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Effect of Harvesting Techniques and Refrigerated Storage on the Quality of Nile Tilapia (*Oreochromis niloticus*) Fillets

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ABSTRACT

This study was performed to investigate the effect of harvesting techniques and refrigerated storage on the quality of the Nile tilapia (*Oreochromis niloticus*) fillets. Two harvesting techniques were conducted; high water column (200 to 100cm) unstressed and appropriately harvested with live transported tilapia (LT), and low water column (up to 30cm) stressed harvested with iced transported tilapia (IT). Tilapia samples harvested by two techniques were filleted and stored at 5±0.5ºC in the refrigerator and analyzed after 0, 3, 6 and 9 days, respectively. Results indicate that the yield of fillets of LT was more than IT samples. For ITFs, a gradual increase was recorded in the values of TBA, pH, and TVBN, associated with a gradual decrease in WHC compared to LTFs. The sum of polyunsaturated fatty acids (PUFAs) decreased gradually, while the sum of both saturated (SFAs) and monounsaturated fatty acids (MUSFAs) increased with increasing the storage period, especially for ITFs relative to LTFs. In conclusion, tilapia fillets of the unstressed, appropriately harvested, and live transported tilapia (LTFs) showed a higher yield, longer shelf life, and better quality than the stressed-harvested and ice- transported tilapia (ITFs). Hence, this study recommends that transferring of harvested fish live in oxygenated water tanks is better than the other technique to prevent high economic losses and increase the profitability of tilapia processing in Egypt and all over the world.

INTRODUCTION

The Nile tilapia as “aquatic chicken” is one of the important produced fish in aquaculture all over the world **(El-Sayed, 2006)**. In Egypt, tilapias are reared essentially in earthen ponds in semi intensive rearing systems. The harvesting process could be a very important aspect for the harvested fish, including the onset of a stress status, which can compromise the marketable, organoleptic and sanitary quality of the final products **(Poli, 2009)**. Moreover, transportation process for live fish is necessary for its marketing as food regardless of the destination or outlet; fish must arrive undeteriorated with very low stress and mortality to ensure the quality of transported fish **(Harmon, 2009)**. Negative impacts of transportation on the quality of fish muscles have been reported in

numerous fish species **(Gatica *et al.*, 2008)**. **Bosworth *et al.* (2004)** postulated that, channel catfish meat quality can be detrimentally affected by the activity and stress during harvesting, transportation and handling. Therefore, failure in properly practicing leads to a decrease in both fish quality and marketing price.

Freshness is one of the important aspects of fish because the customer has a strong tendency to select the super fresh fish **(Ross, 2000)**. Typical shelf life of fish under refrigerated storage conditions ranges from six to twenty days **(Cyprian *et al.*, 2008)** depending on harvest location, species and season, and may lead to heavy economic loss **(Sivertsvik *et al.*, 2002)**. Besides, the shelf life of the fishery products is usually limited by microbial activity as the most important reason for fish spoilage that is influenced most storage temperature **(Gram and Dalgaard, 2002; Simpson *et al.*, 2003)**. The microbial spoilage is often a result of off-flavors and off-odors that caused by bacterial metabolism **(Gram *et al.*, 1990)**. Extending the fish shelf life is significantly advantageous to industry, as it reduces losses during product display and distribution, which may result in marketing enhancements for fresh products and in a regular supply at affordable prices **(Lioutas, 1988)**. Therefore, this work aims to investigate the effect of both harvesting and transportation techniques on the quality and shelf-life of farmed Nile tilapia, *Oreochromis niloticus*.

MATERIALS AND METHODS

Fish samples

The Nile tilapia (*Oreochromis niloticus*) fish samples were obtained from a private fish farm, Kafr El-Shekh Governorate, Egypt. They were harvested by two techniques (a) they harvested from water column (100 - 200cm) and transported as live tilapia (LT) and (b) they harvested from shallow water (~30cm) and transported as iced tilapia (IT) to fish processing laboratory.

Filleting process

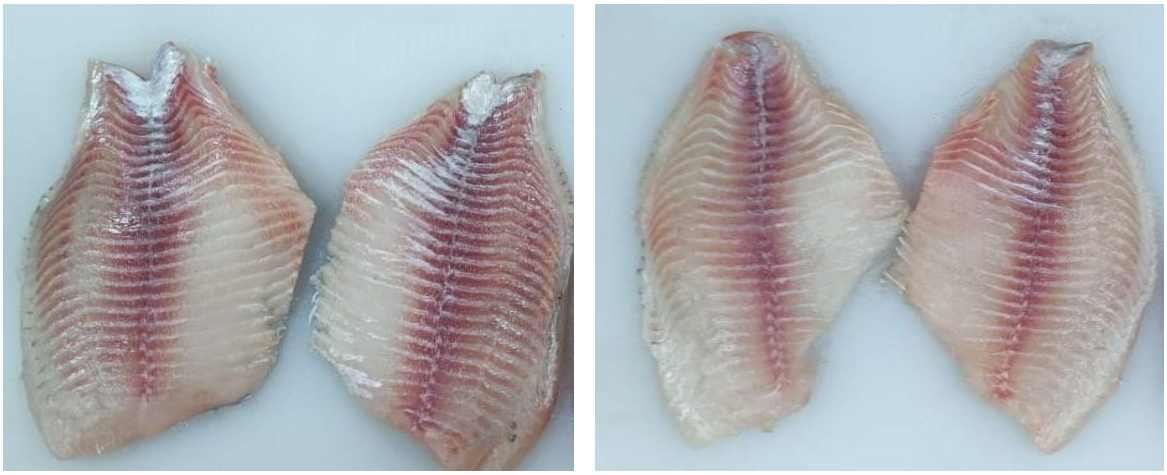
Each fish samples (a & b) were manually filleted and trimmed as shown in Fig.(1). Prepared fish fillets were packed in polyethylene bags and refrigerated at 5±0.5ºC for 9 days. Fish by-products; scales, skin, viscera, bone and blood were discarded.

Yield of edible part

It was estimates as follows: Yield **(**%) = (Fillets wt./body wt.) ×100.

Proximate composition

The proximate analysis; moisture, crude protein (TN×6.25), crude lipid, and ash contents were determined (**AOAC, 2007**).



(a) **(b)**

Fig. 1: Tilapia fillets; (a) Live tilapia (LT), (b) Iced tilapia (IT).

pH value

The pH value of tilapia fillets samples was measured **(Pacheco-Aguilar *et al.*, 1989)** using pH-meter type Orion Research digital Lonalyzer 1501.

Water-holding capacity (WHC)

The water-holding capacity (WHC) of fish fillets samples was determined as ‘‘centrifuge drip’’ **(DelValle and Gonzalez, 1968).** Water-holding capacity was calculated on a wet weight basis as follows: (1 - S/V) * 100.

Where S: the weight of the expelled water, V: the initial weight of sample**.**

Total volatile bases nitrogen (TVB-N)

The TVB-N content was determined according to method described by **(Kirk and Sawyer, 1991)**. The results were expressed as mg TVB-N for 100 gram sample.

Thiobarbituric acid- reactive substances (TBA-RS)

Thiobarbituric acid value was determined by the method described by **(Kirk and Sawyer, 1991).** TBA was expressed as mg Malonaldehyde per kg sample

Fatty acid composition

The preparation of fatty acid methyl esters from the crude fat of tilapia fillets samples was performed according to the procedure of **(Radwan, 1978)**. The atherogenic index (AI) and the thrombogenic index (TI) were calculated based on the data of fatty acid composation **(Ulbricht and Southgate, 1991)** using the following equations:

$AI = [12:0 + (4 \times 14:0) + 16:0] / [\Sigma MUFA + \Sigma PUFA\ (n-6) + (n-3)]$.

$TI = (14:0 + 16:0 + 18:0) / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma PUFA\ \_n-6) + (3 \times \Sigma PUFA\ \_n-3) + (\Sigma PUFA\ \_n-3\ \Sigma PUFA\ \_n-6)]$.

Microbiological examination

Total plate count (TPC) and Psychophilic bacteria of fillets samples were examined **(ICMSF, 1987)** using nutrient agar that incubated at 32°C for 48 h and 7°C for 10 days, respectively.

Statistical analysis

Data were presented as mean±SD. The results were subjected to one-way analysis of variance (ANOVA) using **SPSS** program, Ver. 22. Differences between means were compared using Duncan's (1955) multiple range test at $P < 0.05$ level.

RESULTS

Raw tilapia fillets

Yield of edible part (fillets)

Fig. 2. Shows the yield of tilapia fillets. Results showed that the lower yield (35.67±3.14%) of fillets was found in iced tilpia samples (IT) than live tilapia (LT) (37.88±2.67%) at zero time of harvesting. Also, it was noticed that the mean value of live tilapia fillets (LTFs) yield represent about 2.14% higher than iced tilapia fillets (ITFs).

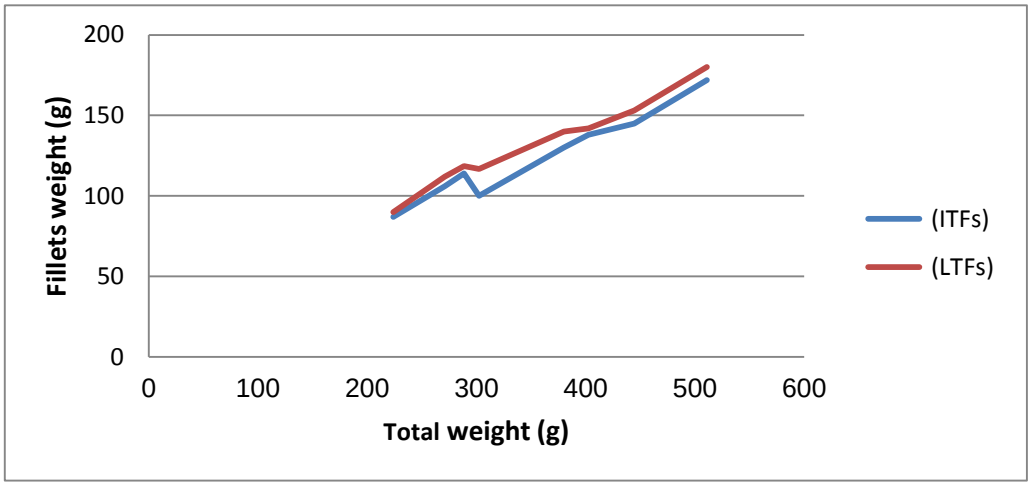


Fig. 2. The yield of both iced tilapia fillets (ITFs) and live tilapia fillets (LTFs).

Effect of refrigerated storage periods at 5 ± 0.5 °C on
Chemical composition

The effect of refrigerated storage periods at 5±0.5 °C on proximate composition (wet wt.) of live (LTFs) and iced (ITFs) tilapia fillets samples is shown in Table (1). The results showed that are statistically significant differences ($P<0.05$) in both moisture and ash content of tilapia fillets stored at 5± 0.5°C of during the 9 days of storage. The initial moisture content for LTFs and ITFs decreased significantly ($P<0.05$) from 77.52±0.13 and 78.01±0.04 to 76.52±0.18 and 76.44±0.08, respectively, at the end of the storage period. While the initial ash content for LTF and ITF increased significantly ($P<0.05$)

from 1.06 ± 0.15 and 1.17±0.01 to 1.22±0.06 and 1.23 ±0.03, respectively, at the same conditions. The protein content of raw LTFs and ITFs samples recorded 20.70±0.3 and 20.20±0.1 %, respectively at zero time. During refrigerated storage periods, it was found a negligible decrease in protein content of all fillets studied. Also, significantly differences ($P<0.05$) in protein content at the end of storage period compared to the initial values. Fat increased significantly ($P< 0.05$) of tilapia fillets stored for 3 days under chilling condition from 2.57±0.04 to 2.87±0.09 % for LTF, but it was 2.90±0.09% increased to 2.80±0.08% for ITF. Table 1. And the end of storage period, the fat content decreased 2.49±.06% and 2.58±0.14%, respectively.

Table 1. Effect of refrigerated storage periods at 5±0.5 ºC on proximate composition (ww) of live (LTFs) and iced (ITFs) tilapia fillets samples.

Storage period (days)	Constituents (%)							
	Moisture		Crude protein		Fat %		Ash	
	LTFs	ITFs	LTFs	ITFs	LTFs	ITFs	LTFs	ITFs
0	77.52±0.13^{b}	78.01±0.04^{a}	20.70±0.3^{a}	20.20±0.1^{ab}	2.57±0.04^{b}	2.90±0.09^{ab}	1.06±0.15^{b}	1.17±0.01^{ab}
3	76.94±0.07^{c}	77.35±0.08^{b}	20.41±0.47^{a}	20.05±0.20^{a}	2.74±0.07^{b}	2.96±00.19^{a}	1.21±0.02^{a}	1.19±0.08^{a}
6	77.24±0.02^{bc}	77.31±0.09^{bc}	20.21±0.17^{ab}	20.01±0.11^{ab}	2.87±.09^{ab}	2.80±0.08^{ab}	1.25±0.05^{a}	1.23±0.06^{a}
9	76.52±0.18^{c}	76.44±0.08^{c}	19.5±0.1^{bc}	19.35±0.26^{c}	2.49±.06^{b}	2.58±0.14^{b}	1.22±0.06^{a}	1.23±0.03^{a}

LTFs: Live tilapia fillets, ITFs: Iced tilapia fillets.

pH value

Effect of refrigerated storage periods at 5±0.5 ºC on pH value of live (LTFs) and iced (ITFs) tilapia fillets samples is presented in Fig. 3 . The pH value of fresh LTFs samples recorded 6.51±0.01 then, it was slightly decreased to 6.16 ± 0.06 after three days of refrigerated storage. After that, the value increased gradually to record 6.66 ± 0.01 after nine days of refrigerated storage. On the other side, the pH value of fresh ITFs samples recorded 6.23 ± 0.01 then, it was slightly increased to 6.82±0.06 after nine days of refrigerated storage.

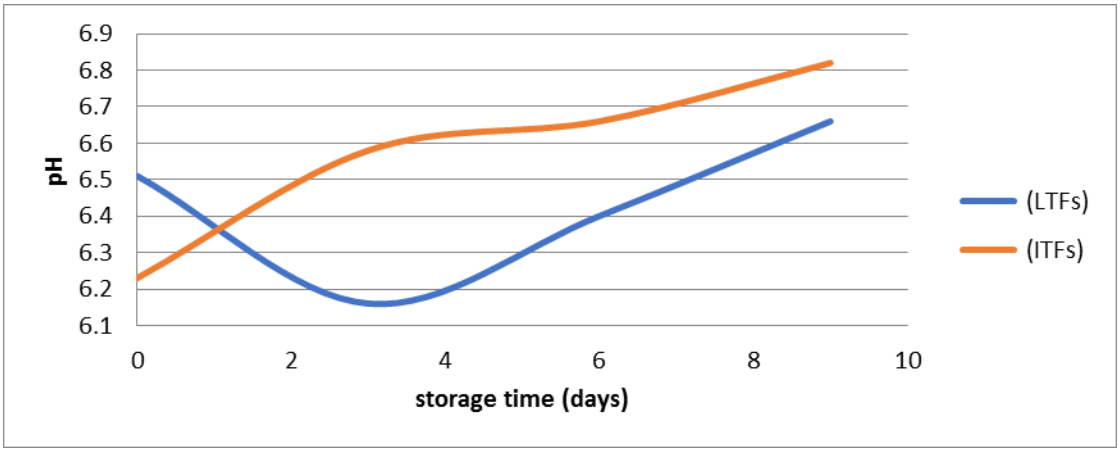


Fig. 3. Effect of refrigerated storage periods at 5±0.5 ºC on pH value of live (LTFs) and iced (ITFs) tilapia fillets samples.

WHC

Fig. 4. Shows the effect of refrigerated storage periods at 5±0.5 ºC on WHC content of live (LTFs) and iced (ITFs) tilapia fillets samples. In the present study, the values of WHC of fresh LTFs and ITFs samples were 19.37±0.03 and 18.68±0.66%, respectively. After that, the values of WHC gradually decreased for both samples with increasing storage periods to reach 15.40 ± 0.34 and 14.44±0.30%, respectively after nine days.

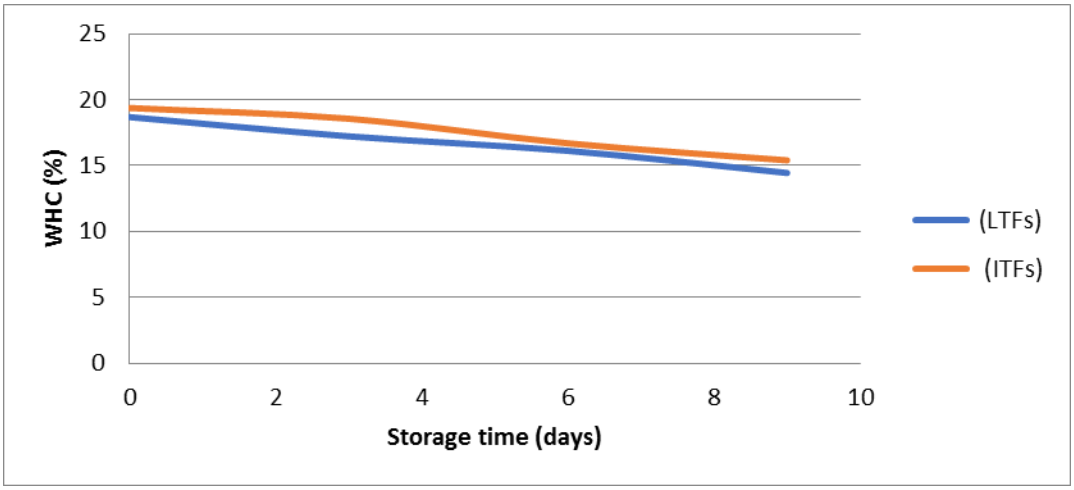


Fig. 4. Effect of refrigerated storage periods at 5±0.5 ºC on WHC value of live (LTFs) and iced (ITFs) tilapia fillets samples.

TVB-N

Fig. 5. Shows the effect of refrigerated storage periods at 5±0.5 ºC on pH value of live (LTFs) and iced (ITFs) tilapia fillets samples**.** In this work, the value of TVB-N content of fresh LTFs was 13.94±0.37mg/100g sample at zero time of storage and gradually increased to reach 31.21±0.73 mg/100g after 9 days of refrigerated storage.

Whereas the its value recorded 15.05±0.29 mg/100g and increased to 36.25 ± 0.47 mg/100g at the same coditions.

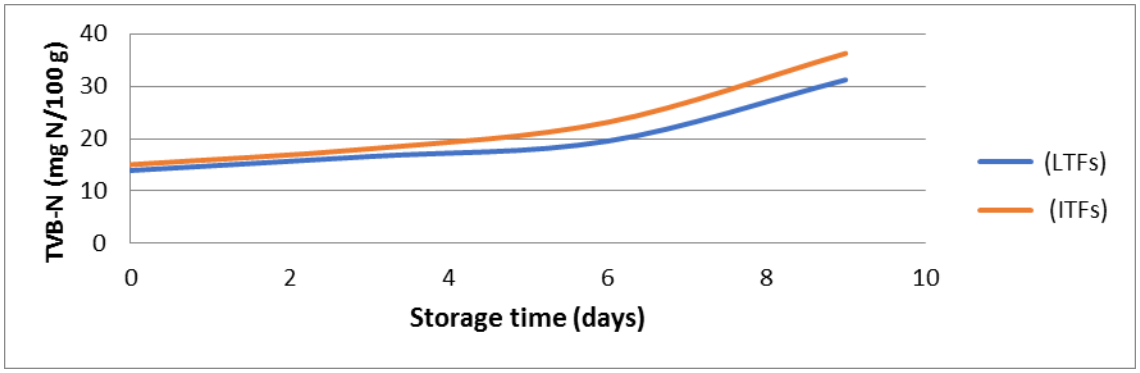


Fig. 5. Effect of refrigerated storage periods at 5±0.5 ºC on TVB-N content of live (LTFs) and iced (ITFs) tilapia fillets samples.

TBARS

The results of TBA-RS values of tilapia fish fillet that was stored for 9 days at 5 ± 0.5 ºC for the current study are presented in Fig.6. Thiobarbituric acid is used as an index to evaluate the degree of lipid oxidation. In this study, the TBA values of fresh ITFs and LTFs recorded 0.25±0.01 and 0.33±0.02 mg MDA/kg sample, respectively. These values increased gradually with prolonged refrigerated storage periods up to nine days to reach 2.22 ± 0.06 and 3.22±0.01 mg MDA/kg sample, respectively. Also, it was found that TBA was higher value in ITFs than other one (LTFs).

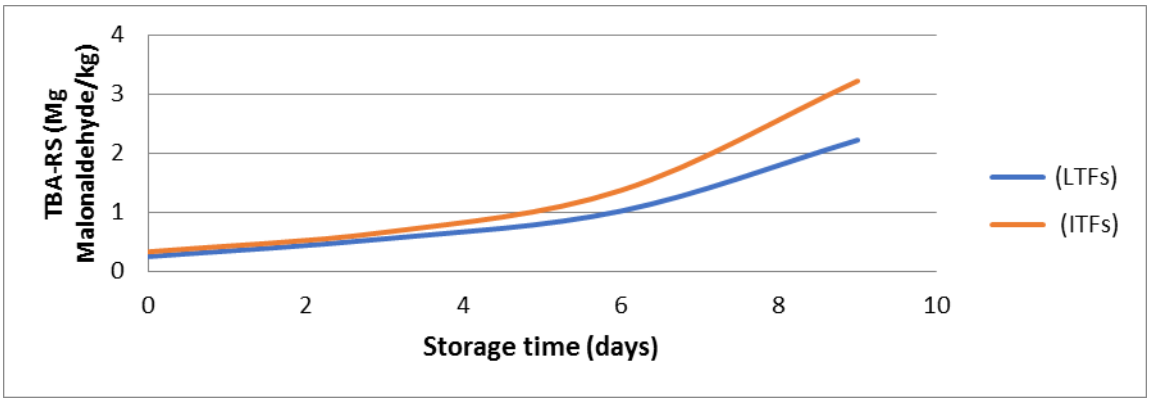


Fig. 6. Effect of refrigerated storage periods at 5±0.5 ºC on TBARS value of live (LTFs) and iced (ITFs) tilapia fillets samples.

Fatty acids composition

The fatty acids (FAs) composition of tilapia fish fillets stored for 6 days at 5±0.5ºC is presented in Table 2. The total fatty acids are classified into the following

three major categories: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). At zero time the percentage of total SFAs, MUFAs and PUFAs in LTFs represented 33.40%; 39.06% and 26.89%, respectively while the corresponding values were 35.69%; 40.22% and 22.95% in ITFs, respectively. The dominant FA was C16:0 of SFAs, C18:1n9t of MUSFAs and C18:2n6c of PUFAs in all samples investigated. Also, the results show that the refrigerated storage periods led to increase the total classes in case of LTFs and to decrease in ITFs at 6 days. LTFs contained high values of Σn 3/Σn 6, PUFA/SFA and PUFA/SFA more than that recorded of ITFs under the same storage conditions. Therefore, the values of both IA and IT were closely associated with prolonged refrigerated storage periods.

Table 2. Fatty acid composition (as % of total FA) of fillets of (LTFs) and fillets of (ITFs) during refrigerated storage at 5 ± 0.5 ºC.

Fatty acid	**LTFs**			**ITFs**		
	0	**3**	**6**	**0**	**3**	**6**
SFAs						
C12:0	0.04	0.04	0.04	0.04	0.05	0.05
C13:0	0.01	0.01	0.01	0.01	0.02	0.01
C14:0	2.74	3.03	3.26	4.08	3.88	3.83
C15:0	0.18	0.18	0.21	0.31	0.32	0.26
C16:0	23.06	23.60	24.40	26.12	24.07	25.19
C17:0	0.37	0.42	0.42	0.74	0.81	0.54
C18:0	5.78	3.93	5.95	3.78	5.47	6.45
C20:0	0.27	0.24	0.30	0.29	0.45	0.02
C21:0	0.66	1.27	0.72	0.43	0.58	0.05
C22:0	0.26	0.21	0.17	0.42	0.17	0.34
C23:0	0.13	0.17	0.17	0.12	0.11	0.00
C24:0	0.09	0.05	0.04	0.03	0.03	0.03
ΣSFA	**33.40**	**33.11**	**35.69**	**36.37**	**35.96**	**35.69**
MUFAs						
C14:1	0.27	0.16	0.10	0.23	0.24	0.16
C15:1	0.07	0.06	0.07	0.10	0.11	0.09
C16:1	4.05	3.93	4.44	5.73	5.37	5.18
C17:1	0.28	0.14	0.01	0.02	0.43	0.02
C18:1n9t	30.51	33.34	33.28	32.92	31.04	31.53
C20:1	1.49	1.40	1.71	0.09	1.42	1.24
C22:1	0.02	0.01	0.03	0.03	0.03	0.02
C24:1	2.37	1.67	1.95	2.07	2.21	1.98
ΣMUFA	**39.06**	**40.71**	**41.59**	**41.19**	**40.85**	**40.22**
PUFAs						
C18:2n9t	0.65	2.19	0.76	2.44	0.11	0.53
C18:2n6t	0.65	1.19	0.38	0.08	0.26	0.01
C18:2n6c	16.14	17.53	17.97	15.09	17.97	16.56
C20:2 n6	0.06	0.04	0.07	0.05	0.05	0.98
C22:2 n6	0.03	0.04	0.04	0.04	0.05	0.09
C18:3n6	0.63	1.10	1.00	0.65	0.71	0.63

C18:3n3	1.21	1.56	1.51	2.13	2.21	1.78
C20:3n6	1.44	1.04	0.46	0.01	0.59	1.18
C20:3n3	0.73	1.15	0.31	0.35	0.78	0.04
C20:4n6	0.27	0.14	0.00	0.01	0.00	1.11
C20:5n-3	1.24	0.02	0.10	0.02	0.37	0.02
C22:6n-3	3.84	0.00	0.01	0.01	0.00	0.02
ΣPUFA	**26.89**	**26**	**22.61**	**20.88**	**23.1**	**22.95**
ΣPUFAn 3	7.02	2.73	1.93	2.51	3.36	1.86
ΣPUFAn 6	19.22	21.08	19.92	15.93	19.63	20.56
Σn 3/Σn 6	0.37	0.13	0.1	0.17	0.17	0.09
DHA/EPA	3.1	0	0.1	0.5	0	1
PUFA/SFA	0.80	0.78	0.63	0.57	0.64	0.62
AI	0.52	0.55	0.59	0.71	0.62	0.65
TI	0.62	0.78	0.92	0.94	0.83	0.98

LTFs: Live tilapia fillets, ITFs: Iced tilapia fillets, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, AI : Atherogenic index, TI: Thrombogenic index.

Microbial aspects

Effect of refrigerated storage periods at 5±0.5 ºC on TBARS value of live (LTFs) and iced (ITFs) tilapia fillets samples is presented in Table 3. The initial total plate count (TPC) was 3.23 log cfu g-1 at zero time for LTFs and increased to 7.49 log cfu g-1 after 9 days of refrigerated storage at 5 ± 0.5 ºC. On the other side, the initial total plate count (TPC) of ITFs was 5.20 log cfu g-1 at zero time and increased to 8.91 log cfu g-1 after 9 days at 5 ± 0.5 ºC. Besides, the psychrophilic bacterial count (PC) is taken the same trend, it increased from 2.60 to 6.90 log cfu g-1 and 3.90 to 8.74 log cfu g-1 for LTFs and ITFs samples, respectivelt under the same conditions.

Table 3. Effect of refrigerated storage periods at 5±0.5 ºC on TBARS value of live (LTFs) and iced (ITFs) tilapia fillets samples.

Storage time (days)	**Total plate counts (TPC) (log CFU g-1)**		**Psychrophilic bacteria (log CFU g-1)**	
	LTF	**ITF**	**LTF**	**ITF**
0	3.23	5.20	2.60	3.90
3	4.08	5.48	2.85	4.78
6	4.42	6.70	3.28	5.86
9	7.49	8.91	6.90	8.74

DISCUSSION

Regarding the effect of harvesting techniques on the yield % of edible part of tilapia fish samples as shown in Fig. 2, the previous studies reported that as a public practice during harvesting of Nile tilapia in Egypt, the height of the water column of fish pond was reduced to its lowest level. It mostly leads to sever conditions of overcrowding and

an increase in the stocking density of the fish that in turn have a harmful effect on the fish health and welfare. The fish skin is considered as the biological and natural barrier encompassing in the ion regulation process **(Ángeles Esteban, 2012; Benhamed *et al*., 2014)** and that provides the protection against the friction that essentially originated from the overcrowding **(Daniel, 1981)** and also it provides the protection against pollution in the aquatic environment and any infectious agents (**Benhamed *et al*., 2014**). The skin injuries are the direct damages or the visible losses of epidermis layer, which would lead to detachment of outer scales, skin discoloration and would be hemorrhage. Not only, it is accompanied with poor fish welfare **(Huntingford *et al*., 2006**) but also it causes production losses **(Ellis *et al*., 2008**). In addition to this study also was focused on the method of fish transportation to the factory because post-harvest transportation is considered one of the most important factors that affect the quality of fish (**FAO, 2005**). Some people transfer fish from the pond a live in oxygenated water tanks (**Olsen *et al*., 2013**), and others transfer fish by putting in boxes and covering the fish with ice **(Bjørnevik and Solbakken, 2010; Ghaly *et al*., 2010**).

The proximate composition of fresh tilapia is presented in Table 1. These values agree with the data shown by **Dergal *et al.* (2013)**. Concerning the content of fat, our results agree with the data stated by **Suloma *et al.* (2008)**; however, lipid content was higher, and in response to that moisture was lower than those reported by other studies. The data of protein content are in line with those obtained by other authors for the same fish species **(Gram and Dalgaard, 2002; and FAO, 2012)**. The content of crude protein in fish muscle can range from11to 24% (wet weight), depending on state of nutrition, specie, the reproductive cycle of the animals, etc. **(Gram and Dalgaard, 2002).** The initial moisture and ash contents significantly decreased ($P < 0.05$) from 79.87 and 0.98% to 78.50 and 0.89%, respectively, at the end of the period of storage (Table 6). Also, a decrease in ash content was reported by **Ozyurt *et al.* (2005)** for the fillets of sea bass during sixty days of frozen storage. The fluctuation of temperature of cold store (temperature abuse) can be a major reason for dehydration **(FAO, 1994**). The temperature abuse in the refrigerator might cause the water vapor migration from the products to the surfaces of the containers. This defect is occasionally found in commercially refrigerated and frozen foods that have been handled improperly. The similar result was reported for frozen tilapia fish by **Arannilewa *et al.* (2005)**.

The highest protein content (20.70±0.3) was recorded for the fresh tilapia sample and the lowest protein content (19.35±0.26) was recorded for fillets samples stored for 9 days in refrigerator (Table 1). The protein content decrease might be attributed to denaturation of the fish, i.e., because of the changes in the proportion of protein breakdown and chemical composition. The protein denaturation includes destruction of its secondary, tertiary and also quaternary structures that reduce proteins to simple polypeptide chains **(Careche and Li-Chan, 1997)**. A number of factors, including

variability of storage conditions and slow chilling, caused this protein denaturation. The rate at which denaturation of protein occurs depends mainly on the chilling temperature. In the current study, protein content of fillets samples was significantly changed ($P < 0.05$). Following death, fish experience rapid degradation of protein as a consequence of the endogenous bacterial enzymes. Also, fish proteins can be impaired by rancidity. During the early stages of the autoxidation, the free radicals and also the relatively stable hydroperoxides are formed that subsequently react with proteins, leading to polymerization of proteins and amino acids destruction **(Danopoulos and Ninni, 1972)**.

Concerning lipid content in this work (Table 1), the lipid oxidation is the major reason for deterioration of many foods that contain oils and fats. The large amounts of polyunsaturated fatty acid (PUFAs) moieties found in the fish lipids highly makes them susceptible to the oxidation by an autocatalytic mechanism **(Smith and Hui, 2004)**. The lipid deterioration limits the shelf life of the fish. Thus, lipid oxidation leads to flavor and nutrition loss, and also creates stiffness and other texture problems **(Aubourg and Medina, 1999)**. The bad result is unpleasant flavor and odour called rancidity. The lipid oxidation plays an important role in the spoilage of fatty and lean fishes. In the current study, the fat content of fillets of tilapia stored for the period of about 9 dayss under the refrigerated storage condition was significantly increased ($P < 0.05$). This was because of the fact that there was a reverse relationship between the lipid and moisture content of tilapia flesh. According to **Ozyurt *et al.* (2005),** the lipid ratio of the fillets of sea bass was 1.22% at the start of the storage and it was reported as 2.28, 2.86, and 3.58% in the 3^{rd}, 6^{th}, and 9^{th} days of storage, respectively. Also, **Tokur (2000)** reported that there was an increase in the lipid content occurred during the frozen (-18°C) storage of rainbow trout fish.

The value pH of the tilapia sample immediately after being caught was reported to be 6.51±0.01 in fillets of (LTFs), but in the fillets of (ITFs) was 6.23±0.01 (Fig.3). These results are in accordance with the results obtained by **Khalafalla *et al.* (2015) and (Moawad *et al.*, 2017)** for the same fish species. Fresh fish pH oscillates between 6.0 and 6.5 **(Fennema, 2000)**, depending on different factors such as fish species, season, diet, level of activity or stress during capture, and storage conditions **(Ocaño-Higuera *et al.*, 2009**). The pH value significantly increased ($P < 0.05$) is due to the increase of the duration of storage period. The highest value of pH (6.82±0.06) was obtained for fish fillets of (ITFs) refrigerated for 9 days. Low pH value is mainly used as an indicator of higher stress before or at the slaughtering time of many animals **(Sigholt *et al.*, 1997**). This is caused by depletion of the energy reserves, essentially glycogen, with the production of lactate. In the present study, the lower initial pH values might outline that fish was subjected to stress before slaughtering. The increament in pH value during chilled storage might be associated with an increase in the volatile basic components. The pH also might be affected by the solute concentration. Since the temperature falls, the

individual solutes reach the saturation point and crystallize out. An increase in the concentration of the solute during chilling leads to a change in the pH (**Fellows, 2000**). All these factors might contribute to a change in pH value. Similar results were reported by **Arannilewa *et al.* (2005),** who investigated the effect of frozen period on the sensory, chemical and microbiological quality of frozen tilapia fish (*Sarotherodum galiaenus*). Slight differences might be because of the differences in the geographical location, catching season, water composition and fish size.

The value of WHC is used as an index to evaluate the degree of denaturation of proteins in the muscle tissue. The muscles ability to retain water is regarded as an essential quality parameter and a high WHC is of great importance both to the industry and the consumers (**Fennema, 1990; Wilson Iii and van Laack, 1999)**. The initial WHC fillets of (LTFs) and (ITFs) were 19.37 ± 0.03 % and 18.68 ± 0.66 %, respectively (Fig.4). These values were significantly decreased with increasing storage time, reaching values of 15.40 ± 0.34%, and 14.44 ± 0.30% after nine days of refrigerated storage in fillets of live transported fish and the fillets of iced transported fish indicating that fillets of iced transported fish was more denaturated due to the high degree of water loss. A similar trend WHC change of Nile tilapia fillets was observed during refrigerated storage by **Moawad *et al.* (2017*)***. However, WHC directly affects product appearance, production efficacy/profitability and consumption quality such as juiciness **(Zhuang *et al.*, 2008)**.

The content of TVB-N was significantly increased during the cold storage from13.94±0.37 to 31.21±0.73mg/100 g of LTFs (Fig. 5). Consequently, the TVB-N content did not reach the unacceptable limit (35 mg N/100 g) for the human consumption **(Egan *et al.*, 1981)**. On the other hand, TVB-N increased during the cold storage from15.05±0.29 to 36.25±0.47 mg/100 g of ITFs. So, the TVB-N content of the fillets of the fish increased significantly during storage in the cold store but had reached the unacceptable limit. Similar results were obtained by **Xue (2000)** for yellowtail, and by **Ola and Oladipo (2004)** for croaker. A similar increase in TVB-N also has been reported in the iced cuttlefish by **Subramanian (2007)**. The increase in TVB-N might be attributed to the production of DMA, TMA, ammonia and other basic nitrogenous components resulted from the decomposition of TMAO by the endogenous enzymes that are present in the fish species **(Egan *et al.*, 1981)**.

The achieved data in Fig. (6), the TBARS amounts that were significantly ($P<0.05$) influenced by the treatments and periods of storage, with interactions being observed between these factors. The TBA-RS values of the examined LTFs and ITFs at zero time were 0.25 ± 0.01 and 0.33 ± 0.02 mg MDA/kg sample, respectively of storage at 5 ± 0.5 ºC. This results agreement with **GutÉRrez GuzmÁN *et al.*, (2015)**. Concerning the permissible limit of TBA value in fish and fish products (4.5 mg MDA/kg) as recommended by Egyptian Organization for Standardization **(EOS, 2005)**.

Connell (1990) stated that TBA values of 1-2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish will normally develop an objectionable odour and taste. The TBA-RS values observed for the fillets of LTFs were lower at the end of the storage period compared to ITFs. This was may be caused by the lower ratio of O_2 that retarded the oxidative process of PUFAs. O_2 reacts with fatty acids to produce hydroperoxide without degrading the odoriferous components **(Church, 1998)**.

The results of fatty acids (FAs) composition of tilapia fillets stored for 9 days at 5 ± 0.5 ºC Table 2. The variation of SFAs, MUFAs and PUFAs of LTFs and ITFs is accordance with those reported by **Navarro *et al.* (2012)**. In the present study, DHA and EPA were the major ω_3 PUFAs while linoleic acid (C18:2 ω_6) and arachidonic acid (C20:4 ω_6) were the predominant ω_6 PUFAs. About half of fish fat is made of oleic fatty acid (C18:1n9) responsible for the soft, juicy texture **(Cvrtila and Kozačinski, 2006)**. PUFA/SFA ratio is recommended to be higher than 0.4, so as to reduce the risk of cardiovascular, autoimmune and other chronic diseases **(Simopoulos, 2002)**. Literature data have revealed that lower ω6/ ω3ratios allow for better utilisation of $\omega_3$fatty acids in the human body (**Wood *et al.*, 2008**). According to health recommendations, n-6/n-3 ratio should be lower than 4, thereby reducing the incidence of chronic food-related illnesses **(Simopoulos, 2002; Cordain *et al.*., 2005)**. The atherogenic index (AI) is the parameter descriptive of the ability of some saturates to exhibit pro-atherogenic effects due to the facilitation of the lipid adhesion onto cells the immune and the circulatory system are composed of, while non-saturates are considered to be anti-atherogenic as they inhibit the formation of plaques and diminish the levels of esterified fatty acids, cholesterol, and phospholipids, therefore preventing micro- and macro-coronary events **(Ulbricht and Southgate, 1991)**. The thrombogenic index (TI) shows the tendency towards blood clotting. Several studies that made use of salmon, hake and saithe fillets have confirmed that freezing and frozen storage may provoke lipid decomposition, PUFA content decrease and SFA content increase **(Saldanha and Bragagnolo, 2007; Karlsdottir *et al.*, 2014; Dawson *et al.*, 2018) .**

The initial value of TPC of LTFs and ITFs at zero time were 3.23 and 5.20 log cfu -1 respectively that increased progressively ($P < 0.05$) with storage time to final values of 7.49 and 8.91 log CFU g-1 respectively Table 3. Our result was in agreement with **(Fu and Labuza 1993; Zambuchini *et al.*, 2008). Fu and Labuza (1993)** found that the duration of the refrigerated storage of fish significantly had an ($P < 0.05$) effect on the bacterial count, which tended to increase with increasing the storage duration. In this respect, International Commission on Microbiological Specifications for Foods **(ICMS 1986)** mentioned that the upper acceptability limit of the total viable count (TVC) of bacteria in fresh fish is 7 $\log_{10}$ cfu/g flesh, and 6 $\log_{10}$ cfu/g is the maximum permissible limit of TVC recommended by the **(EOS, 2005)** in the chilled fish, **(Özogul *et al.*, 2004)** declared that when the aerobic plate count reaches 10^6 cfu/g or mL in a food product, it is

supposed to be at, or near, spoilage. Additionally, fish was assumed "not to be in an enough good condition so as to be stored for long" when total plate count were 10^6 cfu/g. The changes in TVC with the time of storage in all groups were noted. Results obtained the psychrotrophic bacterial count (log CFU/g) during the storage period. The development of psychrotrophic bacteria increased during the storage period, with count above 10^6 cfu g-1 where it recorded 6.90 and 8.74 $\log_{10}$ cfu g-1 for LTFs and ITFs, respectively at the 9^{th} day. Psychrotrophic bacteria are very important among different bacteria that cause spoilage, because they are frequently related to the changes in the sensory attributes such as texture, odor and flavor and can produce different metabolic compounds such as ketones, aldehydes, volatile sulfides and biogenic amines **(Safari and Yosefian, 2006)**. A proposed limit of psychrotrophic bacteria is 10^3 to 10^4 cfu/g, which is consistent with other studies **(Pons-Sánchez-Cascado *et al.*, 2006)**.

CONCLUSION

Based on the results obtained, the Tilapia fillets of the unstressed, appropriately harvested and live transported tilapia (LTFs) have a higher yield and longer shelf life and better quality than the stressed- harvested and ice transported tilapia (ITFs). Also, transferring of harvested fish live in oxygenated water tanks is better than other one to prevent higher economic losses and increase the profitability of tilapia processing in Egypt and all over the world.

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