The role of Lactobacillus acidophilus and *Saccharomyces cerevisiae* as feed additives on the quality evaluation of chilled Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Efficiency of *Lactobacillus acidophilus (L. acidophilus)* and *Saccharomyces cerevisiae* (*S. cerevisiae)* as a potential probiotic for improving the keeping quality of Nile tilapia (*Oreochromis niloticus*) were studied. Four hundred and fifty fingerlings Nile tilapia were divided into three equal groups, each of three main equal replicates. Group (1) fed on basal diet. Group (2) fed on the same diet mixed with $1x10^9$ *L. acidophilus* g^{-1}. Group (3) fed on same diet mixed with $1x10^9$ dried *S. cerevisiae* g^{-1}. The fish were fed on 5% of their body weight for 4 weeks. By the end of the experiment, fish were subjected to organoleptic examination together with some immunological, chemical (pH, total volatile nitrogen & thiobarbeturic acid) and shelf-life and quality tests (total psychrotrophs count with its isolation and identification along 15 days of ice storage). Probiotics used in this study induced no obvious changes on the appearance, consistency and odor of the inspected fish. The consistency and appearance of investigated tilapia were unacceptable at 7, 10, 13 days of ice storage for the control, *L. acidophilus* and *S. cervisiae*; respectively. The odor was unacceptable at 7 day for the control and *L. acidophilus* and at 13 day for *S. cerevisiae*. The pH, TVN and TBA values of the three groups raised by the increasing of storage time. According to the Egyptian Standard, fish flesh in the control, *L. acidophilus* and *S. cerevisiae* of experimented tilapia exceeded the permissible limit for pH at 7, 10 and 13 days and for TVN values at 5, 10 and 13 days of ice storage; *respectively.* On the other hand, the TBA values, in fish flesh of all groups, were within the permissible limit throughout the period of ice storage. The total psychrotrophic counts in the fish-flesh of the harvested Nile tilapia in the *L. acidophilus* and *S. cerevisiae* supplemented groups was lower than that of the control group at all periods of ice storage. Moreover, the three investigated groups showed an increase in the total psychrotrophic counts by the prolongation of the ice storage period which exceed the permissible limit of the Egyptian Standard at 7 days for the control group and 10 days for *L. acidophilus* and *S. cerevisiae.* The variation was in the type of isolates between the 2 probiotic groups and the control, however staphylococci and streptococci were only isolated from the fish-flesh of the control group. It could be concluded that one month application of $1x10^9$ *L. acidophilus* or *S. cerevisiae* g^{-1} improve the fish quality and increase the shelf-life of Nile tilapia.

Key words: Tilapia, *Lactobacillus acidophilus, Saccharomyces cerevisiae,* quality, shelf-life.

INTRODUCTION

Tilapia are one of the most common types of fish produced in Egypt, it represent 40% of total fish production, they easily farming and have an economic low price as well as their flesh are tasty and easy to cook **(*MegaPesca, 2001*).** Fish in large-scale production facilities are exposed to stress conditions, diseases, and deterioration and consequently spoilage of the final product resulted in a serious economic losses and/or public health hazards. Microorganisms are the major cause of spoilage of most seafood products **(*Gram & Dalgaard, 2002*)**.

Healthy fish possess bacteria on the external surface and the internal organs **(*Austin, 1983*),** consequently contamination in edible portions of tilapia could happen leading to fish spoilage and/or public health hazards **(*Al-Harbi & Uddin, 2005 and Herrera et al., 2006*).**

During farming, tilapia is susceptible to the bacterial infection that considered a major cause of fish mortality leading to economic losses **(*Grisez and Ollevier, 1995*).** Sensory evaluation and total psychrotrophic counts are some indicators used for seafood quality determination under chilling storage **(*Paarup et al., 2002, Antoine et al., 2004 and Lopparelli et al., 2004*).** Probiotics are recently recognized as alternatives for antibiotics to control fish diseases and improve fish quality **(*Ahmed et al., 2007 and Aly et al., 2008*).**

The concept of biological disease control, particularly using nonpathogenic bacterial strains, has received widespread attention during the last decade. The probiotics are defined as cultures of live microorganisms that benefit the host (humans and animals) by improving the properties of the indigenous microflora **(*Havenaar et al., 1992*).** The use of probiotics, in human and animal nutrition, is well documented **(*Rink-inen et al., 2003*)** and recently, have been applied to aquaculture **(*Bache`re 2003*).** Extensive investigations in the last decade have shown the benefits of probiotics feed supplement to fish for control the internal microbial load ***(Robertson et al., 2000).*** Such effects have been attributed to bio-chemical, physiological, and antimicrobial effects, as well as competitive exclusion in the intestinal tract **(*Goldin and Gorbach, 1992*).** The mode of action could be through hindering the proliferation of the pathogen in the intestinal tract **(*Verschuere et al., 2000*),** host immuno-regulatory **(*Panigrahi et al., 2004*),** produce antimicrobial metabolites ***(Vine et al. 2004)*** and interfere with attachment of pathogens, as well as the adhesion to fish surfaces ***(Chabrillon et al., 2005).***

The *L. acidophilus* has been considered to be the predominant lactobacillus in the intestinal tract of the healthy humans **(*Ray, 1996*),** and have been widely utilized as a dairy starter culture for their therapeutic activities associated with an intestinal microbial balance. Yeast and yeast by-products have been shown to positively influence non-specific immune responses of some fish species **(*Anderson et al., 1992*).**

The evaluation of general quality and shelf life of the fish and its products is based on the organoleptic, chemical and microbiological tests. The pH of live fish muscle is close to the value 7.0. In fish, refrigerated in aerobic conditions, the rise in pH is mainly due to the production of trimethylamine and other volatile bases due to the spoilage by bacteria. The pH has to be supported by other chemical and sensory criteria **(*Leitão and Rios 2000*).** The total volatile nitrogen (TVN) base was used to determine early stage of spoilage in fish and could be used as a rapid method for the determination of freshness **(*Sengupta et al., 1970*).** Thiobarbituric acid reactive substances (TBA value) were more appropriate for assessment of fish quality **(*McCarthy et al., 1989*).** This test is among those most widely used to quantify lipid deterioration in food because it is simple and fast technique **(*Tarladgis et al., 1960*).**

Malondialdehyde (MDA), an end product of lipid deterioration, is reliable and commonly used biomarkers for assessing lipid quality **(*Sheu et al., 2003*)**.

The present study aimed to evaluate the quality of tilapia reared on feed supplemented with *Lactobacillus acidophilus* or *Saccharomyces cerevisiae*, as a potential probiotics under chilling by ice until it become unsound.

MATERIAL & METHODS

1. Fish:

Four hundred and fifty apparently healthy Nile tilapia (*O. niloticus)* of both sexes (weight 65 ± 5g, each) were obtained from the WorldFish Center. They divided into three equal groups, each of three equal replicates, reared in 9 glass aquaria (60x150x70 cm) and kept for 1 week under observation for acclimatization. The water of the aquaria was daily renewed, and its temperature was maintained at 26±1°C.

2. Experimental design:

Probiotic strains*: Lactobacillus acidophilus* was supplied as a reference strain from the WorldFish Center. *Saccharomyces cerevisiae* were obtained from commercial product available in market (*Vet Yeast*^{TM}) and manufactured by Complimentary Industry Co., Egypt, where 1g of this product containing 1 x 10^9 dried *S. cerevisiae* cells according to the manufacturers.

Preparation of basal and probiotic diets: The dietary ingredients were obtained from specialized factories and prepared locally in pelleted form. The basal diets were prepared by grinding and sieving the corn to granules of 0.5 mm (Thomes-Willey Laboratory Mill Model 4). The ingredients were mechanically mixed by a horizontal mixter (Hobarts model D300T, Troy, Ohio, USA) at a low speed for 30 minutes. The oil (corn & liver cod) was added gradually to assure the homogeneity of the ingredients. The mixing speed was increased for 5 minutes during the addition of water (12% moisture) until clumps began to be formed. The mixture was sterilized and the pellets (0.5 cm, diamter) were prepared using a pellet-machine (CPM California Pellet Mill Co., San Fransisko, California, USA). The pellets were left for 24 hr to dry, under aseptic condition.

The *L. acidophilus* were prepared by the inoculation of the bacterial isolates in tryptic soya broth (TSB) and incubated at 30 ºC for 48 hours. The culture was centrifuged (Beckman, Alaska, Hawaii, USA) at 3000 rpm for 30 minutes. The residues were washed twice with saline. The bacteria (bacterial cell/g) were counted. *S. cerevisiae* were used as a commercial product (*Vet Yeast*^{TM}) after testing its survival and count. The probiotic-supplemented diets were prepared. The tested doses were mixed with the basal diet and pellets were made. The pellets for each diet treatment (1-3) were prepared weekly. Diet (1) was basal diet. Diet (2) was mixed with 1 x 10^9 *L. acidophilus* g^{-1}. Diet (3) was mixed with 1 x 10^9 dried *S. cerevisiae* g^{-1}. Each diet was air-dried at room temperature for 24 hours and stored in a refrigerator (4ºC) until used.

Feeding experiment: Four hundred and fifty Nile tilapia samples were divided into three equal groups (150 samples each), each group of three equal replicates. The basal diet was fed to all fish during the week of acclimatization. The water was daily renewed. Low-pressure electric air pumps provided aeration via air stones and dissolved oxygen (DO) levels was maintained at or near the saturation levels. Water temperature was 25±1°C throughout the trial. The 1st group was fed on basal diet. The 2nd group was fed on diet supplemented with L. *acidophilus* (1 x 10^9 bacteria/g). The 3rd group was fed on diet incorporated with *Saccharomyces cerevisiae* (1 x 10^9 yeast/g). The fish were daily fed at a rate of 5% of the body weight for 4 weeks. The survival rate and immunological tests as well as keeping quality tests were made by the end of 4 weeks of the experiment. All treated and control samples were stored in ice (1 : 1 fish to ice) to keep the temperature within the ice box not more 1°C. Samples were periodically analyzed every two days for quality evaluation until spoiled according to *EOS (2005).*

3. Fish Health parameters:

Body gain and Survival rate:

The fish were weighted and counted after 4 weeks from the start of the experiment to determine the body gain and survival percentage **(**Survival % = No. of fish counted / No. of stocked fish x 100).

Immunological tests:

Whole blood (0.5 ml) was collected from the caudal vein of 20 fish to determine the phagocytic activities of neutrophils and monocytes through measuring the Nitroblue tetrazolium activity (NBT) according to ***Siwicki et al., (1985).*** A further 0.5 ml blood-sample was centrifuged at 1000 xg for 5 min in order to separate the plasma to be used for lysozyme activity test according to ***Parry et al., (1965).***

4. Quality Evaluation:

Sensory evaluation:

The sensory evaluation was done to detect the consumer acceptability. The treated and control groups were evaluated for consistency, appearance and odor by five panelists. They were evaluated by 5 point scale; 1(very bad), 2 (bad), 3 (fair), 4 (good) and 5 (very good). The fish were considered un-

acceptable when their sensory score was below 3, according to ***Shewan et al, (1953).***

Chemical tests:

Hydrogen ion concentration (pH) values were determined by using pH meter according to ***Huss (1995).*** Total volatile base nitrogen (TVN-B) values were determined by Conway's microdiffusion technique recommended by *FAO (1980).* Thiobarbeturic acid (TBA) values were expressed as mg malonaldehyde per 1 kg fish-flesh according to ***Vyncke (1970)***.

Microbiological analysis:

Five random samples from each group were periodically examined to determine total psychrotrophic counts. The samples were prepared for microbiological examination according to the procedure recommended by ***APHA (1992).*** Colonies were purified by streaking on inoculated nutrient agar at 20°C for 2-3 days. The isolates were identified using biochemical tests **(*Bergey et al., 1986*)** and API 20 E strip system.

5. Statistical analysis:

Analysis of Variance (ANOVA) and Duncan's multiple Range Test **(*Duncan 1955*)** was used to determine the differences between treatments. The mean values were significant at the level of ($P<0.05$). Standard errors, of treatment-means, were estimated. All the statistics were carried out using Statistical Analysis Systems (SAS) program **(***SAS 2005***).**

RESULTS & DISCUSSION

The body gain of *O. niloticus*, supplemented with two probiotics for 4 weeks were higher than tilapias of the control group. The increase in the body gain was significant in *S. cerevisiae* supplemented tilapia (Table 1). ***Waché, et al., (2006)*** reported that two strains of *S. cerevisiae* (*S. boulardii* and *D. hansenii)* seemed to stimulate the digestive process in rainbow trout fry. ***Planas et al., (2004)*** mentioned that, the addition of the lactic acid bacteria (LAB) increased the specific maximum growth rate of rotifer (*Brachionus plicatilis*). ***Carnevali et al., (2006)*** noticed that European sea bass fed *L. delbrueckii delbrueckii* showed higher body weight compared to the controls.

The survival rate, in probiotics supplemented groups, showed a non significant increase values at 4 weeks of experiment when compared with the control (Table 1). ***Tovar et al., (2002)*** observed that a *Debaryomyces hansenii* (DH) enriched diet led to an increase in amylase and stimulated the activity of brush border membrane enzymes and was associated with high survival rate of the sea bass larvae. ***Picchietti et al., (2007)*** noticed that feeding of *L. fructivorans* and *L plantarum* increased the number of Ig+ cells and acidophilic granulocytes in the sea bream gut that correlates with improvement of fry survival. The improvement in survival, in our experiment, may be due to the enhancement of the immune response which resulted from the increase in the nitroblue tetrazolium and lysozymes activities.

The nitroblue tetrazolium and lysozyme values were higher in the probiotic treated groups when compared with the control group. The increase was significant with *S. cerevisiae* supplemented group (Table 1). This could explain the promotion of the probiotics for the non-specific immune responses. The lactic acid bacteria (LAB) increases the activities of phagocytes, lysozyme and complement ***(Schiffrin et al., 1997; Panigrahi et al., 2004). Díaz-Rosales et al., (2006)*** observed a high phagocytic ability in fish given a probiotics. The lysozyme has bactericidal hydrolysing β-linked glycoside bonds. It has been shown that the injection of β -glucan induced a significantly elevated lysozyme activity **(*Misra et al., 2006*).**

The organoleptic examinations revealed that, the consistency, appearance and odor of the fish-flesh in the control, *L. acidophilus* and *S. cerevisiae* till 15 days of ice storage showed a similar pattern of decrease in acceptability. The consistency and appearance of experimented tilapia was unacceptable at 7, 10, 13 days of ice storage for the control, *L. acidophilus* and *S. cervisiae*; respectively (Figs.1 & 2). The odor was unacceptable at 7 day for the control and *L. acidophilus* and at 13 day for *S. cerevisiae* (Figs.3). Great interest is developing on seafood safety because of the ever increasing needs to protect consumer health and environment and to revalorize some important food characeristics, such as naturalness and authenticity, without leaving the safety aside **(*Altieri et al., 2005*).** These results suggest that the original tilapia's microflora was mesophilic being able to grow at refrigeration temperatures only after a long lag phase. Similar results were reported by other authors working with freshwater fish in tropical areas, with this slow growth rate being considered one of the main factors explaining the longer shelf life of tropical fish when first stored at refrigeration temperatures **(*Erkan et al., 2006*).** The pattern of sensory evaluation was correlated with the current chemical and microbiological results.

The pH values of the three groups increased by the prolonging the storage time. The pH values exceeded the permissible limit according to *EOS (2005)* of the Egyptian Standard (not more than 6.5) at 7, 10 and 13 days of ice storage for the control, *L. acidophilus* and *S. cerevisiae* supplemented groups; respectively (Table 2). The increases in pH indicated the accumulation of alkaline compounds as ammonia mainly derived from microbial action. Our results were in agreement with the findings of ***Erkan et al., (2006)*** for other fish species stored in ice.

The total volatile nitrogen (TVN) values, in all groups, were raised by the increase in the period of the ice storage. However, in all groups, the TVN values were exceed the permissible limit of the Egyptian Standard according to *EOS (2005)* (not more than 30 mg /100g fish-flesh estimated as nitrogen) at 5, 10 and 13 days of ice storage for the control, *L. acidophilus* and *S. cerevisiae* supplemented groups; respectively (Table 2). ***Pearson (1984)*** concluded that, TVN less than 20mg % indicate fish of good quality while doubtfully acc-

epted around 30mg%. He added that, higher TVB-N values in the range 25-35 mg/100 g indicate that the fishes were slightly decomposed/edible and decomposed/ inedible respectively.

The thiobarbitueric acid (TBA) values, within each groups, were increased by the increase in the period of the ice storage. However, according to the Egyptian Standard (not more than 4.5 mg malonadldehyde/kg fish-flesh) of *EOS (2005),* the TBA values, in the fish flesh of experimented tilapia in all groups, were within the permissible limit of the Egyptian Standard throughout the period of ice storage (Table 2). The unacceptable values of TBA in the flesh of fish are an indicator for fish rancidity. Fish with its high content of unsaturated fatty acids is highly susceptible towards lipid rancidity either oxidative **(*Bragadóttir, 2001*)** or hydrolytic rancidity **(*Ch-urch, 1998*)**. The resulting development of rancidity in fish leads to undesirable ch-anges in flavor **(*Durnford and Shahidi, 1998*)** and appearance **(*Sikorski et al., 1976*).**

The total psychrotrophic counts in the fish-flesh of the harvested Nile tilapia of the *L. acidophilus* and *S. cerevisiae* supplemented groups was lower than that of the control group at all periods of ice storage. This finding could be due to the improved NBT and lysozymes by the effect of probiotics used. Moreover, the three experimented groups showed an increase in the total psychrotrophic counts by the prolongation in the ice storage period which exceed permissible limit of the Egyptian Standard 10^6 CFu/gm, (*EOS 2005*) at 7 days for the control group and 10 days for *L. acidophilus* and *S. cerevisiae* (Table 3).

Fish quality was changed followed the increases in microbiological counts of their flesh, indicating that bacterial load can serve as a useful and objective indicator of gross spoilage **(*Du et al., 2001*).** It was observed that probiotic feed supplement of fish led to a smaller increase in the total psychrotrophic counts during ice storage. Data about the total psychrotrophic count can be used as an index of quality and predict the shelf-life of the tilapia **(*Al-Harbi and Uddin, 2005*).** Under normal refrigerated storage conditions, the shelf-life of these products is limited by enzymatic and microbiological spoilage **(*Ashie et al., 1996*).** The administration of *Lactobacillus delbrüeckii sp. lactis* and *Bacillus subtilis*, singly or in combination, increased phagocytic activity **(*Salinas et al., 2005*).** These results clearly demonstrated the efficiency of probiotic feed additive with combination of ice storage (chilling) for delayed migration and/or inhibition of spoilage flora of fish from the gut, gills and skin to the muscles. Prolonged shelf-life of fish expands the market potential and reduces waste during distribution and retail display **(*Lalitha et al., 2005*).**

Bacteriological examinations in the present study revealed isolation of 144 bacterial strains among all examined fish groups after 0 and 15 days ice storage. At 0 day ice storage, 17, 15 and 13 psychrotrophic strains were isolated from control, *L. acidophilus* and *S. cerevisiae* supplemented groups, respectively. While, at 15 day ice sto-

rage, we could isolate 42, 30 and 27 psychrotrophic strains from the same groups, respectively. Detailed psychrotrophic strains from the control and two probiotics-supplemented groups were reported in Table (4). These microorganisms were found to be regular members of the microbial association in fish flesh **(*Dalgaard,* *2000*)**.

Aeromonas and Pseudomonas spp. contribute to deterioration of iced wild freshwater fish and should be regarded as a potential health concern, particularly for susceptible populations when there is a possibility of cross-contamination **(*Gonzalez et al., 2001*).** Aeromonas may contribute to diarrhoea-related virulence and severe public health hazards **(*Herrera et al., 2006*).** Staphylococcus and streptococcus isolated from the control group only. It was noticed that, probiotic feed additive for fish shown a positive effect on Staphylococcus and streptococcus that could not isolated from the fish flesh fed diet with probiotic. After extended ice storage, the frequency of the psychrotrophic strains in all examined fish groups were increased as seen by ***Gram and Huss, (1996)*** who recognized the specific spoilage bacteria of iced fresh fish regardless of the origin of the fish.

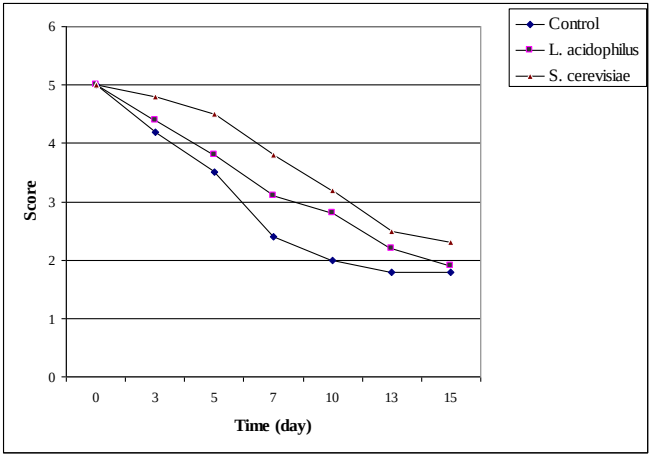

Fig. (1): Consistency evaluation of chilled tilapia reared on feeds supplemented with Probiotics

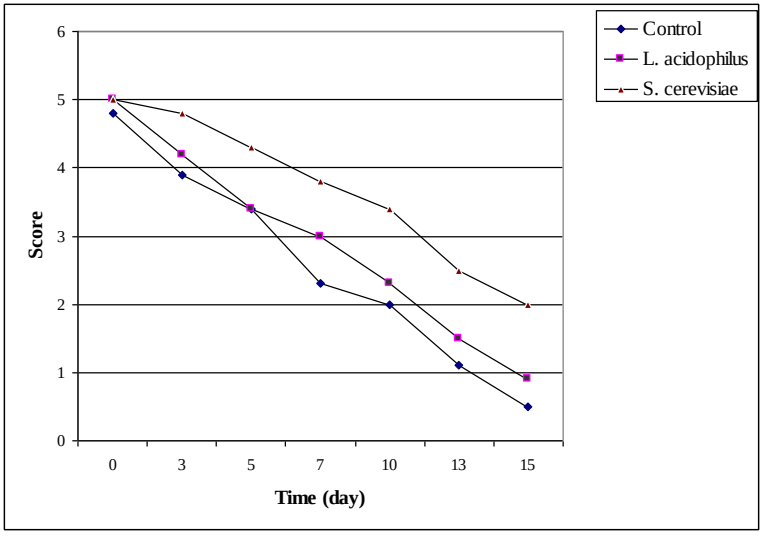

Fig. (2): Appearance evaluation of chilled tilapia reared on feeds supplemented with Probiotics

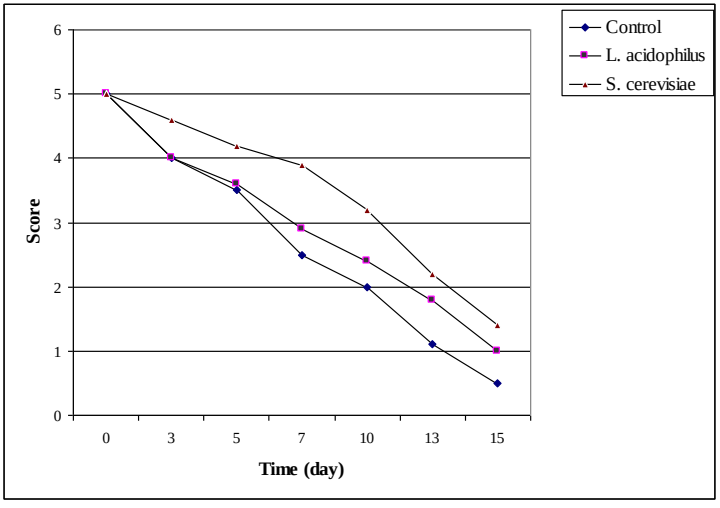

Fig. (3): Odor evaluation of chilled tilapia reared on feeds supplemented with Probiotics

Table (1): Body gain, survival and some immunological tests in *Oreochromis niloticus* supplemented with probiotics for 4 weeks in comparison with the control.

Parameters	Control	*L. acidophilus*	*S. cerevisiae*
Body gain	$62.42^{B} \pm 18.70$	$76.13^{AB} \pm 13.17$	$122.57^{A} \pm 16.02$
Survival	$80.00^{A} \pm 6.67$	$84.44^{A} \pm 8.89$	$88.89^{A} \pm 2.21$
Nitroblue tetrazolium	$0.22^{B} \pm 0.02$	$0.26^{AB} \pm 0.03$	$0.31^{A} \pm 0.01$
Lysozyme	$9.33^{B} \pm 0.88$	$10.67^{AB} \pm 0.88$	$12.93^{A} \pm 0.71$

Table (2): Chemical evaluation of chilled Nile tilapia reared on feeds supplemented with probiotics (Mean ± SE).

Time/ Day	pH values			*TVN-B values*			*TBA values*		
	Control	*L. acidophilus*	*S. cerevisiae*	Control	*L. acidophilus*	*S. cerevisiae*	Control	*L. acidophilus*	*S. cerevisiae*
0	$7.1^{Ae} \pm 0.22$	$7.00^{Ad} \pm 0.15$	$7.2^{Ad} \pm 0.13$	$18.4^{Ac} \pm 0.19$	$18.4^{Ad} \pm 0.10$	$18.1^{Ae} \pm 0.21$	$0.3^{Ac} \pm 0.22$	$0.2^{Ac} \pm 0.15$	$0.2^{Ac} \pm 0.13$
3	$6.00^{Ad} \pm 0.11$	$5.98^{Ac} \pm 0.20$	$6.0^{Ac} \pm 0.21$	$19.2^{Ac} \pm 0.20$	$19.0^{Ad} \pm 0.31$	$18.8^{Ae} \pm 0.15$	$0.8^{Ac} \pm 0.11$	$0.6^{Ac} \pm 0.20$	$0.5^{Ac} \pm 0.21$
5	$6.5^{Ac} \pm 0.37$	$6.04^{Bc} \pm 0.30$	$6.10^{Bc} \pm 0.33$	$31.5^{Ab} \pm 0.14$	$24.6^{Bc} \pm 0.05$	$21.0^{Bd} \pm 0.22$	$2.6^{Ab} \pm 0.37$	$2.5^{Ab} \pm 0.30$	$2.3^{Ab} \pm 0.33$
7	$6.8^{Ac} \pm 0.04$	$6.3^{Bc} \pm 0.03$	$6.20^{Bc} \pm 0.13$	$32.6^{Ab} \pm 0.32$	$28.5^{Bb} \pm 0.40$	$25.6^{Bc} \pm 0.13$	$3.1^{Ab} \pm 0.04$	$2.8^{Ab} \pm 0.03$	$2.6^{Ab} \pm 0.13$
10	$7.2^{Ab} \pm 0.08$	$6.8^{Bb} \pm 0.10$	$6.4^{Cc} \pm 00.14$	$35.9^{Aa} \pm 0.41$	$31.2^{Ba} \pm 0.21$	$28.8^{Bb} \pm 0.15$	$3.6^{Ab} \pm 0.08$	$3.4^{Aa} \pm 0.10$	$3.0^{Ba} \pm 00.14$
13	$7.5^{Ab} \pm 0.30$	$7.2^{Aa} \pm 0.18$	$6.80^{Bb} \pm 0.30$	$36.2^{Aa} \pm 0.11$	$32.6^{Ba} \pm 0.31$	$31.9^{Ba} \pm 0.22$	$4.0^{Aa} \pm 0.30$	$3.8^{Aa} \pm 0.18$	$3.4^{Ba} \pm 0.30$
15	$7.9^{Aa} \pm 0.30$	$7.5^{Ba} \pm 0.28$	$7.2^{Ba} \pm 0.27$	$37.4^{Aa} \pm 0.10$	$33.1^{Ba} \pm 0.15$	$32.7^{Ba} \pm 0.16$	$4.4^{Aa} \pm 0.30$	$4.1^{Aa} \pm 0.28$	$3.6^{Ba} \pm 0.27$

Capital letter = comparison among treatments. Small letter = comparison among time within same treatment.

Table (3): Total psychrotrophic counts in the fish-flesh of Nile tilapia of investigated groups during ice-storage for 15 days (Mean ± SE).

Time/ Day	Control			*L. acidophilus*			*S. cerevisiae*		
	Min.	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**
0	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
3	$2x10^2$	$3x10^4$	$2x10^3 \pm 86$	$< 10^2$	$3x10^4$	$2x10^3 \pm 80$	$< 10^2$	$3x10^4$	$2x10^3 \pm 80$
5	$7x10^3$	$2x10^6$	$8x10^4 \pm 2x10^3$	$2x10^3$	$2x10^5$	$4x10^4 \pm 6x10^2$	$2x10^3$	$2x10^5$	$4x10^4 \pm 6x10^2$
7	$2x10^5$	$4x10^8$	$2x10^6 \pm 5x10^3$	$3x10^5$	$4x10^7$	$10^6 \pm 4x10^3$	$3x10^5$	$4x10^7$	$10^6 \pm 4x10^3$
10	-	-	-	$2x10^5$	$4x10^8$	$10^7 \pm 2x10^4$	$2x10^5$	$4x10^8$	$10^7 \pm 2x10^4$
13	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-

Capital letter = comparison among treatments. Small letter = comparison among time within same treatment.

Table (4): Frequency distribution of psychrotrophic strains isolated from fish-flesh of Nile tilapia in all groups at harvest time (0 time) and after 15 day of ice-storage.

Psychrotrophs	**Control,** No. (%)		***L. acidophilus,*** No. (%)		***S. cerevisiae,*** No.(%)	
	0 day	**15day**	**0 day**	**15 day**	**0 day**	**15 day**
Aeromonas spp.	5 (29.4)	9(21.4)	4 (26.7)	6 (20.0)	3 (23.0)	6 (22.2)
Pseudomonas sp.	3 (17.6)	7(16.7) .7)	3 (20.0)	8 (26.6)	4 (30.1)	6 (22.2)
Streptococcus sp.	1 (5.8)	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Coliforms	0 (0.0)	3 (7.1)	0 (0.0)	2 (6.7)	1 (7.7)	3 (11.1)
Micrococcus spp	3 (17.6)	6(14.3)	3 (20.0)	5 (16.7)	2 (15.4)	5 (18.5)
Flavobacterium spp.	0 (0.0)	7(16.7) (16.7)	3 (20.0)	3 (10.0)	1 (7.7)	3 (11.1)
Yersinia sp.	2 (11.8)	2 (4.8)	0 (0.0)	2 (6.7)	1 (7.7)	1 (3.7)
Shewnella sp.	3 (17.6)	5(11.9) (11.9)	2(13.3)	4(13.3)	1 (7.7)	3 (11.1)
Staphylococcus	0 (0.0)	2 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	17 (100)	42(100)	15(100)	30(100)	13(100)	27(100)

CONCLUSION

It could be concluded that potential probiotics can be used to improve the fish quality and increase the shelf-life of ice-storage tilapia. Application of *L. acidophilus* and *S. cerevisiae* at dose 1 x 10^9 g^{-1}at for one month did not affect the consumer acceptability but was sufficient to improve the fish quality.

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الملخص العربى

فاعلية اللاكتوباسيلس اسيدوفيلس والسكارواميسس سيرفساى كإضافات أعلاف على تقييم جودة أسماك البلطي النيلي المبردة

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قسم الرقابة الصحية على الأغذية – معهد بحوث صحة الحيوان - معمل فرعى الإسماعيلية[1] ، بورسعيد[2] ، الجيزة[3]

يعتبر إنتاج اسماك البلطى في مصر من الصناعات الواعدة ليس فقط للاستهلاك المحلى بل أيضا للتصدير. وتتشابه اسماك البلطي مع باقي الأسماك في احتواء سطحها الخارجي وأعضائها الداخلية على البكتيريا مسببة فساد لتلك الأسماك مما يعد خطورة على صحة المستهلك.

هدفت تلك الدراسة إلى استبيان مدى كفاءة كل من اللاكتوباسيلس اسيدوفيلس والسكارواميسس سيرفساي كبروبيوتك في تحسين جودة وفترة صلاحية اسماك البلطي المستزرعة. استخدم في تلك الدراسة عدد ٤٥٠ من اصبعيات اسماك البلطي النيلي قسمت إلى ثلاثة من المجموعات المتساوية العدد كل مجموعة تم توزيعه على ثلاثة أحواض بالتساوي لضمان صحة التحليل الاحصائى. تم تغذية اسماك المجموعة الأولى على علف صناعي متكامل والمجموعة الثانية نفس العلف مضافا إلية اللاكتوباسيلس اسيدوفيلس أما المجموعة الثالثة أضفنا إلى غذائها السكارواميسس سيرفساي.

استمرت التجربة لمدة أربعة أسابيع حيث ثم تم سحب عينات مع نهاية التجربة. أجرى فحص عضوي حسي للأسماك لاستبيان التماسك ، الشكل الظاهرى والرائحة أضافه إلى قياسات مناعية وكيميائية (الأس الأيدروجينى ، القواعد النيتروجينية الطيارة ، حمض الثيوباربتيورك) كما تم عد وتصنيف للبكتريا المحبة للبرودة بعد تخزين الأسماك لمدة ١٥ يوما.

وقد تبين من الدراسة أن هذه البروبيوتك ليس لها تأثير سلبى على نتائج الاختبارات الحسية. حيث وصل مدى تماسك الشكل الظاهري الى الحدود الغير مرغوبة عند ٧ ، ١٠ ، ١٣ يوم من التخزين فى الثلج للمجموعة الضابطة ومجموعة المعاملة باللكتوباسلس اسيدوفيلس أو السكارواميسس سرفساى على التوالى اما الرائحة فقد وصلت للحد

الغير مرغوب فية عند اليوم السابع لكل من المجموعة الضابطة ومجموعة اللكتوباسلس اسيدوفيلس أما المجموعة المعاملة بالسكارواميسس سرفساى كان التغيير فى اليوم الثالث عشر.وقد ازدادت قيم كل من الأس الأيدروجينى و القواعد النيتروجينية الطيارة وحمض الثيوباربتيورك بزيادة فترة التخزين بالثلج. وطبقا للمواصفات القياسية المصرية فقد تعدت كل من المجموعة الضابطة والمعاملة اللكتوباسلس اسيدوفيلس و السكارواميسس سرفساى للقيم المسموح بها للأس الأيدروجينى عند ٧ ، ١٠ ، ١٣ يوم من التخزين بالثلج. وقد تبين وجود نقص معنوي في الأس الايدروجينى للمجموعة المعاملة بالسكارواميسس سرفساى عن اللكتوباسلس اسيدوفيلس والمجموعة الضابطة حتى اليوم العاشر من التخزين في الثلج. وقد ازداد الأس الايدروجينى عن المستوى المسموح بة في المواصفات القياسية المصرية في المجموعة الضابطة عند اليوم الثالث عشر وللقواعد النيتروجينية الطيارة عند ٧ ، ١٠ ، ١٣ يوم من التخزين في الثلج. أما قيم حمض الثيوباربتيورك فلم تتعدى الحد المسموح بها خلال فترة التخزين بالثلج. وقد حدث انخفاض معنوي في العد الكلى للبكتيريا المحبة للبرودة بلحوم الأسماك المعاملة بكل من اللكتوباسلس اسيدوفيلس و السكارواميسس سرفساى بالمقارنة بالمجوعة الضابطة في معظم فترات التخزين بالثلج علما بأن العدد الكلى لتلك البكتيريا قد ازداد بزيادة فترة التخزين بالثلج والتى فاقت القيم المسموح بها طبقا للمواصفات القياسية المصرية عند اليوم السابع للحوم المجموعة الضابطة واليوم العاشر لكل من مجموعتى اللكتوباسلس اسيدوفيلس و السكارواميسس سرفساى مع تباين أنواع البكتيريا المعزولة من مجموعة لأخرى ولكنة تم عزل ميكروبي الاستافيلوكوكس والاستربتوكوكس من لحوم اسماك المجموعة الضابطة.

وقد خلص البحث إلى أن إضافة اى من اللاكتوباسيلس اسيدوفيلس و السكارواميسس سيرفساي (10^9/ خلية / جم) في عليقه اسماك البلطي لمدة أربعة أسابيع يحافظ على جودتها وفترة صلاحيتها.