EFFECT OF FREEZING ON THE FUNCTIONAL PROPERTIES OF FLESH NILE TILAPIA *OREOCHROMIS NILOTICUS* PROTEIN

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Abstract

Nile tilapia *Oreochromis niloticus* fillets and minced flesh samples were periodically withdrawn and analyzed for water holding capacity (WHC), foaming capacity (FC), emulsification capacity (EC), total soluble protein (TSP), soluble protein nitrogen (SPN) and soluble non protein nitrogen (SNPN) as quality criteria of Nile tilapia *Oreochromis niloticus*. Fillets and machine minced flesh blocks packaged in ice-glaze film or polyethylene bags were evaluated over a 6-months storage period at −20°C. Results showed that WHC, FC and EC gradually decreased in all treatments. Also TSN, SPN and SNPN were slowly decreased in all samples during the storage period. Fillets blocks were much more stable than minced blocks, especially those packaged in ice-glaze film compared with those packaged in polyethylene bags.

INTRODUCTION

Freezing is an excellent method for preserving the organoleptic attributes and protein functionality of flesh fish during prolonged periods of time. Depending on intrinsic factors such as species, season and technological factors such as handling practices prior to freezing, freezing rate, temperature of storage, or presence of protective barriers against oxidation, the practical storage life of frozen fish may vary substantially. Therefore, the quality of fish found on sale is not always good, due to reasons ranging from unsuitable raw material to bad handling practices or storage conditions. This is also a problem for processing industries that have to purchase fish stocks of irregular quality, which may deteriorate at different rates during processing and retail sale. Although good handling and storage practices are broadly known, sometimes, due to technological or economical factors, they cannot be completely followed. The end of practical storage life is reflected as a fibrous, dry product which becomes tough and which has lost important functional properties. (Careche *et al.*, 1999).

Matsumoto (1980) and Owusu-Ansah & Hultin (1992) revealed that the differential insolubilization observed in both sarcoplasmic and contractile proteins may be important in textural deterioration of red hake. Sarcoplasmic proteins are nonstructural proteins and, therefore, not generally considered to contribute to textural deterioration of fish muscle. However, by becoming insoluble either by adsorption onto insoluble structural proteins or by denaturation, water soluble proteins might significantly affect the texture of fish. Sarma *et al.* (1999) investigated the

effects of ice storage on functional properties of pink perch *Nemipterus japonicus* and Indian oil sardine *Sardinella longiceps* proteins, and declared decreased emulsifying capacity EC, relative viscosity (RV) and water holding capacity (WHC), as well as an increase in cook loss (CL) in both fish species; water and salt-soluble proteins also decreased during ice storage. Significant ($P<0.05$) correlations between the various functional properties analyzed indicated their interdependence on changes in soluble proteins. Singh and Balange (2005) demonstrated that moisture, crude protein, total nitrogen and pH decreased during frozen storage, while the total volatile base nitrogen (TVBN), trimethylamine (TMA), peroxide value (PV) and total plate count (TPC) values of surimi increased during frozen storage, quality changes of fish muscle are normally due to autolytic chemical reactions, microbial proliferation, and physical property alterations that consequently cause functionality of end products and reduce shelf life.

The present work was planned to study the effect of storage period at –20°C on function properties of Nile tilapia *Oreochromis niloticus* frame fillets or minced flesh packaged in ice-glaze film or polyethylene bags for 6-months aiming to determine and predict the commercial quality of the fish.

MATERIALS AND METHODS

Preparation of samples:

Sample lots of Nile tilapia (*Oreochromis niloticus)* were immediately obtained after caught from Abbassa fish farm at Sharkia governorate, Egypt. Intact flesh was separated by hand filleting. Half of lots was minced and considered as minced flesh. Fillets and minced flesh were placed in 4×15×30 cm. stainless steel trays and frozen in blocks at –30°C for twelve hours. The blocks were removed from the deep freezer and half of fillets and minced flesh. The blocks were embedded into cold water (ice glazed) then transmitted to deep freezer for one hour at –30°C. The treatments were applied:

1- Fillets blocks were packaged in ice-glazed film.

2- Fillets blocks were packaged in polyethylene bags.

3- Minced blocks were packaged in ice- glazed film.

4- Minced blocks were packaged in polyethylene bags, and stored at –20°C. Sensory evaluations, microbiological count and chemical analyses were carried out at 0, 1, 2, 3, 4, 5 and 6 months of storage. At the end of every freezing period (30 days), samples were withdrawn randomly, aseptically thawed at room temperature, cut into small pieces, mixed and chopped in electric meat chopper and then analyzed in triplicate.

Analytical methods:

The water holding capacity (WHC) was determined using the press method according to Volvinskaga and Kelman (1960). Foaming capacity (FC) was measured in

two grams material blended with 100ml distilled water in an electric blender for 3 min. The blend was poured slowly into a 250ml measuring cylinder and the volume was recorded after 10 sec. FC was calculated as described by Lowhon *et al.* (1972).

Foaming capacity=

$$\frac{Volume \text{ after whipping - Volume before whipping}}{Volume \text{ before whipping}} \times 100$$

Emulsification capacity (EC) was determined according to Beuchat *et al.* (1975). EC was expressed as ml oil emulsified by grams of flesh mince. Total soluble nitrogen (TSN), soluble protein nitrogen (SPN) and Soluble non-protein nitrogen (SNPN) were determined according to the method described by Kline and Stewart (1949).

Statistical Analysis:

Three replications of each trial were performed WHC, FC, EC, TSN, SPN and SNPN data were analyzed using ANOVA and means were separated by Duncan's test (1959) at a probability level of < 0.05 (SAS, 2000).

RESULTS AND DISCUSSION

Water holding capacity (WHC):

One of the most important features of fish flesh quality is its water holding capacity, which is closely related to tenderness and other properties of flesh fish quality as taste, juiciness and color.

Results given in Table (1) show the effect of freezing storage on water holding capacity of fillets and minced *Oreochromis niloticus* blocks packaged in ice-glaze film and polyethylene bags. Data showed that WHC was significantly and gradually decreased ($p<0.05$) throughout the storage period till 90 days, thereafter slight increases were observed during the last 90 days of storage. The decrement in WHC during freezing storage during the first 90 days may be attributed to the mechanical loose of the muscle tissue by the formation of ice-crystals inside the cells.

Generally, the lowest WHC was found for minced blocks packaged in polyethylene, it was 67.90 % at the end of storage period at –20°C compared with the other treatments. The obtained data are in agreement with those reported by Sarma *et al.* (1999), and Singh, and Balange (2005).

Table 1. Average water holding capacity values of *Oreochromis niloticus* fillets and minced blocks, packaged in ice-glaze film or polyethylene bags during storage at –20°C for 6 months.

Parameter		Water holding capacity			
Flesh form		Fillets		Minced	
Packaging		Ice-glaze	Polyethylene	Ice-glaze	Polyethylene
Storage period (Months)	0	72.1 ± 0.01 a	72.1 ± 0.05 a	72.1 ± 0.04 a	72.1 ± 0.07 d
	1	70.1 ± 0.05 b	68.6± 0.07b	69.2 ± 0.06 b	67.9 ± 0.08 b
	2	68.6 ± 0.02 c	66. ± 0.04 c	67.10 ± 0.04 c	64.8 ± 0.06 c
	3	67.3 ± 0.03 d	64.5 ± 0.03 d	65.9 ± 0.05 d	63.2 ± 0.05 d
	4	68.0 ± 0.04 cd	65.4 ± 0.05 cd	66.9 ± 0.06 c	64.4 ± 0.08 c
	5	68.9 ± 0.01 c	66.7 ± 0.03 c	67.1 ± 0.08 bc	65.9 ± 0.05 bc
	6	70.3 ± 0.02 b	68.8 ± 0.04 b	68.6 ± 0.02 b	67.9 ± 0.05 b

[a-d] Means within a column with the same superscript are significantly different ($p<0.05$).

Foaming capacity (FC):

The results presented in Table (2) revealed the effect of storage time at –20°C on foaming capacity (FC) % of *Oreochromis niloticus* frame fillets or minced flesh packaged in ice-glaze film and polyethylene bags. The analysis of FC%, indicated a significant decrease ($p<0.05$) in fillets blocks packaged in ice-glaze film at –20°C (123.40%) at the end of 6-months of storage, and followed in a decreasing order by the fillets blocks packaged in polyethylene bags (120.80%); minced blocks packaged in ice-glaze film (118.20%) and in polyethylene bags (114.60%), respectively. The differences in FC among the treatments may be due to the nature of the protein and the relative abilities of these proteins to denature and lower the surface tension at the air-liquid interface of the foam. These results are in line with those obtained by Kinsella, (1979) and Vaghela and Kilara (1996).

Table 2. Average foaming capacity values of *Oreochromis niloticus* fillets and minced blocks, packaged in ice-glaze film or polyethylene bags during storage at –20°C for 6 months.

Parameter		Foaming capacity (%)			
Flesh form		Fillets		Minced	
Packaging		Ice-glaze	Polyethylene	Ice-glaze	Polyethylene
Storage period (Months)	0	136.4 ± 0.1 a	136.4± 0.2 a	136.4± 0.2 a	136.4 ± 0.3 a
	1	132.7 ± 0.2 b	131.7 ± 0.5 b	130.5 ± 0.3 b	129.6 ± 0.5 b
	2	128.7 ± 0.1 bc	128.8 ± 0.3 bc	127.6 ± 0.2 b	125.9 ± 0.4 c
	3	126.1 ± 0.2 c	125.7 ± 0.2 c	124.5 ± 0.3 c	122.3 ± 0.3 c
	4	124.6 ± 0.3 c	123.4 ± 0.3 d	121.8 ± 0.4 cd	119.6 ± 0.5 d
	5	123.6 ± 0.2 cd	122.0 ± 0.4 d	120.2 ± 0.2 d	117.2 ± 0.3 d
	6	122.4 ± 0.1 d	120.8 ± 0.3 de	118.2 ± 0.2 de	114.6 ± 0.4 e

[a-e] Means within a column with the same superscript are significantly different ($p<0.05$).

Emulsifying capacity (EC):

Results presented in Table (3) show the emulsifying capacity EC levels (ml. Oil/g) as affected by treatments. Results indicated a gradual significant decrease ($p<0.05$) in EC up to 6-months of storage period. Data show that the lowest level of EC was found in minced blocks packaged in polyethylene bags at –20°C, 47.7ml oil/g. at the end of 6-months storage period. The highest value of EC was found in fillets blocks packaged in ice-glaze film at –20°C 50.1ml oil/g. at the end of storage period (6-months). The difference in EC was due to the presence of solubilized proteins. These results are in harmony with those obtained by Li-Chan *et al.* (1985); Vaghela and Kilara (1996) and Sarma *et al.* (1999).

Table 3. Average emulsifying capacity values of (ml. Oil/g.) in *Oreochromis niloticus* fillets and minced blocks, packaged in ice-glaze film or polyethylene bags during storage at –20°C for 6 months.

Parameter		Emulsifying capacity (ml. Oil/g. mince)			
Flesh form		Fillets		Minced	
Packaging		Ice-glaze	Polyethylene	Ice-glaze	Polyethylene
Storage period (Months)	0	56.6 ± 0.01 a	56.6 ± 0.01 a	56.6 ± 0.01 a	56.6 ± 0.02 a
	1	55.2 ± 0.02 b	54.7 ± 0.02 b	53.9 ± 0.01 b	53.3 ± 0.02 b
	2	54.3 ± 0.01 bc	53.5 ± 0.02 c	53.0 ± 0.01 c	52.1 ± 0.02 c
	3	53.2 ± 0.03 c	52.4 ± 0.04 cd	52.0 ± 0.02 cd	51.0 ± 0.03 cd
	4	52.6 ± 0.01 cd	51.5 ± 0.03 d	50.7 ± 0.01d	49.6 ± 0.02d
	5	52.1 ± 0.02 d	50.9 ± 0.05 de	50.0 ± 0.03 de	48.6 ± 0.04 de
	6	51.4 ± 0.01 d	50.1 ± 0.02 e	49.0 ± 0.01 e	47.7 ± 0.02 e

[a-e] Means within a column with the same superscript are significantly different ($p<0.05$).

Total soluble nitrogen (TSN), soluble protein nitrogen (SPN) and soluble non-protein nitrogen (SNPN).

Data shown in tables (4, 5, & 6) indicated a significant slow and gradual decrease ($p<0.05$) in TSN and SPN as well as a significant and gradual increase ($p<0.05$) in SNPN throughout the 6-months storage at –20°C. The data of Careche and Tejada (1990); De-Koning and Mol (1991); Owusu-Ansah and Hultin (1992); Vaghela and Kilara (1996); Sarma (1999); Singh and Balange (2005) supported the present results. The decrease in TSN and SPN could be attributed to a denaturation of protein. During the freezing storage period proteins breakdown into smaller molecules due to the activity of proteolytic enzymes. Data illustrated in tables (4, 5 & 6) revealed that the TSN, SPN and SNPN values for fresh samples were 3.90%, 1.80% and 2.10%, respectively. After 6-months of storage the same parameters reached to 3.40, 3.49, 3.32 and 3.47% for TSN; 1.01, 1.05, 0.92, and 0.92% for SPN and 2.39, 2.44, 2.40, and 2.55% for SNPN for fillets blocks packaged in ice-glaze film or polyethylene bags; minced blocks packaged in ice-glaze film and polyethylene bags, respectively.

It is a matter of interest to announce that dissection and loss of water, especially at surface layers of flesh fish cuts enhanced the denaturation and protein insolubility. The collected data reflected that the intact fillets blocks packaged in ice-glaze can possess good quality during storage period for 6-months at –20°C, compared with quality characteristics of fillets blocks packaged in polyethylene bags and minced blocks packaged in ice-glaze film or polyethylene bags stored at the same conditions.

Based on results obtained, it could be recommended to perform the preservation of fish flesh in form of intact fillet blocks packaged in ice glaze for periods to 6 month.

Table 4. Average total soluble nitrogen values of *Oreochromis niloticus* fillets and minced blocks, packaged in ice-glaze film or polyethylene bags during storage at –20°C for 6 months.

Parameter		Total soluble nitrogen %			
Flesh form		Fillets		Minced	
Packaging		Ice-glaze	Polyethylene	Ice-glaze	Polyethylene
Storage period (Months)	0	3.90 ± 0.002 a	3.90 ± 0.005 a	3.90 ± 0.002 a	3.90± 0.004 a
	1	3.72 ± 0.002 a	3.78 ± 0.003 a	3.69 ± 0.001 a	3.75 ± 0.003 a
	2	3.65 ± 0.001 a	3.69 ± 0.002 a	3.61 ± 0.003 ab	3.68 ± 0.005 a
	3	3.59 ± 0.003 b	a 3.64± 0.004 a	3.53 ± 0.003 b	3.63 ± 0.004 ab
	4	3.53 ± 0.001 bc	3.59 ± 0.002 ab	3.47 ± 0.001 bc	3.57 ± 0.001 b
	5	3.47 ± 0.001 c	3.55 ± 0.001 b	3.43 ± 0.002 c	3.52 ± 0.003 bc
	6	3.40 ± 0.002 c	3.49 ± 0.002 b	3.32 ± 0.001 d	3.47 ± 0.003 bc

[a-d] Means within a column with the same superscript are significantly different ($p<0.05$).

Table 5. Average Soluble protein nitrogen values of *Oreochromis niloticus* fillets and minced blocks, packaged in ice-glaze film or polyethylene bags during storage at –20°C for 6 months.

Parameter		Soluble protein nitrogen %			
Flesh form		Fillets		Minced	
Packaging		Ice-glaze	Polyethylene	Ice-glaze	Polyethylene
Storage period (Months)	0	1.80± 0.001 a	1.80 ± 0.001 a	1.80 ± 0.002 a	1.80 ± 0.002 a
	1	1.54± 0.002 b	1.56 ± 0.002 b	1.49 ± 0.001 b	1.51 ± 0.003 b
	2	1.42 ± 0.001 bc	1.42 ± 0.001 bc	1.36 ± 0.001 bc	1.39 ± 0.001 bc
	3	1.30 ± 0.002 c	1.32 ± 0.003 c	1.24± 0.001 c	1.28 ± 0.003 c
	4	1.21 ± 0.001 cd	1.22 ± 0.001 cd	1.13 ± 0.002 cd	1.15 ± 0.002 cd
	5	1.13 ± 0.001 cd	1.14± 0.001cd	1.05 ± 0.001 cd	1.05 ± 0.001 cd
	6	1.01 ± 0.001 d	1.05 ± 0.001 cd	0.92 ± 0.002 cd	0.92 ± 0.002 cd

[a-d] Means within a column with the same superscript are significantly different ($p<0.05$).

Table 6. Average Soluble non protein nitrogen values of *Oreochromis niloticus* fillets and minced blocks, packaged in ice-glaze film or polyethylene bags during storage at –20°C for 6 months.

Parameter		Soluble non protein nitrogen %			
Flesh form		Fillets		Minced	
Packaging		Ice-glaze	Polyethylene	Ice-glaze	Polyethylene
Storage period (Months)	0	2.10 ± 0.002 b	2.10 ± 0.003 bc	2.10 ± 0.003 bc	2.10 ± 0.004 c
	1	2.18 ± 0.001 b	2.22 ± 0.002 b	2.20 ± 0.001b c	2.24 ± 0.002 bc
	2	2.23 ± 0.002 ab	2.27 ± 0.003 b	2.25 ± 0.001 b	2.29 ± 0.003 bc
	3	2.29 ± 0.001 ab	2.32 ± 0.001 ab	2.29 ± 0.001 b	2.35 ± 0.001 b
	4	2.32 ± 0.001 ab	2.37 ± 0.002 ab	2.34 ± 0.001 ab	2.42 ± 0.002 ab
	5	2.34 ± 0.002 a	2.41 ± 0.001 ab	2.38 ± 0.001 a	2.47 ± 0.001 a
	6	2.39 ± 0.001 a	2.44 ± 0.002 a	2.40 ± 0.002 a	2.55 ± 0.003a

[a-c] Means within a column with the same superscript are significantly different ($p<0.05$).

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تأثير التجميد على الخواص الوظيفية لبروتين البلطي النيلي

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قسم التصنيع ومراقبة الجودة (المعمل المركزى لبحوث الثروة السمكية بالعباسة – مركز البحوث الزراعية – وزارة الزراعة – الدقى – الجيزة)

تم دراسة تأثير التجميد على الخواص الوظيفية لبروتين سمك البلطي النيلي المأخوذ من مزارع المعمل المركزي لبحوث الثروة السمكية بالعباسة – محافظة الشرقية. وقد تم دراسة القدرة على ربط الماء ، سعة الرغوة ، سعة الاستحلاب ، النيتروجين الكلى الذائب ، النيتروجين البروتيني الذائب والنيتروجين الغير بروتيني الذائب كمقياس لجودة شرائح أو مفروم البلطي النيلي المخزن بالتجميد لمدة ستة أشهر على صورة بلوكات مغلفة إما بطبقة رقيقة من الثلج (التزجيج) أو بغلاف من البولي إثيلين.

وقد أوضحت النتائج : حدوث انخفاض تدريجي في كل من مستويات القدرة على ربط الماء ، سعة الرغوة وكذلك سعة الاستحلاب خلال فترة التخزين. كذلك أظهرت النتائج أيضاً انخفاضا بسيطا في كل المعاملات المختلفة السابقة خلال فترة التخزين على – ٢٠°م لمدة ستة أشهر. من هذه النتائج تبين ان بلوكات شرائح البلطي النيلي كانت أكثر ثباتاً من بلوكات مفروم البلطي النيلى ، خاصة بلوكات الشرائح المحاطة بطبقة رقيقة من الثلج (التزجيج) مقارنة بتلك المغلفة بالبولي إثيلين.