fresh or fresh like seafood products. This high demand for seafood in restaurant, supermarkets, delicatessens and other outlets has improved market stability (Beuchat *et al.*, 1975).

Quality and safety of refrigerated and freezed foods have been enhanced by preventing growth or destroying aerobic spoilage bacteria and foodborne pathogens during storage and handling using additives and preservatives (Gilliand and Ewell, 1983; Lindgren and Dobrogosz, 1990; Kim *et al.*, 1995a). Bio preservatives such as bifidobacteria may control food spoilage bacteria through production of lactic acid and acetic acid as well as other antibiotic substances (Larioia and Martin, 1991; Ray, 1992; Vazquez *et al.*, 2005). Previous works have shown that potassium sorbate effective in suppressing growth of aerobic spoilage bacteria and increase shelf life of seafoods (Godavari *et al.*, 1987; Kondaiah *et al.*, 1985; Mendonca *et al.*, 1989a; Unda *et al.*, 1990; Zhuang *et al.*, 1996).

This study, therefore, was mainly initiated to evaluate the possibility of extending the shelf life of refrigerated and frozen fish treated with combination of bifidobacteria and food-grade organic salts.

MATERIALS and METHODS

Lot of fresh Bolti fish (*Tilapia nilotica*) 25 kg used in this experiment were purchased from El-Abour market, Cairo. Fish fillets were obtained after evisceration and removal of scales and skin of fresh fish. Fish flesh were then cut into parts and then packed in ice-box with ice ratio 1:2. The samples were directly transported to the laboratory without delay and then classified into two main groups, which they

subdivided into two subgroups. The 1st group was used as a control one while the 2nd group was divided into three treated groups each was treated with Bifidobacteria culture, potassium sorbate 1.5% and combination of 1.5% potassium sorbate and Bifidobacterial culture. One of the main group was kept at cold storage at 4±1°C (crushed ice 1:2) through the storage period (14 days). Melted ice was drained daily and was replaced with ice when needed. Every 24 hours intervals samples were withdrawn and assed for examinations.

The 2nd main group were packed in polyethylene bag and were immediately stored at home freezer (-18°C) for 24 weeks. Two hundred gm of fish fillets were examined periodically every 2 weeks. Each sample was thawed at 10°C overnight prior to examination.

Preparation of bifidobacteria cultures:

Bifidobacterium infantis (ATCC 15697) was obtained from Food Technology and Dairy Department, National Research Center, Dokki, Cairo. Stock cultures were maintained in sterile skim milk medium (SM; 10% skim milk. 0.5% yeast extract and 0.5% glucose) at 37°C for 24 hours in an anaerobic jar.

Bifidobacteria were enumerated by pour plating appropriate serial dilutions in 0.1% peptone water with neomycin-paramycin-nalidixic acid-lithium chloride agar (Teraguchi *et al.*, 1978; Laroia and Martin, 1991) followed by incubation at 37°C for 48 hours in an anaerobic jar (Gas-Pack; BBL) prior to counting colonies. Bifidobacteria cultures in skim milk (6.0×10^7 to 1.0×10^8 CFU/ml) were added at a given percentage (v/v) to fresh *Tilapia nilotica*, final skim milk pH was 4.4-4.8 alone or was thoroughly mixed with solution of 1.5% potassium sorbate (organic acid). Fish fillets were dipped for 2 minutes in 500 ml of the treatment solution with gentle swirling using a sterile glass rode to ensure complete contact with treatment solution. Fillets were then removed from the treatment solution with sterile tongs and allowed to drain for 2 minutes on a sterile metal net. After draining the excess solution, samples were placed into sterile polyethylene bags and stored according to its group of experiment which was subjected to quality evaluation tests as the following:

A- Sensory evaluation:

The sensory evaluation was carried out according to the technique reported by More and Iriter, (1970). The items examined were appearance, consistency, tenderness, flavour, and overall eating quality.

The quality of fish fillet were graded excellent, very good, good, medium, fair, poor and the score system points 7, 6, 5, 4, 3, 2, and 1 respectively.

B- Chemical examination:

Deterioration criteria assayed in samples of fish fillets by determining the following:

1- pH value was carried out according to the method of AOAC (1990) by using pH meter (Jenco, Digital pH meter 609).

2- Total Volatile Basic Nitrogen (TVBN) (mg/100gm) was determined according to the method described by FAO (1980).

C- Bacteriological examination:

The bacteriological methods were carried out according to APHA (1992).

1- Aerobic Plate Count.

2- Total Enterobacteriaceae count.

3- Enumeration of total coliforms count (MPN/gm).

4- Detection of *E. coli.*

5-*Staphylococcus aureus* count.

RESULTS

Table 1: Sensory evaluation of control and treated samples stored at 5°C. (Mean of 3 samples).

Time	Control		1		2		3	
	Mean	Quality	Mean	Quality	Mean	Quality	Mean	Quality
0 time	7.0	Excellent	7.0	Excellent	7.0	Excellent	7.0	Excellent
2nd day	5.0	Good	7.0	Excellent	7.0	Excellent	7.0	Excellent
4th day	3.8	Medium	5.0	Good	5.5	Good	7.0	Excellent
6th day	3.0	Fair	4.0	Medium	4.5	Medium	6.0	V. good
8th day	2.0	Poor	3.0	Fair	4.0	Medium	5.1	Good
10th day	-	-	2.0	Poor	3.0	Fair	4.3	Medium
12th day	-	-	-	-	2.5	Poor	3.8	Fair
14th day	-	-	-	-	-	-	3.0	Fair

1= Examined samples treated with Bifidobacterial culture
2= Examined samples treated with Potassium sorbate 1.5%
3= Examined samples treated with combination of Bifidobacterial culture and potassium sorbate 1.5%

Table 2: pH values and Total Volatile Basic Nitrogen (TVBN) (mg/100gm) of control and treated samples stored at 5°C.

Time	pH				TVBN (mg/100gm)			
	Control	1	2	3	Control	1	2	3
0 time	6.0	5.8	5.7	5.6	12.0	12.0	12.0	12.0
2nd day	6.1	5.8	5.7	5.6	21.0	17.0	16.0	14.0
4th day	6.4	5.8	5.8	5.7	29.0	23.6	22.0	19.0
6th day	6.5	6.0	5.9	5.8	34.3	29.0	28.0	23.0
8th day	6.7	6.2	6.0	5.9	40.0	35.2	33.0	25.0
10th day	-	6.7	6.2	6.0	-	40.0	38.1	27.0
12th day	-	-	6.6	6.1	-	-	40.0	28.3
14th day	-	-	-	6.2	-	-	-	33.0

1= Examined samples treated with Bifidobacterial culture
2= Examined samples treated with Potassium sorbate 1.5%
3= Examined samples treated with combination of Bifidobacterial culture and potassium sorbate 1.5%

Table 3: Microbiological quality of control and treated samples stored at 5°C.

Time	APC CFU/g				Enterobacteriaceae count CFU/g				Coliforms count (MPN/g)				*Staphylococcus aureus* count CFU/g			
	Control	1	2	3	Control	1	2	3	Control	1	2	3	Control	1	2	3
0 time	2×10^4	2×10^4	2×10^4	2×10^4	10^3	10^3	10^3	10^3	92	92	92	92	3×10^2	3×10^2	3×10^2	3×10^2
2nd day	4.6×10^4	3.1×10^4	2.6×10^5	2.2×10^4	6.2×10^3	4.2×10^3	2.5×10^3	1.5×10^3	92	75	70	40	2.5×10^2	1.5×10^2	1.5×10^2	10^2
4th day	8×10^4	5×10^4	3.9×10^4	3.0×10^4	9.3×10^3	6.1×10^3	5×10^3	2.3×10^3	92	45	40	31	2×10^2	10^2	10^2	10^2
6th day	7.2×10^5	2.1×10^5	1.5×10^5	7.5×10^4	5.1×10^4	1.5×10^4	10^4	9.7×10^3	110	35	31	25	1.5×10^2	10^2	10^2	10^2
8th day	6.2×10^6	10^6	9.8×10^5	2.1×10^5	9.2×10^5	4.2×10^4	3.1×10^5	10^4	120	25	25	23	1.5×10^3	10^2	10^2	10^2
10th day	3×10^7	3.1×10^6	1.5×10^6	4.2×10^5	3.9×10^5	9.1×10^4	5.1×10^4	1.5×10^4	140	23	21	11	3×10^3	10^2	10^2	$<10^2$
12th day	2×10^8	10^7	9.7×10^6	8.3×10^5	7.9×10^5	1.2×10^5	9.2×10^4	3.2×10^4	170	21	11	9	4×10^3	$<10^2$	$<10^2$	$<10^2$
14th day	10^9	10^8	9.8×10^7	7×10^6	9×10^6	7.9×10^5	1.3×10^5	8.7×10^4	210	11	9	<3	9×10^3	$<10^2$	$<10^2$	$<10^2$

1= Examined samples treated with Bifidobacterial culture
2= Examined samples treated with Potassium sorbate 1.5%
3= Examined samples treated with combination of Bifidobacterial culture and potassium sorbate 1.5%

Table 4: Sensory evaluation of control and treated samples stored at -18°C (Mean of 3 samples).

Time	Control		1		2		3	
	Mean	Quality	Mean	Quality	Mean	Quality	Mean	Quality
0 time	7.0	excellent	7.0	Excellent	7.0	Excellent	7.0	Excellent
2 weeks	6.0	V. good	6.0	V. good	6.0	V. good	6.4	V. good
4 weeks	5.0	Good	6.0	V. good	6.0	V. good	6.3	V. good
6 weeks	5.0	Good	6.0	V. good	6.0	V. good	6.2	V. good
8 weeks	4.0	Medium	6.0	V. good	6.0	V. good	6.1	V. good
10 weeks	4.0	Medium	5.0	Good	6.0	V. good	6.0	V. good
12 weeks	3.5	Fair	5.0	Good	5.0	Good	6.0	V. good
14 weeks	3.0	Fair	4.0	Medium	5.0	Good	6.0	V. good
16 weeks	2.0	Poor	3.7	Fair	4.0	Medium	5.0	Good
18 weeks	1.3	V. poor	3.5	Fair	4.0	Medium	5.0	Good
20 weeks	-	-	3.2	Fair	3.8	Fair	4.0	Medium
22 weeks	-	-	2.9	Fair	3.0	Fair	3.5	Fair
24 weeks	-	-	2.3	Poor	2.5	Poor	3.0	Fair

1= Examined samples treated with Bifidobacterial culture
2= Examined samples treated with Potassium sorbate 1.5%
3= Examined samples treated with combination of Bifidobacterial culture and potassium sorbate 1.5%

Table 5: pH values and Total Volatile Basic Nitrogen (TVBN) (mg/100gm) of control and treated samples stored at -18°C.

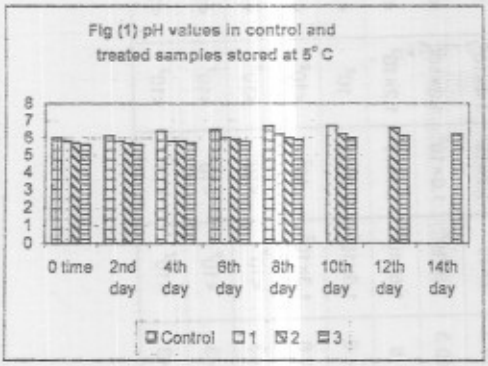
Time	pH				TVBN (mg/100gm)			
	Control	1	2	3	Control	1	2	3
0 time	5.90	6.50	5.50	5.5	12.00	12.00	12.00	12.00
2 weeks	5.93	5.60	5.60	5.63	15.44	9.80	8.90	8.70
4 weeks	6.03	5.70	5.60	5.65	17.23	11.30	9.70	9.20
6 weeks	6.06	5.80	5.7	5.71	20.57	11.60	11.20	11.00
8 weeks	6.13	5.90	5.8	5.75	22.46	14.30	11.50	11.20
10 weeks	6.23	6.00	5.9	5.78	25.30	17.01	14.20	11.50
12 weeks	6.36	6.10	6.00	5.80	27.74	17.90	17.00	14.00
14 weeks	6.43	6.20	6.10	5.90	30.00	19.70	17.60	15.22
16 weeks	6.50	6.30	6.20	6.00	32.93	21.80	19.20	17.00
18 weeks	-	6.40	6.30	6.10	-	24.30	24.00	20.60
20 weeks	-	6.50	6.40	6.20	-	26.90	26.00	22.30
22 weeks	-	6.60	6.50	6.30	-	28.60	28.00	27.00
24 weeks	-	6.70	6.60	6.40	-	31.60	31.00	30.00

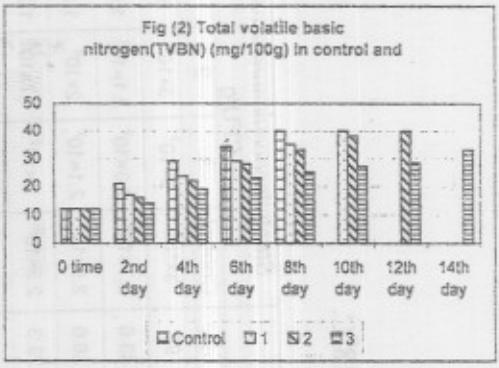
1= Examined samples treated with Bifidobacterial culture
2= Examined samples treated with Potassium sorbate 1.5%
3= Examined samples treated with combination of Bifidobacterial culture and potassium sorbate 1.5%

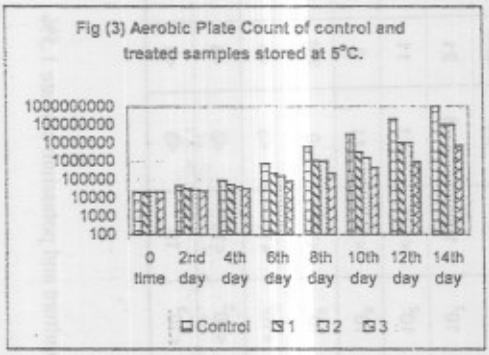
Table 6: icrobiological quality of control and treated samples stored at -18°C.

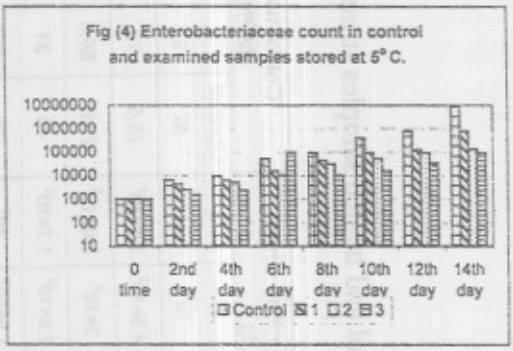
Time	APC CFU/g				Enterobacteriaceae count CFU/g				Coliforms count (MPN/g)				*Staphylococcus aureus* count CFU/g			
	Control	1	2	3	Control	1	2	3	Control	1	2	3	Control	1	2	3
0 time	8×10^4	6×10^4	4×10^4	2×10^4	10^3	10^3	10^3	10^3	92	92	92	92	3×10^2	3×10^2	3×10^2	3×10^2
2 weeks	5.8×10^4	5.1×10^4	3.1×10^4	8.8×10^3	7.6×10^2	5.5×10^2	4.5×10^2	3.1×10^2	76.6	70	66.6	43.6	2.7×10^2	2.3×10^2	2.1×10^2	1.6×10^2
4 weeks	5.3×10^4	4.1×10^4	3×10^4	6.2×10^3	5.2×10^2	5×10^2	3×10^2	2.6×10^2	76.6	66.6	43.6	28.6	2.5×10^2	2.1×10^2	2×10^2	1.5×10^2
6 weeks	4.6×10^4	3.8×10^4	2.2×10^4	2.6×10^3	4.3×10^2	3.5×10^2	2.5×10^2	1.7×10^2	40	31	28.6	22.3	2.3×10^2	2×10^2	1.7×10^2	1.3×10^2
8 weeks	3.1×10^4	3.1×10^4	1.5×10^4	1.1×10^3	3.3×10^2	2.5×10^2	1.5×10^2	10^2	25	23.3	22.3	21.6	2.1×10^2	1.8×10^2	1.5×10^2	1.2×10^2
10 weeks	1.5×10^4	2.5×10^4	1.2×10^4	10^3	1.5×10^2	1.3×10^2	10^2	10^2	23	22.3	21.6	14.3	2×10^2	1.7×10^2	1.4×10^2	10^2
12 weeks	8.3×10^3	1.9×10^4	0.9×10^4	7.6×10^2	10^2	10^2	10^2	10^2	21.6	21	11	10.3	1.8×10^2	1.6×10^2	1.3×10^2	$<10^2$
14 weeks	6.3×10^3	9.5×10^3	7.6×10^3	6.8×10^2	10^2	10^2	10^2	$<10^2$	11	11	9	5	1.5×10^2	1.4×10^2	1.2×10^2	$<10^2$
16 weeks	7.4×10^3	5.6×10^3	4.8×10^3	4.4×10^2	10^2	10^2	10^2	$<10^2$	11	9	7.6	4.6	1.6×10^2	1.4×10^2	10^2	$<10^2$
18 weeks	3.5×10^3	1.5×10^3	1.1×10^3	3.5×10^2	10^2	10^2	10^2	$<10^2$	9	7.6	5	2.6	1.3×10^2	10^2	$<10^2$	$<10^2$
20 weeks	2.5×10^3	1.3×10^3	1.3×10^3	2.9×10^2	10^2	10^2	$<10^2$	$<10^2$	<3	<3	<3	<3	$<10^2$	$<10^2$	$<10^2$	$<10^2$
22 weeks	1.5×10^3	10^3	10^3	2.9×10^2	$<10^2$	$<10^2$	$<10^2$	$<10^2$	<3	<3	<3	<3	$<10^2$	$<10^2$	$<10^2$	$<10^2$
24 weeks	10^3	7.2×10^2	5.2×10^2	2.1×10^2	$<10^2$	$<10^2$	$<10^2$	10^2	<3	<3	<3	<3	$<10^2$	$<10^2$	$<10^2$	$<10^2$

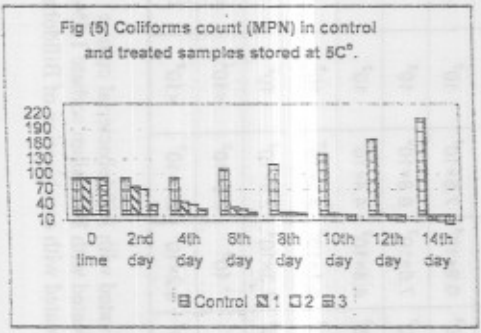
1= Examined samples treated with Bifidobacterial culture
2= Examined samples treated with Potassium sorbate 1.5%
3= Examined samples treated with combination of Bifidobacterial culture and potassium sorbate 1.5%

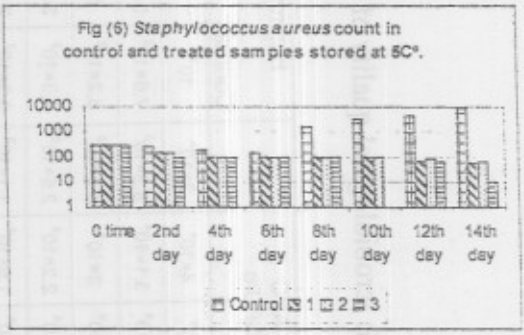












1= Examined samples treated with Bifidobacterial culture
2= Examined samples treated with Potassium sorbate 1.5%
3= Examined samples treated with combination of Bifidobacterial culture and potassium sorbate 1.5%

Fig. (7) pH values of control and treated samples stored at -18°C.

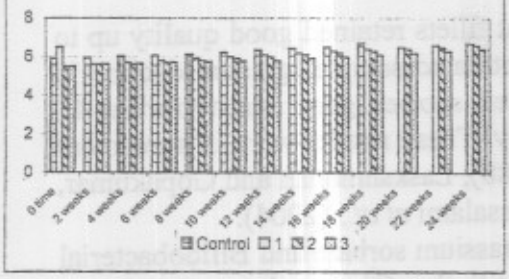


Fig. (8) Total Volatile Basic Nitrogen (TVBN) (mg/100gm) in control and treated samples stored at -18°C.

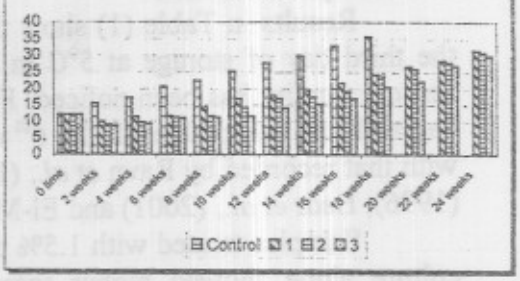


Fig. (9) APC of control and treated samples stored at -18°C.

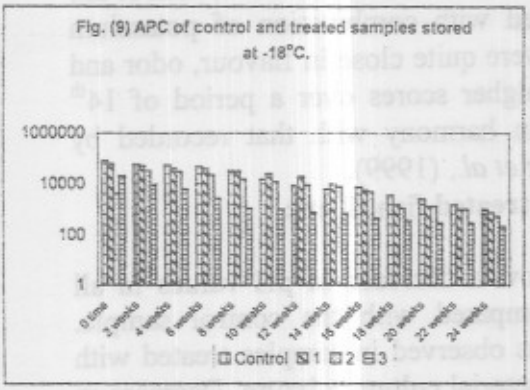


Fig. (10) Enterobacteriaceae count of control and treated samples stored at -18°C

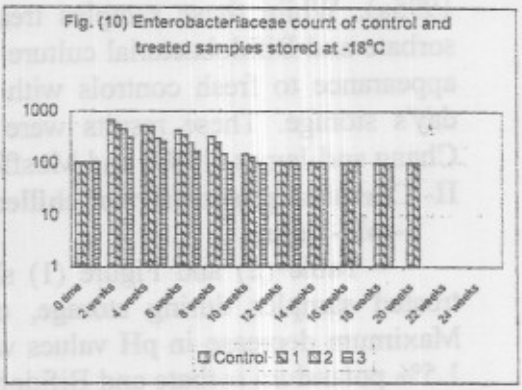


Fig. (11) Coliforms count (MPN) of control and treated samples stored at -18°C.

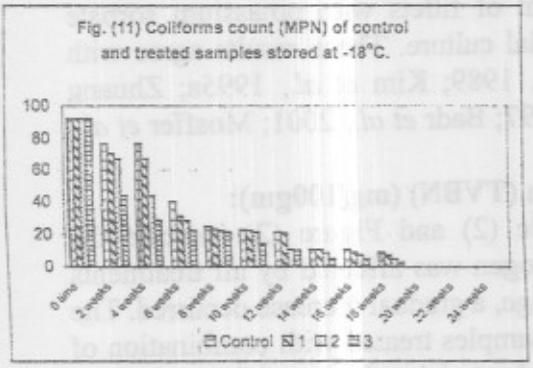
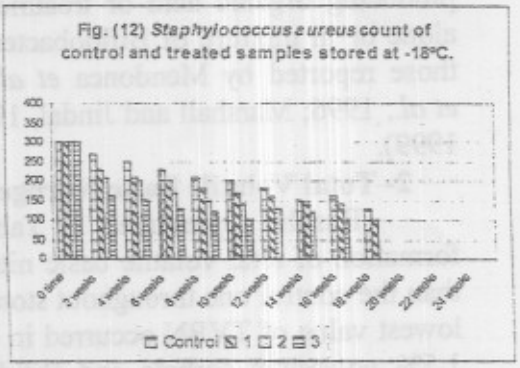


Fig. (12) *Staphylococcus aureus* count of control and treated samples stored at -18°C.



1= Examined samples treated with Bifidobacterial culture
2= Examined samples treated with Potassium sorbate 1.5%
3= Examined samples treated with combination of Bifidobacterial culture and potassium sorbate 1.5%

DISCUSSION

Chilled fish fillets at 5°C±1:

I- Sensory evaluation:

Results in Table (1) show that fillets retained good quality up to the third day of storage at 5°C in untreated sample; gradual decline in sensory quality has been noticed. Fillets showed good quality for the 4th day and just acceptable till the 6th day. These results were in agreement with that recorded by Ravn *et al.*, (1988); Laskshmanan and Gopakumar, (1996); Badr *et al.*, (2001) and El-Mossalami *et al.*, (2004).

Samples treated with 1.5% potassium sorbate and Bifidobacterial culture alone showed higher score till the 6th day than control one (Chang and James, 1994; Farid *et al.*, 1998; Badr *et al.*, 2001; Kim *et al.*, 1995a). While those samples treated with combination of potassium sorbate and Bifidobacterial culture were quite close in flavour, odor and appearance to fresh controls with higher scores over a period of 14th day's storage. These results were in harmony with that recorded by Chang and James (1994) and Mosffer *et al.*, (1999).

II- Chemical parameters of chilled treated fish fillets:

1- pH-Value:

Table (2) and Figure (1) show a decrease in pH values in all treated samples during storage, compared with the control sample. Maximum decrease in pH values was observed in samples treated with 1.5% potassium sorbate and Bifidobacterial culture mixture. Decrease in pH values may be due to the microbial enzyme activity and autolysis producing organic acid or treatment of fillets with potassium sorbate alone or in mixture of Bifidobacterial culture. These results agree with those reported by Mendonca *et al.*, 1989; Kim *et al.*, 1995a; Zhuang *et al.*, 1996; Marshall and Jindal, 1997; Badr *et al.*, 2001; Mosffer *et al.*, 1999).

2- Total Volatile Basic Nitrogen (TVBN) (mg/100gm):

Results represented in Table (2) and Figure (2) indicate the formation of total volatile basic nitrogen was affected by all treatments than the control one throughout storage, a gradual increase occurred. The lowest value of TVBN occurred in samples treated with combination of 1.5% potassium sorbate and Bifidobacterial culture, while maximum TVBN was found in control samples followed by samples treated with Bifidobacterial culture, 1.5% potassium sorbate and combination of 1.5% potassium sorbate and Bifidobacterial culture.

Connel (1990) reported that the content of TVBN is a useful indicator of freshness of lean fish and suggested 30-40 mgN/100g. However, the increment in TVBN during storage in ice could be the result of decomposition and degradation of nitrogen substance which may be due to the activity of microorganisms. These results are in line with those obtained by Woyewoda and Bilgh (1986); Khuntia *et al.*, (1993); Badr *et al.*, (2001).

III- Microbiological examination of chilled treated fish fillets:

Results in Table (3) and Figure (3) indicate maximum APC was observed in control samples followed by the fish fillets treated with Bifidobacterial culture, potassium sorbate 1.5% alone and combination of potassium sorbate 1.5% and Bifidobacterial culture respectively.

It is worthily to mention that the decrease of APC in those treated with 1.5% potassium sorbate may be due to its bacteriostatic effect (Buncic *et al.*, 1995; Badr *et al.*, 2001) and extend shelf life of fish during storage at 5°C±1.

APC was higher in control and treated samples with Bifidobacterial culture and 1.5% potassium sorbate during storage for 12 days at 5°C±1. These results coincide with those given by Mendonca *et al.*, (1989a); Khuntia *et al.*, (1993); Buncic *et al.*, (1995); Kim *et al.*, (1995a); Zhuang *et al.*, (1996); Marshal and Jindal, (1997); Badr *et al.*, (2001).

Chang *et al.*, (1995) reported that APC in refrigerated (4°C) Tilapia fillets were directly affected by potassium sorbate.

With respect to those treated with Bifidobacterial culture, the observed decrease was due to the antimicrobial property of Bifidobacterial culture which produce lactic, acetic acid, hydrogen peroxide and possibility unknown compounds.

APC of fillets with Bifidobacterial culture alone rapidly increased after 3 days of storage, but fillets were not considered spoiled by the sensory panel until after 6 days.

Aerobic food spoilage organisms in refrigerated food can reduce shelf life and microbiological quality (Reddy *et al.*, 1970; Shewan, 1971; Gilliland and Specks, 1975; Post *et al.*, 1985; Ingham, 1989; Berry *et al.*, 1991). The combination of lactic acid producing bacteria (BIfidobacterial culture) and food additives could be considered as a food preservative to repress growth of such microorganisms (Reddy *et al.*, 1970; Gilliland and Ewell, 1983; Lindgren and Dobrogoze, 1990).

Table (3) revealed that APC in untreated fish rapidly increased for 14 days. While counts from a treatment with combination of

potassium sorbate and lactic acid culture caused a significant decrease during the 6 days of storage at pH 5.8. However, undesirable microorganisms grew visibly after 9 days. This was in agreement of Kim and Hearnberger (1994) and Chang *et al.*, (1995) who stated that combination of Bifidobacteria with potassium sorbate efficiency inhibited growth of Gram negative bacteria in refrigerated catfish fillets.

From the achieved results in Table (3) and Figure (4), Enterobacteriaceae count at zero time were 10^3 increased after 2, 4, 6, 8, 10, 12 and 14 days of storage at 5°C, which become 3.9×10^5 (signs of rejection was noticeable), Coliforms could be detected with slight increase from 92 till reached 210 CFU/ml at the end of storage period (Table 3 and Figure 5). *E. coli* failed to be detected in control and treated samples. These findings were in agreement with those reported by Rose (1968); Abd Almenom (1986) and Daoud and El-Mossalami (2002) who stated that Gram negative bacteria are more susceptible to cold. There is a marked decrease in total bacterial, Enterobacteriaceae, Coliforms and Staphylococcal counts in samples treated with 1.5% potassium sorbate than other control samples during storage at 5°C for 18 days. These results coincide with those given by Mendonca *et al.*, (1989b); Khuntia *et al.*, (1993); Buncic *et al.*, (1995); Kim *et al.*, (1995b); Zhuang *et al.*, (1996); Marshal Jindal (1997) and Badr *et al.*, (2001).

It is worthily, to mention that in samples treated with Bifidobacterial culture, there was a marked decrease in all bacterial counts (plate count, Enterobacteriaceae, Coliforms and Staphylococcal count), as Bifidobacterial culture having a pronounced inhibiting effect on Gram negative and Gram positive bacteria (Reddy *et al.*, 1984; Schaack and Marth, 1988; Wijsman *et al.*, 1989; Harris *et al.*, 1991; Okereke and Montville, 1991; Kebbary, 1995 Badawi and El-Sonbaty, 1997; Abou-Dawood, 2002).

Bifidobacteria may control food spoilage bacteria and foodborn pathogens through production of lactic and acetic acids as well as other antibiotic substances (Laroia and Martin, 1990; Modler *et al.*, 1990; Hughes and Hoover 1991; Ray 1992).

These results agree with Martin and Chou (1992); Biavati *et al.*, (1992) and Mehanna *et al.*, (2002) who observed that 88% of Bifidobacterial strains tested decrease their viability after 7 days of storage under acidic conditions (skim milk at pH 4.0) and less than 10% survived after 15 days. Others recorded a decrease in 0.5 log cycles after 4months at refrigeration (Robinson, 1990; Gomes *et al.*, 1995; Mehanna *et al.*, 2002).

Blanchette *et al.*, (1996) and Effat (2002) found that the presence of lactic and acetic acids inhibit the growth of Bifidobacteria after 4 weeks of refrigeration storage.

A previous report on treated fillets with potassium sorbate or combined with Bifidobacteria attributed antimicrobial effects primarily to potassium sorbate (Kim and Hearnsbergers 1994).

From our results, an additive interaction occurred when Bifidobacteria were combined with potassium sorbate, the initial pH values of fillets treated with 1.5% potassium sorbate and Bifidobacteria, either alone or combined were 5.7, and 5.6 Units, lower than untreated control fillets pH (6.0). Hence, inhibitory effect results in our study were likely due to changes in pH and to the specific action of potassium sorbate. These results were in agreement of Chang *et al.*, (1995) and disagreed with Kim and Hearbsberger (1992b) who stated that treated catfish fillets with organic acid didn't necessarily have lower pH than controls, but remained inhibitory to growth of aerobes (Kim and Hearnsberger, 1994). Quality and safety of refrigerated foods have been enhanced by preventing growth or destroying aerobic spoilage bacteria and foodborne pathogens during storage and handling using food additives and biopereservatives (Gilliland and Ewell, 1983; Lindgren and Dobrogosz, 1989; Kim and Hearnsberger, 1994). However, biopreservatives such as lactic acid bacteria suppress aerobic bacteria that cause food spoilage (Raccach and Baker, 1978; Gilliland and Ewell, 1983; Schaack and Marth, 1988).

Frozen fillets at -18°C±1:

I- Sensory evaluation:

The data obtained during experiment in Table (4) showed that organoleptic scores had occurred in frozen samples during the successive weeks of storage at home freezer (-18°C) where it reached a suggestive limit of 3 at the 14th week of storage.

The organoleptic scores of treated fillets with Bifidobacterial culture decreased gradually to suggestive limits 2.9 at the 22nd week of storage at home freezer (-18°C), while reached 2.3 at the end of experiment 24th week of storage.

The organoleptic scores of treated Tilapia fillets with 1.5% potassium sorbate gradually decreased till reached a suggestive limits 3.0 at the 22nd week of storage at home freezer, while scores of 2.5 at the 24th week of storage (end of the experiment).

Within each treatment, acceptability decreased with increasing storage time with the exception of combination of 1.5% potassium

sorbate and Bifidobacterial culture treatment was the best in maintaining the acceptability till the end of storage period (24 weeks). These values agreed well with the microbiological shelf life. These results agreed with those of Mocking and Machava (1986); Benner *et al.*, (1994) and Kim *et al.*, (1995b).

The difference in keeping quality time is due to the nature of the initial microflora present on fish at the time of capture as well as the effect of freezing on the microbial load. Moreover, the use of 1.5% potassium sorbate, Bifidobacterial culture and/or combination of them on treated samples had antimicrobial effect on the surface contaminations of fish samples. This held the view reported by Jadhav and Magar (1970); Choi *et al.*, (1986); Joseph *et al.*, (1989) and Cano-Munoz (1991).

II- Chemical parameters of frozen treated fish fillets:

1- pH-Value:

The data obtained during this investigation recorded in Table (5) and Figure (7) regarding the pH values of the fresh samples was 5.9. Gradual increase in the pH values of frozen samples occurred during successive weeks of storage at home freezer (-18°C), where it reached a limit of 6.23 at the 10th week and 6.7 at the 18th week.

The mean pH value of Tilapia fish fillets samples treated with Bifidobacterial culture subjected to a slight decrease (5.6) immediately after treatment then showed a gradual increase where it arrived a level of 6.1, 6.6 and 6.7 at the 12th, 22nd and 24th weeks of storage respectively at home freezer (-18°C).

The mean pH values of Tilapia fillets samples treated by 1.5% potassium sorbate (Table 5 and Figure 7) subjected to a slight decrease (5.6) immediately after treatment, then showed a gradual increase where it arrived a level of 6.0, 6.5, and 6.6 at 12th, 22nd and 24th weeks of storage respectively at home freezer (-18°C).

It is of importance to recognize that the maximum pH limit given by EOS (1991) had been encountered in the frozen samples stored for 10-12weeks at -18°C is 6.2. On the other hand, such limit had been observed at 10, 14, 16, and 20 weeks of storage at home freezer for control, treated samples with Bifidobacterial culture, 1.5% potassium sorbate and combination of them respectively at the same temperature.

The present data reported here, indicated that maximum pH limit stipulated by EOS (1991) has been recognized in the frozen untreated fillets samples after 10 weeks of storage at home freezer (-18°C) whereas treated samples showed such limit within 14th, 16th and 24th week with

Bifidobacterial culture, potassium sorbate, or mixture of 1.5% potassium sorbate and Bifidobacterial culture of storage at the same degree of temperature.

In this regard, Eitenmiller *et al.*, (1982) considered that, the pH value was not a good diagnostic indicator for the quality of fish as well as unsatisfactory to indicate early stages of spoilage in frozen fishes.

Meanwhile, Galli *et al.*, (1993) held the opinion that gradually increased pH values of fishes during freeze storage indicated bacterial growth and possible spoilage of fish. A pH value of more than 6.6 had been reported for spoiled fish and could be attributed to the decomposition of fish protein to the level of amino acids followed by their decarboxylation and deamination and production of volatile basic compounds such as ammonia.

From the above mentioned discussion, it could be safely concluded that the treatment of fish by 1.5 potassium sorbate and/or Bifidobacterial culture were the best treatment lead to extending the keeping quality of frozen fishes comparison with the untreated frozen fishes.

2- Total Volatile Basic Nitrogen (TVBN) (mg/100gm):

It is evident from the results recorded in Table (5) and Figure (8) that the estimated TVBN values reached 32.92 mg/100gm of the examined control samples at 16^{th} week of storage whereas in treated samples they were 31.6, 31 and 30 mg/100gm of samples treated with Bifidobacterial culture, potassium sorbate 1.5% and combination of both at 24 week of storage respectively.

Meanwhile, EOS (1991) stipulated the maximum limit of TVBN for frozen fish to be not more than 30 mg/100g of fish flesh. The gradual increase of TVBN in present data may be attributed to several volatile odour bearing compounds like the volatile basic nitrogenous compounds produced in fish as a result of bacterial spoilage which are not normally found in live muscles. The increase TVBN is logarithmically parallel with microbial growth; therefore it may provide useful data for the evaluation of fish freshness. This substitutes the finding reported by Botta *et al.*, (1984).

In this respect, Ehira *et al.*, (1984) and Person (1984) also stated that the fish at the point of incipient deterioration contain 30 mg of TVBN per 100 g fish.

Such findings spot light on the efficiency of treatment with a mixture of 1.5% of potassium sorbate and bifisobacterial culture in

extending the keeping quality of treated fish up to 24 weeks in comparison to untreated samples.

III- Microbiological examination of frozen treated fish fillets:

From the results obtained in Table (6) and Figure (9), it is evident that the minimum aerobic plate counts of experimentally control frozen fish fillets at zero time was 8×10^4 CFU/g. The storage of such fish at home freezer for 18th weeks caused slight decrease in aerobic plate count during the first 4 weeks constituting 3.5×10^3 CFU/g.

Nevertheless, treated fish fillets with Bifidobacterial culture, the aerobic plate counts decreased reaching 6×10^4 at zero time of the experiment, followed by the decrease of such counts to 7.4×10^2 CFU/g after 24 weeks of storage.

Concerning the treatments of fish fillets with potassium sorbate, the aerobic plate counts decreased reaching 4×10^4 CFU/g at zero time of experiment followed by the decrease of such counts to 5.2×10^2 CFU/g, after 24 weeks of storage at home freezer.

In comparison of the aerobic plate counts of experimentally frozen fish with 1.5% potassium sorbate or Bifidobacterial culture at home freezer (-18°C), the reduction in total bacterial numbers during the first 4 weeks of freezing storage period could be attributed to the disappearance of mesophilic organisms that could not adapt to the cold environment. At this point psychrotrophic spoilage flora had been established and began to multiply, as well as the total plate count decreased rapidly until signs of spoilage appear (18 weeks). This substitutes the finding reported by Acuff *et al.,* (1984). In this respect, Frazier (1967) reported that, freezing kills some but not all microorganisms present in fish where psychrotrophs can survive freezing and are ready to grow on thawing.

On the other hand, ESO (1991) stated that, the permissible limit for the total bacterial count for frozen fish was no more than 10^6 CFU/g fish muscles. Comparatively, it is obvious that the incipient spoilage in fish fillets took place after 18 weeks as well as, the incipient deterioration in frozen fish fillets treated with potassium sorbate, Bifidobacterial culture occur after 24 weeks alone or in mixture.

The microbial activity is one of the main caused of quality deterioration of fish, so the spoilage pattern of fish depends upon the initial bacterial count, in addition to those acquired during handling and storage (Cobb and Vanderzant, 1971). However, Thatcher and Clark (1978) stated that, the high viable counts of frozen fish indicates

contamination of materials from unsatisfactory sanitation during handling, processing as well as inadequate chilling and/or freezing.

From the present data it could be concluded that, the extended shelf life of treated frozen fillets with 1.5% potassium sorbate and Bifidobacterial culture had higher inhibitory effect that each of them, these results were in harmony with Harris *et al.*, (1991); Okereke and Montville, (1991) and Chang *et al.*, (1995) who stated that combination of organic acids and Bifidobacterial culture was effective in suppressing aerobic spoilage bacteria on catfish fillets and should be considered as a potential method for shelf life extension.

The Enterobacteriaceae counts in experimentally control frozen fillets at zero time was 10^3 CFU/g. This was followed by a gradual decrease in the Enterobacteriaceae counts to 10^2 CFU/g after 12 weeks storage (Table 6 and Figure 10)

With respect to Enterobacteriaceae counts in experimentally treated fish with Bifidobacterial culture a gradual decrease was observed till reached 10^2 CFU/g at 22 weeks till 24 weeks (end of the experiment). These results agree with that recorded by Kebary (1995) and Korshunove *et al.*, (1999).

Dealing with the treated fillets with 1.5% potassium sorbate, the Enterobacteriaceae counts decreased to 10^2 CFU/g from 10 weeks till the end of the experiment (24 weeks). These results agree with that reported by Buncic *et al.*, (1995) and Badr *et al.*, (2001).

It is concluded, that the data present revealed the noticeable significant decrease of Enterobacteriaceae count till reach $<10^2$ at the end of experiment.

The counts of Enterobacteriaceae may have potential indicator for not only of health hazard but also as an indicator of spoilage (Gorczyca *et al.*, 1985). Members of family Enterobacteriaceae are potential public health importance as it causes diseases for humans during lowering of their resistance. Also this group contains most members of food poisoning microorganisms (Edwards and Ewing 1972; Collins 1984).

Freezing kills a proportion (usually 60-90% of the Enterobacteriaceae bacteria present) and cold storage results in a further less dramatic and gradual decrease in their numbers, in addition other organisms are injured (Weber and Schmidt, 1989).

From the present data, it could be concluded that the treatment of fish fillets with 1.5% potassium sorbate and Bifidobacterial culture lead to lower in Enterobacteriaceae count to a great extent. As the antimicrobial effect of organic acids (potassium sorbate) could be enhanced by the combination with lactic acid bacteria producing natural organic acids as lactic and acetic acid (El-Shenawy and Marth (1988); Chang and James 1994).

Korshounov *et al.,* (1999) stated that Bifidobacterial strains capable of exhibiting the growth of all indicator bacterial strains (*Escherichia coli, Klebsiella, Staphylococcus aureus, Enterococcus faecalis, Pseudomnas aeruginosa*).

The given results in Table (6) and Figure (11) showed that the MPN of coliforms in frozen untreated Tilapia fillets during frozen storage at home freezer at zero time were 92 CFU/ml. The count gradually decreased and correlated the lowest levels after 18 weeks (9 CFU/ml). In general, the MPN of coliforms decreased gradually till reached <3 CFU/ml in all treated samples at the end of storage time (end of experiment) at home freezer (-18°C). Nearly similar results were obtained by Wu and Chen, (1980); El-Sayed (1991) and Yehia (1996). But higher numbers were obtained by Abdel-Galil *et al.,* (1988); Mahmoud (1990) and Mahmoud (1994).

Comparatively, the obtained results of the experimentally frozen fish and treated frozen fish were within the permissible limits (100 colonies/ml) recommended by ESO (1991) for frozen fishes. Used of the coliforms count as an index of pollution in frozen food has been criticized because of the susceptibility of this group of microorganisms to freezing injury resulting in gradual disappearance in their numbers in frozen food during continued storage (Licciardello and Hill, 1978). Presence of coliforms in frozen fish in the present study may be attributed to neglected sanitary measures during production and handling. This agrees with that reported by (Licciardello and Hill, 1978). In this respect, Farouk (1989); El-Sayed (1991); Seback (1998) stated that the presence of coliforms in fish serves as an index of sanitation and proper handling conditions.

E. coli could not be detected in this study. The present results were coincide with that of Raj Liston, (1963); Joseph *et al.,* (1989) and Soliman and Shalaby (2001). They reported that *E. coli* was absent in fish after freezing. While samples treated with 1.5% potassium sorbate, Bifidobacterial culture alone or in combination there is noticeable decrease in coliforms count was due to the effect of bacteriocidal effect

of potassium sorbate (Badr *et al.*, 2001) and the antimicrobial substances and acid production of Bifidobacterial culture (Badawi and El-Sonbaty, 1997; Nour and Abosrea 2005).

From the achieved results in Table (6) and Figure (12), coagulase-positive *Staphylococcus aureus* in Tilapia fillets were present in count of 3×10^2 CFU/g at zero time of control samples. Similar findings were obtained by Mohamed (1990); Saad *et al.*, (1991) and El-Shater (1999). The frequent contamination on examined fish samples with *Staphylococcus aureus* is undoubted imparted from skin, mouth and nose of fish handlers (Polledo *et al.*, 1986), beside dirty utensils (Banwarl, 1989). From the recorded results, *Staphylococcus aureus* counts decreased by freezing till reach $<10^2$ CFU/g at the 18th week of storage. It is evident that the freezing resulted in loss of *Staphylococcus aureus* viability as manifested by the drop of viable counts. This observation is consistent with that finding of Raj Liston (1963) who found that a temperature of -18°C for 393 days decreased the number of *Staphylococcus aureus* contaminating sea food by ten folds. In this respect Jackson (1974) reported that certain function such as multiplication and cell division could obviously not occur below the minimum growth temperature. Moreover, Ingram and Mamckery (1967) recorded that during the process of freezing many organisms are mechanically crushed or injured by extracellular ice crystals. Similar findings had been recorded by Niazi *et al.*, (1988) who recorded that the exposure of toxigenic *Staphylococcus aureus* to freezing storage resulted in gradual loss in its viability by time.

Regarding the effect of 1.5% potassium sorbate and Bifidobacterial culture on fish fillets stored at the same temperature, the viable counts at Zero time was 3×10^2 CFU/g, recording noticeable decrease in its count reaching $<10^2$ at the 10th week of storage. These results are in agreement of Niazi *et al.*, (1988) who reported that after 24 weeks of storage no viable organisms could be detected in minced meat. Joseph *et al.*, (1989) reported that coagulase positive Staphylococci were absent in frozen fish after freezing.

Concerning, Staphylococcal counts in fish fillets treated with a mixture of Bifidobacterial culture and 1.5% potassium sorbate, there is a recorded decrease at zero time which was 3×10^2 CFU/g with a remarkable decrease till reach $<10^2$at the beginning of 18th week of storage. This reduction was due to the bacteriocidal effects of potassium sorbate and the effect of freezing with addition of the antimicrobial activity associated with Bifidobacterium (Anand *et al.*, (1984); Lindgren

and Dobrogosz (1990); Kurmann and Rasic (1991); Kebary (1995); Badawi, (1997); Korshunov *et al.*, (1999); Ahmed *et al.*, (2002); Vazquez *et al.*, (2005).

The combination of potassium sorbate 1.5% and Bifidobacterial culture was shown to be effective on improving sensory, chemical, microbiological quality and increasing the shelf life of Bolti fish fillets during the storage period of both samples stored at 4°C or those samples stored at -18°C.

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