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# Effect of Freezing Methods on Protein Fractions of Nasser Lake Fish Meat During Storage

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## Abstract:

The comparative studies of Nile Tilapia *(Oreochromis niloticus*) and Nile Perch (*Lates niloticus)* were caught from Nasser Lake. We aim to find the association between freezing and protein fractions. The comparative studies of two species. Were investigated during storage at -18˚C for 6 months. The Protein and protein fractions for Nile Tilapia (Total protein, Myofibrillar, Sarcoplasm, Connective tissue and Denaturated protein) were studied during this period by freezing at -20˚C freezing at -30 ℃ and the best results were at glazing with rosemary extract were noted that from ( (18.02,12.44,3.96,0.29 and 1.33%) to (16.27,10.33, 3.68, 0.30 and 2.78%), respectively. As for Nile perch from (21.54, 13.77, 5.40, .94 and 1.43%) to (18.50, 11.30, 3.43, 0.90 and 3.31%) at glazing, correspondingly. Chemical, physical parameters and fatty acids changed during storage were investigated. Also, the amino acids increased during storage period. From the results drawn from this study, we find that the freezing by glazing rosemary in maintaining the quality of the stored fish especially on the protein fractions.

**Keywords:** Tilapia, Nile perch, Myofibrillar, Sarcoplasmic, Glazing.

## 1- Introduction

Lake Nasser in south Egypt has served as the nation's main source of fresh water since the Aswan High Dam was finished in the 1960s. Inside Egyptian borders, it runs for more than 350 kilometres, and Lake Nasser is a significant fishery for Egypt **(Awatef and Abu Elhassan, 2022).** Nile Tilapias are cultured in approximately 135 countries worldwide, making them the second most commercially important Whitefish species after salmonids, accounting for approximately 40% of the global trade volume **(FAO, 2018). Ayanda et al. ( 2019)** calculated that Protein (17.60) %, Fat (1.89) %, Ash (3.84) % and Moisture (76.40) % in Tilapia**.** In Lake Nasser, the predominant species for sale as fresh fish are the Tilapias, followed by Nile perch, which constitute 25.07% of the fish in the lake **(GAFRD, 2016)**. The proximate composition of Nile perch was (19.25) % Protein, % (3.13) Fat, (1.29) % Ash, (76.42) % Moisture **(Tasabeeh et al., 2016).**

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Rosemary is a spice plant that is frequently used to flavour cuisine. Due to its strong antioxidant potential, it is well known as a natural antioxidant **(Yang et al., 2016; Moczkowska et al., 2020).** Freezing at low temperatures provided small ice crystals which increased light scattering and absorption across all wavelengths in the visible region **(Ottestad et al., 2011)**. Anzymatic and chemical deterioration is slowed by lower temperatures, these processes still happen when food is kept frozen. The flavor, look, and nutritional value of fish will be impacted by browning and autoxidation reactions during frozen storage **(Dawson et al., 2018).** Fish, with a temperature of less than –18.0 °C is considered to be "deep frozen**" (Bøgh-Sørensen, 2006).** Traditionally, the storage temperature of frozen fish is between –18.0 and –30.0 ºC **(FAO/WHO, 2003).** Glazing is largely used in the fish industry to protect fish from the deterioration of sensory characteristics, and can be defined as the application of a layer of ice in frozen products surface by means of a dipping process, or by spraying in a water bath **(Zoldos et al., 2011**).

In fish, different muscles have various types of protein composition. First of all, there are three kinds of muscles such as cardiac, smooth, and striated muscles but the major one is striated muscles present in fishes. Secondly, on the base of solubilization, fish muscle proteins are categorized into three types such as, sarcoplasmic proteins myofibrillar protein and stroma protein **(Ochiai and Ozawa, 2020).** Myofibrillar, sarcoplasmic, and stroma proteins (connective tissue), constitute the 70–80 %, 20–30 %, and 3 % of total muscle proteins, respectively. **(Venugopal 2009)** The myofibrillar proteins play a crucial role in the process of food processing, consumption and storage **(Li et al., 2022).** Myofibrillar and sarcoplasmic protein denaturation occurs in freezing and thawing. The rate of freezing produces a significant effect in the myofibrillar fraction. A slow freezing will develop a more severe protein denaturation than a fast freezing. Consequently, the myofibrillar protein denaturation, related to the freezing rate, is proposed to contribute to the generation of thaw loss **(Zhang , 2020)**. The present study was mainly conducted to study the effect of freezing methods on protein fractions for Nile Tilapia and Nile perch during storage (6 months) at -18℃, and determination of chemical properties, amino acids and fatty acid composition of studied fish.

## 2- Materials and Methods

### 2.1. Preparation of Samples and Glazing Solution:

In the Nasser Lake, Aswan, Egypt, 60 KG of Nile Tilapia (Oreochromis niloticus) and Nile Perch (Lates niloticus) were coughed
. They were removed the bowels and washed by running water. Were divided to three groups; group (slow freezing) transported in frozen (-20℃) for 12 h for group (Fast freezing) transported in frozen (-30℃) for 7 h. Group (Glazing) were glazed by dipping with rosemary extract at -18℃. All the groups were stored at (-18℃) for six months. The glazing solution was prepared by dissolving rosemary extract (1.5% (w/v) in potable water. The water solution of rosemary extract was kept at 0°C before used for glazing for fish. Treated fish samples were labeled and aerobically packaged as triplicates inside fiber dishes, then stored at -18℃ inside the deep freezer, the extraction of active ingredients from dry rosemary plant was done according to the technique developed by **(Sarabi et al., 2017).**

### 2.2. Physical Analysis:

pH determinate using a digital pH meter (HANNA, H1902 m Germany) according to **Han et al. ( 2005),** the samples were analyzed for titratable acidity according to **Pearson (1971).** Drip loss and cooking loss were determined according to **Mousakhani-Ganjeh et al (.2015).**

**2.3. Chemical Analysis:**

Chemical analysis (moisture, Crude lipid, and ash) were determined **(AOAC, 2015)**

- Moisture content was determined by heating samples at 105 ± 1 °C in a hot air oven.
- Crude lipid was determined using Soxhalet apparatus (petroleum ether).
- Ash was determined in samples using Muffle Furnace at 555 °C Samples were weighed into ashing dish which has been ignited in Muffle until light gray ash was obtained. Ash could cool in desiccators and weighed after obtained at room temperature.

**2.3.1. Determination of total protein:**

The total protein content was estimated by the Biuret procedure **(Gornall et al .,1949; Torten and Whitaker 1964).** Fish sample (1 g) was dispersed in 20 mL of 0.5 M NaOH, heated in the boiling water using water bath (DAIHAN, Korea) for 10 min and cooled in an ice-water bath. After cooling the solution was filtered through Whatman No. 1 filter paper. Then, 15 mL of the filtrate was centrifuged with 15 mL of anhydrous ether at 2278×gfor 10 min using centrifuge (model: Pro-Research.K2015R 218218-1. United kingdom) After centrifugation,1 mL of the superannuated was taken and mixed with 3 mL of Biuret reagent for 30 minutes and the absorbance was measured at 540 nm by using spectrophotometer (model: T60UV, PG instruments . United Kingdom) Bovine serum albumin was used as a standard .

**2.3.2 Determination of sarcoplasmic protein***:*

Sarcoplasmic protein were determined according to**Malva et al. ( 2018)** with some modifications. Samples (1.0 gram) were homogenized with 0.03 M phosphate buffer pH 7 for 2 min the homogenate was centrifuged at 3000 rpm for 20 min at 4°C After centrifugation, (sarcoplasmic proteins) 1 mL of the superannuated was taken and mixed with 3 mL of Biuret reagent and the absorbance was measured at 540 nm Bovine serum albumin (Sigma–Aldrich, StLouis, MO, USA) was used as a standard.

**2.3. 3 Determination of myofibrillar proteins:**

Myofibrillar proteins were determined according to **Niu et al. ( 2015).** With some modifications as following, the pellet recovered was suspended in 10 volumes of NaCl solution the obtained sediments were washed with NaCl solution (10mL, 12%) three times, and Shacked for 2 min to extract more water-soluble substances. The supernatant was collected, and the myofibrillar protein concentration was determined by the Biuret assay method.

**2.3. 4 Determination of denaturated protein:**

Denaturated protein was determined according to **(king.1966).**The denaturated protein were extracted by10 mL 0.1 N NaOH solution centrifuged at 3000 rpm for 15 min at 4°C after centrifugation ( model: Pro-Research.K2015R 218218-1. United kingdom) at 3000 rpm for 15 min at 4°C, the supernatant was determined by the Biuret assay method. Connective tissues were calculated by difference as the following equation: connective tissue = Total protein - (myofibrillar protein + sarcoplasmic protein + denaturated protein).

**2.4. Determination of amino acids :**

Amino acids were calculated by (mg/g) using HPLC according to (**Laurens et al, .2012; Jagic et al., 2013)**.

1.0 g of the sample was mixed with 5 mL H2O and 5 mL of HCl (Note: final concn. of HCl is 6 M) and then heated at 100°C for 24hrs and then filtered. Finally 1 mL of the filtrate was injected to HPLC.

### 2.5. Determination of amino acids and fatty acids:

Fatty acids were determined by GC-MS according to **Christie ( 2010)**.

The methyl esters of fatty acids separated using HP 6890 GC (at Agriculture Research Center, Cairo, Egypt.) Capillary column gas liquid chromatography with a dual falme ionization were carried out on (30 m x 0.32 mm x 0.25 µm) DB-225 capillary column, stationary phase (50% cyanopropyl phenyl + 50% dimethyl polysiloxane). Column temperature was 150 ºC, the temperature was programmed by increasing the temperature from 150-170 ºC at the rate of 10 ºC/minute, then increased from 170-192 ºC at the rate of 5 ºC /minute, holding for five minutes and then increased from 192-220 ºC during 10 minutes, holding three minutes. The injector and detector temperatures were 230 ºC and 250 ºC, respectively. Carrier gas: Hydrogen flow rate 40 ml/minute, nitrogen at the rate 3 ml/minute, and air flow rate was 450 ml/minute. Peak identifications were established by comparing the retention times obtained with standard methyl ester. The areas under the chromatographic peak were measured with electronic integrator

### 2.6. Statistical Analysis:

The Statistical analysis was carried out using IBM SPSS Statistics 16, PC statistical software. LSD Multiple Range Test was applied to assess significant differences between means at 5% levels of probability. Also, statistical analysis by one-way ANOVA test was applied significant differences at 5 % and 1% levels **(Steel et al., 1997).**

## 3. Results and discussions

### 3.1. Change in physical properties:

After freezing the pH values were significantly ($p \leq 0.05$) decreased in Nile Tilapia and Nile Perch as in Table (1). At the fourth month of freezing in groups frozen at -20°C values of pH was the lowest (6.53), (6.56), followed by groups frozen at -30°C (6.63), (6.60), and glazed groups 6.73 and 6.90 at the conclusion of the freezing period caused by the lactic acid formed by glycolytic reaction. These results are in agreement with (**Jiang et al., 2018).** Alternatively, the end of period the pH values were increased due to an increase in volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes **(Obemeata et al., 2011).**The Nile Perch fish were glazed by rosemary was the highest value of pH (7.00**)**, followed by Nile Tilapia treated by freezing at (-20)℃ (6.96) there were due to containing phenolic components (**Topuz et al., 2014).**

Table (2) shows changes in the acidity values of the two types of fishes. In general, after freezing the acidity were significantly ($p \leq 0.05$) increased in the two types of fishes due to the activity of lipolytic bacteria **(Romotowska et al., 2016)** ,The acidity at Nile Perch was higher than Nile Tilapia at the end of freezing period, The fishes were treated by glazing with rosemary are less content of acidity than others in Nile Tilapia and Nile Perch (4.16%-3.40%) and fishes were frozen at -20℃ (5.94%-4.46%) were the highest. similar results were found by **(Wang et al., 2020).**

The denaturation of myofibrillar protein during frozen storage was a contributing factor to the drip loss growth's tendency to continue increasing significant ($p \leq 0.05$), and results showed in Table (3)**.** The values of drip loss for both species (Nile Tilapia and Nile Perch) during freezing, recording their highest percentage at all freezing treatments. The groups frozen at -20° C had the highest values (7.01%) (8.13%), followed by the groups frozen at -30° C (5.95%), (7.62%), and the glazed groups the lowest values (4.66%), (4.58%), respectively, at the end of the freezing

period for both species, Nile Tilapia and Nile Perch, respectively. Was in the same line with **(Wang et al., 2020).**

Due to the denaturation of myofibrillar protein during frozen storage, which increases the loss of nutrients and water during cooking, the content of cooking loss in two types of fish in Table (4) show highly significantly ($p \leq 0.01$) increased overall during all freezing treatments during storage., The end of the freezing period for both the species Nile Tilapia and Nile perch saw the lowest values at the glazed group (28.02%), (17.26%) than the group was frozen at -30° C (34.13%), (18.00%) ,and the greatest values at freezing at -20° C groups (36.20%) and (19.65%), respectively. These results are in agreement with **Li et al.( 2018) and Wang et al.( 2020).**

### 3.2. Changes in chemical properties for fish meat:

The moisture content of Nile Perch and Nile Tilapia in Table (5) generally reduced significantly ($P \leq 0.05$), across all treatments. The groups that were frozen at -20°C had the lowest moisture content (71.88%) (73.79%), followed by the groups that were frozen at -30°C (73.00%), (73.95%), and the glazed groups (72.31%), (74.48%), which generally modifies the water content and distribution within the meat tissue, leading to a significant increase in water loss and lower WHC following thawing, Obtained results agreed with **Alizadeh et al. 2007; Leygonie et al.,2012.** The glazed group was a highest of moisture in the end of freezing period duo to the glaze may offer a more uniform surface compared to unglazed surface and thus have a smaller total surface area for evaporation **(Coban, 2012).**

Generally, frozen groups have lower lipid values than fresh ones. Moreover, lipid oxidation during storage in fish (Nile Tilapia and Nile Perch) changed the chemical makeup of fish muscle and caused the lipids value to drop. There were very minor differences between the fresh and frozen groups in Table (6). Similar results were obtained by **Shi *et al (*** **2019**) but these results are disagreement with **Popelka et al. (2014).** At the end of the freezing period, the lipid content of the two species of Nile perch and Nile Tilapia was lowest in groups frozen at -20°C (1.34%),,(2.00%) , followed by groups frozen at -30°C (1.59%), (2.22%), and glazed groups (2.00%), (2.78%). due to the glazing that was used to prevent oxidation and damage during the frozen storage. These results are in agreement with **Gandotra et al., 2012 , and Solval et al., 2014**.

Because minerals were lost in drip during the thawing, the level of ash significantly ($P \leq 0.05$) decreased in both species during storage in Table (7). At the end of storage, it was discovered that the ash depending on the freezing treatment generally, the samples that were treated by glazing with rosemary extract are more ash than others at the end of the storage period. (4.02%) for Nile Perch and (4.00%) for Nile Tilapia. However, the lowest content when they were frozen at (–20°C) 3.01% and 3.02%, respectively .similar results with **Gandotra et al. ( 2012.**

### 3.3. Change in total protein and protein fractions:

By the end of the storage period, it could notice that there was slight variation of total protein with a storage period at -18 C° in Tables (8and 9). Several factors are of importance in relation to the protein changes such as changes in lipids and fatty acids, lipid oxidation, enzymatic breakdown of trimethylamine oxide **(Popelka et al., 2014).** There were seemed to be highly significant at ($P \leq 0.01$) lesser at the end of storage at all treatment (at-20℃,-30℃ and glazing with

rosemary extract). While, Nile Perch at the group was glazed has the upper percentage (18.50%) at the end of period after that the freezing at -30° C group (18.32%), at the same time as the freezing at -20℃ presented lowest percentage of total protein (17.02%). Nile perch samples were relatively more affected by freezing at all treatment than Nile Tilapias were determinated to be (16.27% 16.04% and 14.14 %,), respectively .These results are in agreement with **(Zhang, 2020)**.

Results in Tables (8 and 9) showed that Sarcoplasmic protein (S.P)of both species (at -20℃, at-30℃ and glazing) for the control fishes, There were no significant changes observed, which reduce (S.P) in Nile Tilapia (3.01- 3.40- 3.96 %), in Nile Perch (5.27- 5.28 -5.40%). The results showed also that a (S.P) content at freezing by -20C° in Nile Tilapia and Nile Perch were highly significant at ($P \leq 0.01$) the lowest content (2.68%) and (2.99%) at the end of storage period, these results are in agreement with (**Popelka et al., 2014).** On the other hand the freezing by glazing (S.P) percentage (3.68%), (2.99%) at Nile Tilapia and Nile Perch than the other method of freezing duo to glazing treatment similar results with **(Shi *et al.*, 2019).**

The Myofibrellar protein (M.P) at Nile Tilapia and Nile Perch were highly significant at ($P \leq 0.01$) decreased by frozen period by denaturation and this may be caused by the dissociation of myosin subunits and actin denaturation during frozen storage, in Tables (8 and 9) at the end of storage by glazing had higher (M.P) percentage for Nile Tilapia and Nile Perch (10.33 %) ,(11.30 %) than the other methods ,at -30℃ ( 9.88 -11.23%) while the freezing at -20℃ had a lowest (M.P) percentage( 7.95 -10.44%), respectively. these results are in agreement with **(Shi et al., 2019.)**

As a results of decrease of (S.P) and (M.P) of fish during freezing period followed by the denaturated protein (D.P) had highly significant at ($P \leq 0.01$) increased as shown in tables (8 and 9), the end of period where the highest value of (D.P) was (3.49 %) for Nile Perch followed by Nile Tilapia (3.31%) at fishes were treated by freezing at (-20℃). Fishes were treated by glazing with rosemary extract were the least in (D.P) at two species Nile Tilapia and Nile Perch (2.69%), (3.31%) ,respectively. duo to glazing could protect the protein from denaturation **(Shi et al, .2019;.Wang et al.,2020).**

In general, the connective tissue protein (C.T.P) often significant at ($P \leq 0.05$) decreased at all treatments of the two species Nile Tilapia and Nile perch because effect of freezing caused physical damage to the connective tissue **(Valencia et al., 2008).** At Tables (8 and 9).In Nile Tilapia at freezing by -30° C (0.40%) was the highest while Nile perch at glazing has the highest value (0.90%) Also, these results are in agreement with **(Kiessling et al., .2004).**

**Table 1:** Effect of freezing storage on pH of Nile Tilapia and Nile perch

| Species | Fresh | Treatment | *Storage period at -18 C°* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Nile Tilapia | 7.2±.00 | Freezing at -20℃ | 7.2±.00$^{a}$ | 7.0±.00$^{a}$ | 6.8±.10$^{b}$ | 6.76±.07$^{b}$ | 6.53±.06$^{bc}$ | 6.7±.06$^{a}$ | 6.96±.12$^{ac}$ |
| | | Freezing at -30℃ | 7.1±.06$^{b}$ | 7.03±.07$^{a}$ | 6.8±.10$^{b}$ | 6.83±.07$^{b}$ | 6.4±.06$^{c}$ | 6.83±.10$^{a}$ | 7.06±.06$^{a}$ |
| | | Glazing | 7.06±06$^{b}$ | 7.03±.07$^{a}$ | 7.1±.10$^{a}$ | 6.96±.07$^{a}$ | 6.73±.06$^{a}$ | 6.73±.06$^{a}$ | 6.83±.06$^{bc}$ |
| Nile perch | 7.1±.00 | Freezing at -20℃ | 7.1±.00$^{ac}$ | 6.83±.06$^{a}$ | 6.93±.07$^{a}$ | 6.7±.00$^{a}$ | 6.56±.12$^{a}$ | 6.76±.06$^{b}$ | 6.83±.06$^{c}$ |
| | | Freezing at -30℃ | 7.03±.06$^{bc}$ | 6.83±.15$^{a}$ | 6.6±.10$^{b}$ | 6.5±.43$^{a}$ | 6.2±.26$^{bc}$ | 6.7±.00$^{c}$ | 6.9±.00$^{b}$ |
| | | Glazing | 7.13±.06$^{a}$ | 6.86±.06$^{a}$ | 6.80±.10$^{a}$ | 6.86±.12$^{a}$ | 6.3±.10$^{b}$ | 6.9±.00$^{a}$ | 7.0±.00$^{a}$ |

**Table 2:** Effect of freezing storage on Acidity (%) of Nile Tilapia and Nile perch

| Species | Fresh | Treatment | *Storage period(month) at -18* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Nile Tilapia | 1.0±.18 | Freezing at -20℃ | 1.27±.08$^{a}$ | 1.24±.01$^{b}$ | 1.54±.00$^{a}$ | 2.36±.60$^{a}$ | 2.50±.82$^{a}$ | 4.46±.13$^{a}$ | 5.94±1.03$^{a}$ |
| | | Freezing at -30℃ | 1.27±.08$^{a}$ | 1.13±.00$^{b}$ | 1.70±.32$^{b}$ | 1.84±.19$^{b}$ | 2.42±.26$^{b}$ | 3.06±.11$^{b}$ | 5.13±1.22$^{b}$ |
| | | Glazing | 1.24±.01$^{a}$ | 1.51±.13$^{a}$ | 1.59±.23$^{a}$ | 1.71±.09$^{b}$ | 2.36±.88.$^{c}$ | 2.65±.57$^{c}$ | 4.16±.13$^{c}$ |
| Nile perch | 1.27±.08 | Freezing at -20℃ | 1.28±.05$^{a}$ | 1.74±.07$^{c}$ | 1.97±.13$^{a}$ | 1.59±.09$^{b}$ | 3.54±2.72$^{a}$ | 3.71±.47$^{a}$ | 4.46±.13$^{a}$ |
| | | Freezing at -30℃ | 1.16±.05$^{b}$ | 1.66±.07$^{b}$ | 1.99±.48$^{a}$ | 1.82±.22$^{a}$ | 2.87±.35$^{b}$ | 3.86±.00$^{b}$ | 4.16±.26$^{ab}$ |
| | | Glazing | 1.31±.03$^{a}$ | 1.63±.07$^{a}$ | 1.97±1.02$^{a}$ | 1.36±.09$^{c}$ | 2.03±.10$^{c}$ | 3.10±1.05$^{c}$ | 3.40±.82$^{c}$ |

Means with different letters (a, b, c) in the same Column different significantly at p≤0.05, while those with similar letters are not significant by different

**Table 3:** Effect of freezing storage on drip loss (%) of Nile Tilapia and Nile perch.

| Species | Treatment | *Storage period(month) at -18 C°* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Nile Tilapia | Freezing at -20℃ | 1.77±.08$^{a}$ | 2.07±.08$^{a}$ | 4.40.22$^{a}$ | 4.85±.24$^{a}$ | 5.00±2.00$^{a}$ | 5.87±.43$^{a}$ | 7.01±.28$^{a}$ |
| | Freezing at -30℃ | 1.58±.04$^{ac}$ | 1.81±.04$^{a}$ | 3.67.02 $^{a}$ | 4.10±.26$^{b}$ | 4.88±.19$^{a}$ | 5.24±.04$^{b}$ | 5.95±.19$^{b}$ |
| | Glazing | 1.52±.16$^{bc}$ | 1.93±.16$^{a}$ | 2.52±.05$^{a}$ | 3.22±.32$^{c}$ | 3.90±1.27$^{a}$ | 4.01±.29$^{c}$ | 4.66±.28$^{c}$ |
| Nile perch | Freezing at -20℃ | 2.04±.21$^{a}$ | 3.16±.21$^{a}$ | 4.95±.18$^{a}$ | 5.25± .06$^{a}$ | 6.03±.32$^{a}$ | 7.45±.11$^{a}$ | 8.13±.15$^{a}$ |
| | Freezing at -30℃ | 1.81±.13$^{ac}$ | 2.97±.15$^{a}$ | 4.07±.68$^{ac}$ | 4.93± .47$^{a}$ | 5.33±.06$^{b}$ | 6.12±.15$^{b}$ | 7.62±.39$^{a}$ |
| | Glazing | 1.52±.20$^{bc}$ | 1.92±.08$^{b}$ | 2.99±.73$^{bc}$ | 3.52± .27$^{b}$ | 3.97±.35$^{c}$ | 4.05±.09$^{c}$ | 4.58±.60$^{b}$ |

Means with different letters (a, b, c) in the same Column different significantly at p≤0.05, while those with similar letters are not significant by different.

**Table 4:** Effect of freezing storage on cooking loss of Nile Tilapia and Nile perch.

| Species | Fresh | Treatment | *Storage period(month) at -18 C°* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Nile Tilapia | 33.13±1.92 | Freezing at -20℃ | 33.07±.13$^{b}$ | 31.04±.14$^{b}$ | 29.20±.41$^{b}$ | 27.62±.21$^{b}$ | 29.60±.59$^{a}$ | 27.60±.23$^{a}$ | 36.29±.63$^{a}$ |
| | | Freezing at -30℃ | 34.55±.08$^{a}$ | 32.59±.60$^{a}$ | 28.90±.13$^{a}$ | 26.71±.25$^{c}$ | 30.16±.42$^{a}$ | 26.93±.50$^{a}$ | 34.13±.22$^{a}$ |
| | | Glazing | 30.28±.10$^{c}$ | 20.74±.15$^{c}$ | 33.46±2.3$^{c}$ | 32.62±.32$^{a}$ | 23.01±6.96$^{a}$ | 20.55±.30$^{b}$ | 28.02±1.0$^{b}$ |
| Nile perch | 22.39±1.87 | Freezing at -20℃ | 18.44 ± .54$^{ab}$ | 16.81±.11$^{b}$ | 15.95±.09$^{a}$ | 13.77±.42$^{a}$ | 15.68±.32$^{a}$ | 16.32±.57$^{ac}$ | 19.65±.49$^{a}$ |
| | | Freezing at -30℃ | 19.17± .22$^{a}$ | 17.49±.50$^{a}$ | 14.99±.13$^{b}$ | 13.06±.11$^{b}$ | 15.04±.95$^{a}$ | 17.53±.46$^{a}$ | 18.00±.03$^{ab}$ |
| | | Glazing | 15.52 ± .29$^{b}$ | 11.85±.19$^{c}$ | 12.63±.55$^{c}$ | 13.66±.15$^{a}$ | 14.47±.46$^{a}$ | 15.75±.32$^{bc}$ | 17.26±1.46$^{c}$ |

Means with different letters (a, b, c) in the same Columns different significantly at p≤0.05, while those with similar letters are not significant by difference

**Table 5:** Effect of freezing storage on Moisture (%) of Nile Tilapia and Nile perch.

| Species | Fresh | Treatment | *Storage period(month) at -18 C°* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Nile Tilapia | 79.17±.32 | Freezing at -20℃ | 79.30±.18$^{a}$ | 79.21±.14$^{a}$ | 77.01±.03$^{ac}$ | 77.59±.16$^{a}$ | 76.97±.06$^{a}$ | 73.11±.22$^{b}$ | 71.88±.82$^{a}$ |
| | | Freezing at -30℃ | 79.23±.34$^{a}$ | 78.99±.14$^{ab}$ | 78.21±.16$^{ab}$ | 79.93±.21$^{a}$ | 77.31±.16$^{a}$ | 74.38±.61$^{a}$ | 73.00±.10$^{a}$ |
| | | Glazing | 78.98±.024$^{a}$ | 78.77±.12$^{b}$ | 77.62±.54$^{b}$ | 77.43±.14$^{ab}$ | 76.92±.09$^{a}$ | 75.15±.17$^{a}$ | 72.31±.61$^{a}$ |
| Nile Perch | 80.59±.16 | Freezing at -20℃ | 80.16±.42$^{a}$ | 79.20±.17$^{a}$ | 78.88±.73$^{a}$ | 77.26±.09$^{c}$ | 76.87±.00$^{b}$ | 74.31±.18$^{c}$ | 73.79±.45$^{ab}$ |
| | | Freezing at -30℃ | 79.86±.30$^{a}$ | 79.32±.07$^{a}$ | 78.20±.04$^{a}$ | 78.14±.05$^{b}$ | 77.84±.04$^{a}$ | 75.61±..34$^{b}$ | 73.95±.053$^{a}$ |
| | | Glazing | 79.88±.13$^{a}$ | 79.43±.07$^{a}$ | 79.19±.51$^{a}$ | 78.43±.03$^{a}$ | 77.78±.31$^{a}$ | 76.27±.10$^{a}$ | 74.48±.23$^{a}$ |

Means with different letters (a, b, c) in the same Column different significantly at p≤0.05, while those with similar letters are not significant by different

**Table 6:** Effect of freezing storage on lipids (%) of Nile Tilapia and Nile Perch.

| Species | Fresh | Treatment | *Storage period(month) at -18 C°* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Nile Tilapia | 2.61±.13 | Freezing at -20°C | 2.52±.10$^{a}$ | 2.34±.13$^{a}$ | 2.24±.11$^{b}$ | 1.94±.10$^{c}$ | 1.82±.16$^{b}$ | 1.65±.10$^{a}$ | 1.34±.23$^{b}$ |
| | | Freezing at -30°C | 2.44±.23$^{a}$ | 2.27±.19$^{ab}$ | 2.24±.13$^{b}$ | 2.22±.02$^{b}$ | 2.00±.11$^{ab}$ | 1.56±.11$^{a}$ | 1.59±.34$^{a}$ |
| | | Glazing | 2.59±.03$^{a}$ | 2.58±.09$^{a}$ | 2.55±.11$^{a}$ | 2.53±.12$^{a}$ | 2.27±.24$^{a}$ | 2.05±.08$^{a}$ | 2.00±.12$^{a}$ |
| Nile Perch | 3.49±.13 | Freezing at -20°C | 3.20±.17$^{a}$ | 3.19±.18$^{a}$ | 3.06±.09$^{a}$ | 2.42±.14 $^{bc}$ | 2.30±.05$^{c}$ | 2.09±.08$^{c}$ | 2.00±.14$^{bc}$ |
| | | Freezing at -30°C | 3.35±.08$^{a}$ | 3.29±.07$^{a}$ | 3.11±.03$^{a}$ | 3.13±.11$^{b}$ | 2.79±.06$^{b}$ | 2.44±.06$^{b}$ | 2.22±.06$^{b}$ |
| | | Glazing | 3.34±.21$^{a}$ | 3.44±.14$^{a}$ | 3.27±.16$^{a}$ | 3.26 ±.07$^{a}$ | 3.14±.17$^{a}$ | 2.00±.02$^{a}$ | 2.78±.17$^{a}$ |

Means with different letters (a, b, c) in the same Column different significantly at p≤0.05, while those with similar letters are not significant by different

**Table 7:** Effect of freezing storage on Ash (%) of Nile Tilapia and Nile Perch.

| Species | Fresh | Treatment | *Storage period(month) at -18 C°* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Nile Tilapia | 4.43±.15 | Freezing at -20°C | 4.22±.03$^{a}$ | 4.05±.35$^{a}$ | 3.57±.25$^{c}$ | 3.51±.25$^{b}$ | 3.21±.23$^{b}$ | 3.62±.08$^{c}$ | 3.01±.25$^{cb}$ |
| | | Freezing at -30° C | 4.25±.23$^{a}$ | 4.89±.30$^{b}$ | 4.24±.05$^{ab}$ | 4.11±.05$^{a}$ | 4.05±.21$^{a}$ | 3.73±.11$^{b}$ | 3.23±.18$^{b}$ |
| | | Glazing | 4.38±.09$^{a}$ | 4.23±.11$^{a}$ | 4.44±.11$^{a}$ | 4.26±.11$^{a}$ | 4.43±.35$^{a}$ | 4.16±.14$^{a}$ | 4.00±.19$^{a}$ |
| Nile Perch | 4.80±.19 | Freezing at -20°C | 4.34±.09$^{a}$ | 4.53±.33$^{a}$ | 4.24±.05$^{b}$ | 4.23±.24$^{a}$ | 4.21±.02$^{a}$ | 4.04±.08$^{b}$ | 4.03±.24$^{a}$ |
| | | Freezing at -30° C | 4.56±.32$^{a}$ | 4.50±.08$^{a}$ | 4.46±.04$^{a}$ | 4.28±.095$^{a}$ | 4.22±.09$^{a}$ | 3.66±.21$^{c}$ | 3.6± .16$^{b}$ |
| | | Glazing | 4.56. 32$^{a}$ | 4.62±.07$^{a}$ | 4.51±.02$^{a}$ | 4.38±.05$^{a}$ | 4.27±.10$^{a}$ | 4.25±.16$^{a}$ | 4.02±.07$^{a}$ |

Means with different letters (a, b, c) in the same Column different significantly at p≤0.05, while those with similar letters are not significant by different

**Table 8:** Effect of freezing storage on Protein, Myofibrillar Protein, Sarcoplasmic Protein, Denaturated and Connective tissue (%) of Nile Tilapia

| | | | *Storage period(month) at -18* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Fresh** | **Treatment** | **Control** | **1** | **2** | **3** | **4** | **5** | **6** |
| **T.P** | **18.±.2** | **Freezing at-20℃** | **18.11±.33$^{a}$** | **17.35±.41$^{c}$** | **17.54±.09$^{c}$** | **17.36±.15$^{abc}$** | **16.08±.26$^{c}$** | **15.62±.60$^{c}$** | **14.41±.22$^{c}$** |
| | | **Freezing at-30℃** | **17.97±.53$^{c}$** | **17.90±.12$^{b}$** | **18.34±73$^{ab}$** | **17.13±.10$^{abc}$** | **17.27±.42$^{b}$** | **16.50±.26$^{ab}$** | **16.04±.23$^{ab}$** |
| | | **Glazing** | **18.02±.56$^{ab}$** | **18.22±.26$^{ab}$** | **17.94±.05$^{ac}$** | **17.26±.41$^{abc}$** | **17.48±.14$^{ab}$** | **16.99±.11$^{ab}$** | **16.27±.31$^{ab}$** |
| **M.p** | **12.62±.24** | **Freezing at-20℃** | **12.12±.119$^{cb}$** | **11.23±.29$^{b}$** | **10.99±.35$^{b}$** | **10.68±.46$^{bc}$** | **9.96±.14$^{bc}$** | **9.01±.07$^{c}$** | **7.95±.17$^{c}$** |
| | | **Freezing at-30℃** | **12.22±.10$^{b}$** | **12.00±.09$^{a}$** | **11.862±.16$^{a}$** | **11.10±.13$^{c}$** | **11.00±.25$^{b}$** | **10.89±.00$^{a}$** | **9.88±.19$^{b}$** |
| | | **Glazing** | **12.44±.220$^{a}$** | **12.22±.11$^{a}$** | **12.00±.12$^{a}$** | **11.88±.10$^{a}$** | **11.10±.12$^{a}$** | **10.66±.09$^{b}$** | **10.33±.10$^{a}$** |
| **S.P** | **4.0±.16** | **Freezing at-20℃** | **3.01±.18$^{c}$** | **3.10±.14$^{b}$** | **3.58±.08$^{c}$** | **3.15±0.38$^{ab}$** | **3.77±0.11$^{a}$** | **3.24±.019$^{c}$** | **2.86±.09$^{b}$** |
| | | **Freezing at-30℃** | **3.40±.08$^{b}$** | **4.01±.12$^{a}$** | **3.87±.12$^{b}$** | **3.66±0.06$^{a}$** | **3.44±.21$^{b}$** | **2.88±.21$^{b}$** | **2.63±.21$^{c}$** |
| | | **Glazing** | **3.96±.08$^{a}$** | **3.96±.19$^{a}$** | **4.18±.02$^{a}$** | **4.11±0.11$^{a}$** | **3.37±.10$^{a}$** | **3.95±.15$^{a}$** | **3.68±.17$^{a}$** |
| **D.P** | **1.27±.10** | **Freezing at-20℃** | **1.33±.13$^{a}$** | **1.77±.09$^{a}$** | **1.63±.10$^{a}$** | **2.02±.10$^{a}$** | **2.02±.10$^{b}$** | **2.64±.10$^{ab}$** | **3.13±.11$^{a}$** |
| | | **Freezing at-30℃** | **1.33 ±.07$^{a}$** | **1.40±.10$^{b}$** | **1.61±.06$^{a}$** | **1.70±.11$^{b}$** | **2.22±.14$^{a}$** | **2.40±.09$^{b}$** | **2.78±.11$^{c}$** |
| | | **Glazing** | **1.33±.07$^{a}$** | **1.39±.01$^{b}$** | **1.45±.04$^{b}$** | **1.55±.12$^{bc}$** | **1,68±.21$^{c}$** | **2.49±.20$^{c}$** | **2.69±.04$^{bc}$** |
| **C.T.P** | **.11 ±.02** | **Freezing at-20℃** | **1.65±.47$^{a}$** | **1.25±.34$^{a}$** | **0.74±.12$^{c}$** | **0.55±03$^{b}$** | **0.73±.14$^{b}$** | **0.11±.03$^{c}$** | **0.09±.00$^{b}$** |
| | | **Freezing at-30℃** | **01.02±.21$^{b}$** | **0.49±.23$^{c}$** | **1.00±23$^{a}$** | **0.67±.02$^{a}$** | **0.61±09$^{c}$** | **0.46±.10$^{b}$** | **0.40±.12$^{a}$** |
| | | **Glazing** | **0.29±.09$^{c}$** | **0.65±.03$^{b}$** | **0.91±.11$^{b}$** | **0.68±.05$^{a}$** | **1.33±.22$^{a}$** | **0.60±.10$^{a}$** | **0.30±.06$^{a}$** |

**T.P:** Total protein, **M.P:** Myofibrillar protein, **S.P:** sarcoplasmic Protein, **D.P:** Denaturated Protein, **C.T.P:** Connective tissue Protein
Means with different letters (a, b, c) in the same Column different significantly at p≤0.05, while those with similar letters are not significant by different.

On wet weight basis.

**Table 9:** Effect of freezing storage on Protein, Myofibrillar Protein, sarcoplasmic Protein, Denaturated and Connective tissue (%) of Nile perch.

| | | | *Storage period(month) at -18C°* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Fresh** | **Treatment** | **Control** | **1** | **2** | **3** | **4** | **5** | **6** |
| **T.P** | **21.31** | **Freezing at-20°C** | **21.09±.27$^{b}$** | **20.24±.04$^{c}$** | **20.24±.24$^{ab}$** | **19.50±.43$^{c}$** | **18.26±.25$^{c}$** | **17.42±.52$^{c}$** | **17.02±.12$^{c}$** |
| | | **Freezing at-30°C** | **20.04±.37$^{bc}$** | **20.68±.02$^{a}$** | **20.72±.21$^{a}$** | **20.51±.31$^{ab}$** | **19.80±.07$^{ab}$** | **18.69±.57$^{ab}$** | **18.32b±.48$^{ab}$** |
| | | **Glazing** | **21.54±.15$^{ab}$** | **20.47±.13$^{bc}$** | **20.13±.12$^{ac}$** | **20.36±.32$^{ab}$** | **19.82±.06$^{ab}$** | **19.10±.17$^{a}$** | **18.50±.20$^{ab}$** |
| **M.p** | **14.04±.01** | **Freezing at-20°C** | **13.89±.12$^{a}$** | **13.40±.18$^{a}$** | **13.35±.10$^{a}$** | **12.76±.02$^{a}$** | **12.28±.10$^{a}$** | **11.10 ±.07$^{a}$** | **10.44±.11$^{c}$** |
| | | **Freezing at-30°C** | **13.52±.01$^{a}$** | **13.23±.25$^{a}$** | **12,86±.53$^{b}$** | **12.65±.20$^{a}$** | **12.25±.01$^{a}$** | **11.00±.13$^{b}$** | **11.23±.20$^{a}$** |
| | | **Glazing** | **13.77±.01$^{a}$** | **13.36±.40$^{a}$** | **13.04±.10$^{ab}$** | **12.87±.20$^{a}$** | **12.36±.01$^{a}$** | **11.49±.20$^{a}$** | **11.30±.20$^{b}$** |
| **S.P** | **5.56±.25** | **Freezing at-20°C** | **5.27±.16$^{b}$** | **5.31±.18$^{a}$** | **4.71±.20$^{b}$** | **4.44±.08$^{a}$** | **4.20±.19$^{a}$** | **3.44±.17$^{a}$** | **2.85±.20$^{a}$** |
| | | **Freezing at-30°C** | **5.28±.21$^{b}$** | **5.35±.13$^{a}$** | **5.13±.15$^{a}$** | **4.83±.18$^{a}$** | **4.09±.10$^{a}$** | **3.65±.22$^{a}$** | **2.97±.11$^{a}$** |
| | | **Glazing** | **5.40±.05$^{a}$** | **4.29±.05$^{b}$** | **4.99±.20$^{ab}$** | **4.58±.20$^{a}$** | **4.00±.00$^{a}$** | **3.24±.12$^{a}$** | **2.99±.10$^{a}$** |
| **D.P** | **0.48±.10** | **Freezing at-20°C** | **0.98±.01$^{a}$** | **1.00±.11$^{a}$** | **1.68±.19$^{a}$** | **1.76±.11$^{a}$** | **2.20±.21$^{a}$** | **2.37±.00$^{b}$** | **3.49±.11$^{a}$** |
| | | **Freezing at-30°C** | **0.44±.17$^{a}$** | **1.08±.11$^{b}$** | **1.50±.04$^{a}$** | **1.84±.09$^{a}$** | **2.44±.10$^{ab}$** | **3.12±.11$^{a}$** | **3.43±.05$^{ab}$** |
| | | **Glazing** | **1.43±.20$^{a}$** | **1.62±.03$^{c}$** | **1.23±.05$^{b}$** | **1.37±.03$^{b}$** | **2.26±.08$^{b}$** | **3.28±.09$^{a}$** | **3.31±.09$^{c}$** |
| **C.T.P** | **1.23±.02** | **Freezing at-20°C** | **0.95±.01$^{a}$** | **0.53±.10$^{c}$** | **0.50±.12$^{c}$** | **0.54±.01$^{b}$** | **0.42±.02$^{c}$** | **0.51±.00$^{c}$** | **0.30±.10$^{c}$** |
| | | **Freezing at-30°C** | **0.80±.11$^{b}$** | **0. 23±.20$^{a}$** | **1.23±.12$^{a}$** | **1.19±.34$^{b}$** | **1.02±.22$^{b}$** | **0.92±.12$^{b}$** | **0.63±.04$^{b}$** |
| | | **Glazing** | **0.94±.10$^{a}$** | **1.20±.23$^{a}$** | **0.87±.11$^{b}$** | **1.31±.43$^{a}$** | **1.20±.23$^{a}$** | **1.09±.65$^{a}$** | **0.90±.11$^{a}$** |

**T.P:** Total protein, **M.P:** Myofibrillar protein, **S.P:** sarcoplasmic Protein, **D.P:** Denaturated Protein, **C.T.P:** Connective tissue Protein.
Means with different letters (a, b, c) in the same Column different significantly at p≤0.05, while those with similar letters are not significant by differen
On wet weight basis.

### 3.4. Change in Amino Acids during freezing storage:

The amino acids (A.A) determined to be 131.28 mg/g in fresh Nile Tilapia was found to be 156.22 mg/g in the fresh Nile Perch as in Tables (10 and 11). Similar results were reported by **(Elagba et al., 2010)**. Longer storage time leads to increased protein oxidation in frozen muscles, This lead to increase in free amino acid level in frozen samples due to enzymatic activity during long-term storage **(Calanche et al,. 2019).** At The end of storage period the the lowest amino acids content was at group were frozen at -20℃ 146.15mg/g due to the concentration of amino acids decreased and the concentration in drip increased with increasing storage time **(wldyka and Dawson 1968),** While in the glazed group were recorded and 168.43mg/g, While group were frozen at -30℃ recorded 225.94 mg/g, These results are in agreement with **(Li et al., 2018; Shi et al., 2019).** Phenylalanine and Leucine were major essential acid in Nile Tilapia (17.28-17.59 mg/g) and Nile perch (21.84-20.91mg/g) Also, Glutamic acid were major not essential amino acid in two kind of fishes which was in Nile Tilapia (12.73mg/g) and in Nile Perch (16.08mg/g). Similar results were found by **(Wang et al., 2015).**

**Table 10:** Changes in amino acids (mg/g, dry weight) of frozen Nile Tilapia with stored at -18℃.

| | *Storage period (month) at -18˚c* | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3 months | | | 6 months | | |
| **Amino acids** | **Fresh** | **Freezing at -20C˚** | **Freezing at -30˚C** | **Glazing** | **Freezing at -20C˚** | **Freezing at -30˚C** | **Glazing** |
| **Aspartic acid** | **10.55±2.33** | **14.92±.19$^{a}$** | **12.66±.6$^{b}$** | **10.413±.20$^{c}$** | **16.15±.77$^{b}$** | **23.68±.99$^{a}$** | **18.32±.98$^{c}$** |
| **Glutamic acid** | **16.08±1.22** | **23.95±.53$^{a}$** | **20.33±.34$^{b}$** | **15.57±.87$^{c}$** | **26.37±.23$^{c}$** | **39.09±.21$^{a}$** | **32.21±.90$^{b}$** |
| **Serine** | **4.54±.89** | **6.99±.77$^{a}$** | **6.21±.65$^{b}$** | **4.19±.98$^{c}$** | **4.80±1.98$^{c}$** | **8.47±.53$^{a}$** | **7.13±.65$^{b}$** |
| **Glycine** | **11.73±.93** | **14.25±3.45$^{a}$** | **13.218±.67$^{a}$** | **12.23±.65$^{b}$** | **6.94±.63$^{b}$** | **19.84±.45$^{a}$** | **4.94±.45$^{c}$** |
| **Alanine** | **10.84±1.67** | **15.57±.3.00$^{a}$** | **12.47±.11$^{b}$** | **10.29±.4$^{c}$** | **4.49±.88$^{c}$** | **12.98±.78$^{a}$** | **5.10±.01$^{b}$** |
| **Arginine** | **7.62±.46** | **10.431±.89$^{a}$** | **9.87±.69$^{b}$** | **6.91±..4$^{c}$** | **4.80±.49$^{c}$** | **14.67±.67$^{a}$** | **5.04±.92$^{b}$** |
| **Tyrosine** | **6.59±.92** | **9.57±1.09$^{a}$** | **8.28±.35$^{b}$** | **6.53±.23$^{c}$** | **4.26±.19$^{b}$** | **6.82±.27$^{a}$** | **4.53±.20$^{b}$** |
| **Cysteine** | **ND** | **ND** | **ND** | **ND** | **4.41.00** | **3.42±.90** | **6.39±.87** |
| **Proline** | **9.30±.25** | **11.054±.34$^{a}$** | **8.56±1.23$^{b}$** | **4.09±.34$^{c}$** | **5.83±.24$^{b}$** | **6.06±.47$^{a}$** | **8.00±.39$^{a}$** |
| **Nonessential Amino Acid** | **77.17±8.67** | **106.69±6.98$^{a}$** | **95.591±5.64$^{b}$** | **66.23±5.05$^{c}$** | **117.69±5.41$^{c}$** | **165.78±5.27$^{a}$** | **144.12±5.37$^{b}$** |
| **Histidine** | **7.78±2.09** | **11.42±.94$^{a}$** | **9.33±.65$^{b}$** | **7.31±.01$^{c}$** | **21.96±.56$^{b}$** | **22.85±.22$^{a}$** | **22.53±.66$^{c}$** |
| **Threonine** | **3.99±.67** | **6.431±.1.23$^{a}$** | **4.77±.23$^{b}$** | **4.441±.01$^{c}$** | **7.49±.12$^{b}$** | **15.23±.93$^{a}$** | **5.77±.29$^{c}$** |
| **Valine** | **3.23±.49** | **3.98±.38$^{a}$** | **2.71±.33$^{b}$** | **2.34±.05$^{c}$** | **3.13±.48$^{b}$** | **4.20±.86$^{a}$** | **3.14±.55$^{b}$** |
| **Methionine** | **14.14±2.66** | **17.39±.45$^{a}$** | **15.40±.47$^{b}$** | **13.64±.44$^{c}$** | **2.98±.98$^{c}$** | **4.48±.20$^{a}$** | **3.18±.34$^{b}$** |
| **Phenylalanine** | **21.84±1.09** | **27.09±2.66$^{a}$** | **22.42±09$^{b}$** | **12.82±.33$^{c}$** | **6.05±.24$^{c}$** | **9.25±.33$^{a}$** | **7.81±.34$^{b}$** |
| **Isoleucine** | **3.97±.84** | **5.74±1.23$^{a}$** | **4.50±.57$^{b}$** | **3.39±.34$^{c}$** | **5.10±.27$^{b}$** | **6.77±.38$^{a}$** | **5.77±.34$^{b}$** |
| **Leucine** | **20.91±1.12** | **28.74±11.1$^{a}$** | **22.62±.11$^{b}$** | **19.84±.37$^{c}$** | **11.41±1.04$^{c}$** | **14.62±.13$^{a}$** | **15.35±.99$^{b}$** |
| **Lysine** | **10.20±2.45** | **13.97±2.99$^{a}$** | **4.92±.23$^{b}$** | **3.96±.99$^{c}$** | **9.98±.49$^{c}$** | **13.56±.23$^{a}$** | **15.22±.98$^{b}$** |
| **Essential Amino acids** | **79.05±11.41** | **104.49±9.88$^{a}$** | **83.37±2.68$^{b}$** | **64.04±2.2$^{c}$** | **48.1± 3.91$^{c}$** | **70.96±3.28$^{a}$** | **51.77±4.49$^{b}$** |
| **Total** | **156.22±20.08** | **211.18±16.86$^{a}$** | **178.96±8.32$^{b}$** | **145.69±7.25$^{c}$** | **146.15±9.32$^{c}$** | **225.99±8.55$^{a}$** | **168.43±9.86$^{b}$** |

Means with different letters (a, b, c) in the same row different significantly at p≤0.05, while those with similar letters are not significant by different, ND: Not detected.

**Table 11:** Changes in amino acids (mg/g, dry weight) of frozen Nile Perch with stored at -18℃.

| | | 3 months | | | 6 months | | |
|---|---|---|---|---|---|---|---|
| **Amino acids** | **Fresh** | **Freezing at -20C°** | **Freezing at -30°C** | **Glazing** | **Freezing at -20C°** | **Freezing at -30°C** | **Glazing** |
| **Asparticacid** | **8.91±.98** | **10.36±.31$^{a}$** | **8.44±.31$^{b}$** | **7.40±.10$^{c}$** | **22.29±.27$^{a}$** | **19.55±.12$^{b}$** | **18.31±.09$^{c}$** |
| **Glutamicacid** | **12.73±1.20** | **15.53±.06$^{a}$** | **12.87±.01$^{b}$** | **10.58±.10$^{c}$** | **40.27±.26$^{a}$** | **31.55±.45$^{b}$** | **31.60±.45$^{b}$** |
| **Serine** | **3.72±.57** | **4.52±.06$^{a}$** | **3.87±.03$^{b}$** | **3.24±.08$^{c}$** | **76.71±.30$^{a}$** | **64.23±.11$^{c}$** | **64.69±.20$^{b}$** |
| **Glycine** | **8.46±.92** | **11.73±.10$^{a}$** | **9.82±.02$^{b}$** | **9.47±.22$^{bc}$** | **9.90±.11$^{a}$** | **9.58±.03$^{b}$** | **7.27±.00$^{c}$** |
| **Alanine** | **4.42±.78** | **10.62±.33$^{a}$** | **9.29±.11$^{b}$** | **7.06±.45$^{c}$** | **10.53±.64$^{a}$** | **7.90±.29$^{b}$** | **5.43±.90$^{c}$** |
| **Arginine** | **5.86±.26** | **7.83±.39$^{a}$** | **6.63±.34$^{b}$** | **5.61±.29$^{c}$** | **14.49±.18$^{a}$** | **12.47±.35$^{b}$** | **5.98±.36$^{c}$** |
| **Tyrosine** | **5.72±1.30** | **6.61±.10$^{a}$** | **5.74±.87$^{b}$** | **4.90±.12$^{c}$** | **7.00±.28$^{a}$** | **6.05±.00$^{b}$** | **4.49±.18$^{c}$** |
| **Cystine** | **ND** | **ND** | **ND** | **ND** | **5.75±.83$^{c}$** | **9.98±.97$^{b}$** | **10.52±1.87$^{a}$** |
| **Proline** | **3.08±.38** | **8.78±.12$^{b}$** | **9.74±01$^{a}$** | **5.24±.09$^{c}$** | **5.90±.09$^{a}$** | **5.63±.70$^{b}$** | **4.93±.12$^{c}$** |
| **Nonessential Amino Acid** | **69.01±6.39** | **75.98±1.47$^{a}$** | **63.57±1.7$^{b}$** | **53.50 ±1.45$^{c}$** | **192.66±2.96$^{a}$** | **166.94±3.02$^{b}$** | **145.95±4.17$^{c}$** |
| **Threonine** | **2.37±.27** | **4.67±.09$^{a}$** | **3.29±.03$^{b}$** | **1.95±.12$^{c}$** | **6.54±.23$^{b}$** | **5.00±.87$^{c}$** | **7.99±.21$^{a}$** |
| **Histidine** | **5.34±1.03** | **8.34±.11$^{a}$** | **6.81±.01$^{b}$** | **5.75±.05$^{c}$** | **2.69±2.30$^{a}$** | **2.55±.10$^{b}$** | **2.37±.09$^{b}$** |
| **Valine** | **2.70±.33** | **3.57±.12$^{a}$** | **2.83±.00$^{b}$** | **2.34±.34$^{c}$** | **4.16±.23$^{a}$** | **4.08±.98$^{b}$** | **4.07±.56$^{b}$** |
| **Methionine** | **10.02±2.09** | **15.18±.14$^{a}$** | **12.17±.59$^{b}$** | **10.59±.34$^{c}$** | **4.56±.94$^{a}$** | **2.90±.23$^{c}$** | **3.55±.62$^{b}$** |
| **Phenylalanine** | **17.28±3.19** | **22.31±.90$^{a}$** | **19.11±.44$^{b}$** | **14.96±87$^{c}$** | **7.11±.03$^{b}$** | **7.60±.98$^{a}$** | **6.71±.98$^{c}$** |
| **IsoLeucine** | **3.04±.68** | **4.17±.09$^{a}$** | **3.53±.22$^{b}$** | **2.41±.23$^{c}$** | **8.23±.23$^{a}$** | **7.46±.98$^{b}$** | **5.68±.67$^{c}$** |
| **Leucine** | **17.59±1.33** | **20.77±.71$^{a}$** | **18.35±.00$^{b}$** | **14.33±.33$^{c}$** | **16.51±.14$^{a}$** | **14.84±.67$^{b}$** | **12.94±.09$^{c}$** |
| **Lysine** | **3.93±.38** | **10.18±.23$^{c}$** | **3.82±.44$^{a}$** | **18.22±.00$^{b}$** | **17.72±.67$^{a}$** | **12.92±.23$^{b}$** | **11.63±.19$^{b}$** |
| **Essential Amino acids** | **62.27±5.3** | **89.19±2.3$^{a}$** | **72.74±1.73$^{b}$** | **70.55±2.28$^{bc}$** | **67.52±4.77$^{a}$** | **57.35±5.04$^{b}$** | **54.94±3.41$^{bc}$** |
| **Total** | **131.28±11.69** | **165.17±3.77$^{a}$** | **136.31 ±3.43$^{b}$** | **124.05±3.73$^{c}$** | **260.18±7.73$^{a}$** | **224.29±8.06$^{b}$** | **200.89±7.58$^{c}$** |

Means with different letters (a, b, c) in the same row different significantly at p≤0.05, while those with similar letters are not significant by different, ND: Not detected.

### 3.5. Change in Fatty Acids during freezing storage:

Tables (12 and 13) show the total fatty acids composition of the studied fishes , Frozen storage reduced the polyunsaturated fatty acids (PUFA) and increased the saturated fatty acids (SFA) which indicated a substantial loss of nutritional value in fish muscles ***(*Mendoza et al., 2014).*** After 3 months of storage period changes in the (SFA) content in group were frozen at -20℃ were higher than SFA content in group were frozen at -30℃ followed by glazed group to be in Nile Tilapia 40.17 mg/g, 39.59 mg/g and 33.21 mg/g respectively. Also, in Nile Perch 59.73 mg/g, 52.11 mg/g and 49.91 mg/g, correspondingly. In the end storage SFA content in Nile Tilapia 49.33, 47.31 and 37.76 mg/g respectively. While SFA content in Nile perch 69.39, 58.03 and 50.26 mg/g respectively. These results agreement with **(Emir Coban, 2013; Shi et al., 2019).** Changes in the unsaturated fatty acids UFA content in group were frozen at -20℃ was higher than UFA content in group were frozen at -30℃ followed by glazed group after 3 months of storage period to be in Nile Tilapia were 49.45, 40.35 mg/g and 35.06 mg/g respectively. Also, in Nile Perch were 59.07 mg/g, 56.78 mg/g and 55.09 mg/g, correspondingly. At The end of storage period (UFA) content was in Nile Tilapia, and mg/g respectively. While UFA content in Nile Perch 73.33, 61.92 and 57.21mg/g respectively, due to the glazing used to prevent oxidization **(Solval et al., 2014).Table 12:** Effect of freezing storage on Fatty Acids of Nile Tilapia (mg/g).

**Table 12:** Effect of freezing storage on Fatty Acids of Nile Tilapia (mg/g).

| | *Storage period( month) at -18°c* | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3 months | | | 6 months | | |
| Fatty acid / Treatment | Fresh | Freezing at -20C° | Freezing at -30°C | Glazing | Freezing at -20C° | Freezing at -30°C | Glazing |
| Lauric acid (C12:0) | 0.36±.01 | 0.66±.03$^{b}$ | .68±.00$^{a}$ | ND | 2.25±.12$^{c}$ | 2.82±.76$^{a}$ | 2.29±.35$^{b}$ |
| Tridecanoic acid (C13:0) | 3.39± 1.09 | 5.53±.99$^{a}$ | 1.85 ±.78$^{c}$ | 2.59 ±.94$^{b}$ | .38±.06 $^{a}$ | 0.37±.10$^{a}$ | ND |
| Tridecanoic acid (C13:1) | 2.81±.90 | 9.55±2.23$^{a}$ | 2.33±.33$^{c}$ | 4.5± .89$^{b}$ | 0.28±.08$^{a}$ | 0.25±.10$^{b}$ | ND |
| Myristic acid (C14:0) | 2.93±.23 | 4.99±.89$^{a}$ | 4.91±68$^{b}$ | 4.46±.22$^{c}$ | 4.67±.56$^{a}$ | 3.48± .36$^{c}$ | 4.25 ±.13 $^{b}$ |
| Myristoleic acid (14.1) | 0.73±.10 | 1.27±.24$^{a}$ | 1.24±.48$^{b}$ | 1.23±.35$^{c}$ | 3.1±.11$^{a}$ | 2.32±.35$^{c}$ | 2.64±.9$^{b}$ |
| Myristolinoleic (C14:2) | 3.87±.27 | 1.07±.34$^{a}$ | ND | 1.03±.09$^{b}$ | ND | 0.74±.06$^{a}$ | 0.77 ± .12$^{a}$ |
| Pentadecanoic acid (C15:0) | ND | 0.73±.02$^{a}$ | 0.76±.05$^{a}$ | ND | 1.99±.38$^{a}$ | 1.25±.66$^{b}$ | ND |
| Cis-10-Pentadecenoic (C15:1) | ND | 0.89±.09$^{a}$ | 0.93±.01$^{a}$ | ND | 1.22±.45$^{a}$ | 1.12 ±.13$^{ab}$ | 0.61 ±.08$^{c}$ |
| Palmitic acid (C16:0) | 10.39±2.34 | 18.51±3.10$^{a}$ | 17.38±2.76$^{ab}$ | 14.02±1.76$^{c}$ | 19.67±3.09$^{a}$ | 18.51 ± 2.87$^{b}$ | 15.69 ± 2.98$^{c}$ |
| Palmitoleic acid (C16:1) | 3.11± .34 | 8.93 ±1.32$^{a}$ | 6.96 ±2.09$^{bc}$ | 6.21 ± .15$^{c}$ | 12.32±2.34$^{a}$ | 10.23±2.74$^{b}$ | 9.29± 1.56$^{c}$ |
| Palmitoleic acid (C16:1, n7) | 3.26 ±.34 | 1.47 ±.23$^{b}$ | 2.59±.78$^{a}$ | 2.64 ± .98$^{a}$ | 1.65 ±.86$^{b}$ | 2.24±.36$^{a}$ | 1.02 ±.34$^{c}$ |
| Heptadecanoic acid (C17:0) | ND | 0.82± .12$^{b}$ | 1.99±.45$^{a}$ | ND | 1.21± .26$^{b}$ | 4.23±.19$^{a}$ | 1.25±.09$^{b}$ |
| Cis-10-Heptadecanoic acid (C17:1) | ND | 0.81±.05$^{b}$ | 1.16±.28$^{b}$ | ND | 1.27± .98$^{b}$ | 1.75±.28$^{a}$ | 0.77± .02$^{c}$ |
| Stearic acid (C18:0) | 3.07± .87 | 4.17± 1.22$^{c}$ | 6.09 ±.23$^{a}$ | 5.27± 1.98$^{b}$ | 9.68± 1.87$^{a}$ | 7.62 ± 1.54$^{b}$ | 6.68± 2.63$^{c}$ |
| Oleic acid (C18:1n9c) | 3.07±.98 | 13.07±1.87$^{a}$ | 10.89±2.93$^{b}$ | 7.18±2.09$^{b}$ | 21.86±3.7$^{a}$ | 10.71±1.87$^{b}$ | 9.77±1.98$^{c}$ |
| Oleic acid (C18:1n9t) | 14.03±2.73 | 3.43±1.08$^{b}$ | ND | 7.5±1.76$^{a}$ | ND | 3.17±.98$^{a}$ | 3.87±1.09$^{a}$ |
| Linoleic acid (C18:2n6c) | 7.77±1.63 | 2.91±.28$^{c}$ | 3.99 ±.46$^{b}$ | 4.56±.9$^{a}$ | 1.16 ±.09$^{c}$ | 1.81 ±.57$^{b}$ | 3.18 ±.69$^{a}$ |
| Linoleic acid (C18:2n6t) | 3.13±.37 | 0.87±.01$^{ab}$ | 0.51±.10$^{c}$ | 0.88 ±.06$^{a}$ | ND | ND | .21±00 |
| α- Linolenic acid (C18:3n3) | 3.17 ± .94 | ND | 1.12±.45$^{b}$ | 1.22±.37$^{a}$ | 1.87±.91$^{c}$ | 2.7±.67$^{a}$ | 2.25±.48$^{ab}$ |
| Arachidic acid (C20:0) | 2.51±.12 | 1.15±.28$^{c}$ | 2.38 ±.45$^{b}$ | 1.44±.23$^{a}$ | 0.25±.06$^{c}$ | 0.45.±.07$^{b}$ | .78±.04$^{a}$ |
| Gadoleic acid (C20:1) | ND | 1.43±.45$^{a}$ | 1.25±.34$^{b}$ | 0.52±.01$^{c}$ | 1.95±.56$^{a}$ | 1.83±.31 $^{b}$ | ND |
| EPA | 5.55±.87 | 4.41±1.03$^{a}$ | 3.12±.94$^{c}$ | 4.01±.34$^{b}$ | 1.87±.36$^{b}$ | 4.37±.12$^{a}$ | 1.87±76$^{b}$ |
| Behenic acid (C22:0) | 0.71 ±.09 | 1.15±.37$^{c}$ | 1.52±.29$^{a}$ | 1.21±.23$^{b}$ | 3.92±.98$^{a}$ | 1.77±.76$^{b}$ | 1.53±.95$^{c}$ |
| Arachidonic acid (C20:1) | 0.82± .05 | 1.89±.22$^{a}$ | 1.85±.36$^{a}$ | ND | 2.77±.23$^{a}$ | 1.48±.65$^{b}$ | 0.7±.09$^{c}$ |
| Lignocereic acid (C24:0) | 0.62±.03 | 3.17±.87$^{c}$ | 3.55±.52$^{b}$ | 4.21±.92 | 5.29±.29$^{b}$ | 6.81 ±.1.02$^{a}$ | 5.47 .45$^{b}$ |
| DHA | 5.36±.12$^{a}$ | 3.83±.25$^{c}$ | 4.24 ±.13$^{b}$ | 4.79 ±.25$^{a}$ | 2.16 ±.33$^{c}$ | 2.57±.23$^{b}$ | 3.52±.27$^{a}$ |
| Saturated FAs | 23.98±.75 | 40.17±1.40$^{a}$ | 39.59±1.00$^{b}$ | 33.21±1.09$^{c}$ | 49.33±1.01$^{a}$ | 47.31±1.70$^{b}$ | 37.76±2.00$^{c}$ |
| Unsaturated fatty acids | 45.95±.80 | 48.41±1.48$^{a}$ | 34.82±.50$^{c}$ | 37.47±1.00$^{b}$ | 49.45±.97$^{a}$ | 40.35±2.17$^{b}$ | 35.06±1.67$^{c}$ |
| Total fatty acids | 69.93±1.55 | 88.58±2.88$^{a}$ | 74.41±1.49$^{b}$ | 70.68±2.09$^{c}$ | 98.78±1.98$^{a}$ | 87.66±3.87$^{b}$ | 72.82±3.67$^{c}$ |

Means with different letters (a, b, c) in the same row different significantly at $p \leq 0.05$, while those with similar letters are not significant by difference, ND = Not detected.

**Table 13:** Effect of freezing storage on Fatty Acids of Nile perch (mg/g).

| *Storage period( month) at -18°c* | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3 months | | | 6 months | | |
| Fatty acid Treatment | Fresh | Freezing at -20C° | Freezing at -30°C | Glazing | Freezing at -20C° | Freezing at -30°C | Glazing |
| Lauric acid (C12:0) | 0.5±.09 | 1.48 .22$^{a}$ | 0.29±.01$^{c}$ | 0.41±.11$^{b}$ | 4.31 ± . 76$^{b}$ | 5.13±.29$^{a}$ | 3.37±.34$^{c}$ |
| Tridecanoic acid (C13:0) | 21.43 | 6.31 ±1.02$^{b}$ | 2.93 ±.55 $^{c}$ | 12.74 ±2.33 $^{a}$ | 0.55 ±.09$^{a}$ | 0.22±.01$^{b}$ | ND |
| Tridecanoic acid (C13:1) | 8.59 | 10.9 ± 1.98$^{b}$ | 2.63 ±.77 $^{c}$ | 21.1 ±3.09$^{a}$ | 0.13±.00$^{b}$ | 0.44±.10$^{a}$ | ND |
| Myristic acid (C14:0) | 5.24±.45$^{c}$ | 8.49±.76$^{a}$ | 7.36±.33$^{b}$ | 6.36±.12$^{c}$ | 15.21±.1.22$^{a}$ | 12.05±.20$^{b}$ | 10.15± .23$^{c}$ |
| Myristoleic acid (14.1) | 1.16±.88 | 7.77±.1.09$^{a}$ | 6.03±.37$^{b}$ | 4.27±.37$^{c}$ | 13.01±1.33$^{b}$ | 15.12±.27$^{a}$ | 9.11±.50$^{a}$ |
| Myristolinoleic (C14:2) | 7.11±1.22 | 3.5±.12$^{a}$ | 2.65±.56$^{b}$ | 2.84±.24$^{cb}$ | ND | ND | 1.5±.23 |
| Pentadecanoic acid (C15:0) | ND | 3.48$^{a}$ | 0.82$^{b}$ | ND | 3.59$^{a}$ | 1.23$^{b}$ | 0.65$^{c}$ |
| Cis-10-Pentadecenoic (C15:1) | ND | 4.63 ± .56$^{a}$ | 4.01 ±1.09$^{b}$ | ND | 7.56 ± 1.78$^{a}$ | 4.89 ± .87$^{b}$ | 3.77 ± .97$^{c}$ |
| Palmitic acid (C16:0) | 17.12 ± 3.2 | 26.35± 2.87$^{a}$ | 26.07 ± 3.76$^{b}$ | 22.67 ± 2.36$^{c}$ | 27.84 ± 3.87$^{a}$ | 22.27 ±1.23$^{b}$ | 21.11 ±3.98$^{c}$ |
| Palmitoleic acid (C16:1) | 3.76± .97 | 11.77± 2.62$^{a}$ | 13.03± 2.98$^{c}$ | 11.02± 1.24$^{ab}$ | 18.05± 2.46$^{a}$ | 16.89± 3.07$^{b}$ | 15.42± 2.98$^{c}$ |
| Palmitoleic acid (C16:1, n7) | 5.95 ±.23 | ND | ND | ND | ND | 1.14±.37$^{b}$ | 1.67±.19$^{a}$ |
| Heptadecanoic acid (C17:0) | ND | 2.97 ±.87$^{a}$ | 1.49 ±.25$^{b}$ | 1.42 ± .65$^{b}$ | 4.24 ±1.87$^{a}$ | 3.18 ±.27$^{b}$ | 3.08±.74$^{c}$ |
| Cis-10-Heptadecanoic acid (C17:1) | ND | 1.99±.86 $^{a}$ | 1.75± .64$^{ab}$ | 1.27±.37$^{c}$ | 2.82 ±.97$^{a}$ | 2.61 ± .96$^{b}$ | 1.76 ± 28$^{c}$ |
| Stearic acid (C18:0) | 2.44 ± .73 | 6.55 ± 1.96$^{a}$ | 5.26 ±2.09$^{ab}$ | 4.08±.89$^{c}$ | 10.48±3.87$^{a}$ | 8.19±1.56$^{b}$ | 8.24±1.32$^{b}$ |
| Oleic acid (C18:1n9c) | 7.92±.98 | 12.54±1.65$^{b}$ | 16.72±2.94$^{a}$ | 6.43±2.10$^{c}$ | 20.96±3.00$^{a}$ | 14.38±1.93$^{b}$ | 11.03±2.98$^{c}$ |
| Oleic acid (C18:1n9t) | 7.14±1.23 | 2.45±.29$^{c}$ | 4.11±1.18$^{a}$ | 4.92± .86$^{a}$ | ND | 2.3±.26$^{a}$ | 1.91±.42$^{b}$ |
| Linoleic acid (C18:2n6c) | 3.11±.84 | 1.55±.06$^{b}$ | 2.01±.86$^{a}$ | 1.32±.27$^{b}$ | 4.84±1.01$^{b}$ | 2.88±.73$^{c}$ | 7.62±2.05$^{a}$ |
| Linoleic acid (C18:2n6t) | 2.01 ±.25 | ND | ND | 0.71 ± .19 | ND | ND | ND |
| α- Linolenic acid (C18:3n3) | 2.42 ±.82 | 1.97 ±.57$^{a}$ | 1.45±.84$^{b}$ | 1.21± .73$^{c}$ | 0.66±.10$^{b}$ | 0.52±.12$^{bc}$ | 1.47 ±.14$^{a}$ |
| Arachidic acid (C20:0) | 1.92±.23 | 0.53±.03$^{c}$ | 0.76±.27$^{b}$ | 1.17±.34$^{a}$ | ND | 0.66±.02$^{b}$ | 1.06±.23$^{a}$ |
| Gadoleic acid (C20:1) | 0.55±.10 | ND | 1.18±.12 | ND | 3.52 ±1.09$^{a}$ | 1.32±.53$^{b}$ | 1.02±.16$^{c}$ |
| EPA | 3.26±.26 | 2.49 ±.19$^{b}$ | 2.29 ±.55$^{c}$ | 4.27±.87$^{a}$ | 2.04±.37$^{c}$ | 3.36±.12$^{ab}$ | 3.39±.66$^{a}$ |
| Behenic acid (C22:0) | 0.68±.04 | 1.64±.65$^{b}$ | 4.16±.98$^{a}$ | 1.00 ±.11$^{c}$ | 0.78±.04$^{b}$ | 2.89±.54$^{a}$ | 0.11±.1$^{bc}$ |
| Arachedonic acid (C20:1) | 0.51±.02 | 0.84±.12$^{b}$ | 1.21±.21$^{c}$ | ND | 1.78 ± .23$^{a}$ | 0.75±.02$^{c}$ | 0.93±.24$^{b}$ |
| Lignocereic acid (C24:0) | 1.42±.73 | 1.98±.25$^{b}$ | 2.97±.87$^{a}$ | 1.06±.46$^{c}$ | 2.39±.25$^{ab}$ | 2.21±.65$^{b}$ | 2.49±.12$^{a}$ |
| DHA | 3.92 ±.99 | 3.28±.67$^{b}$ | 3.58±1.08$^{a}$ | 2.46$^{c}$ ±.56$^{c}$ | 2.23±.44$^{c}$ | 3.11± .26$^{b}$ | 3.83±.90$^{a}$ |
| Saturated FAs | 50.75±1.99 | 59.73±2.07$^{a}$ | 52.11±2.09$^{b}$ | 49.91±1.06$^{bc}$ | 69.39±3.80$^{a}$ | 58.03 ±3.01$^{b}$ | 50.26±2.79$^{c}$ |
| Unsaturated fatty acids | 50.23±2.00 | 59.07±2.20$^{a}$ | 56.78±1.97$^{b}$ | 55.09±1.50$^{bc}$ | 73.33±5.07$^{a}$ | 61.92±3.08$^{b}$ | 57.21±1.99$^{c}$ |
| Total fatty acids | 100.98± 3.99 | 118.8± 4.27$^{a}$ | 108.89±3.98$^{b}$ | 104.96± 2.56$^{bc}$ | 142.72±8.87$^{a}$ | 119.95±6.09$^{b}$ | 107.47±4.78$^{c}$ |

Means with different letters (a, b, c) in the same row different significantly at p≤0.05, while those with similar letters are not significant by different, ND = Not detect

## 4. Conclusions:

In the summary the application of glazing with rosemary extract approaches to extend the shelf-life of frozen Nile Tilapia and Nile perch fish during 6 months at -18° C through testing chemical, physical properties and protein fractions. Based on the results there were significant differences between the different freezing methods (freezing at -20℃, freezing at -30℃ and glazing with rosemary extract1.5%). Total protein, sarcoplasmic proteins, myofibrils, and stroma decreased in both species, while the percentage of denaturated protein increased during frozen storage, it was the best among them in the method of using rosemary extract, then freezing at (-30℃) and then freezing at (-20℃). Nile perch were more affected by the rosemary extract because this variety contains more lipids content, so the antioxidant effect of rosemary extract. We find through the results that fatty acids and amino acids are very affected by freezing in all treatment of freezing, it was noted that they increase gradually during the storage period, but at the end of storage period Nile Tilapia were less amino acids at freezing (-20° C), due to the increase in the drib loss at this time. We also discover that the methods of freezing in glazing with rosemary extract in maintaining the quality of the stored fish by freezing, followed by freezing at -30℃ and then freezing at freezing at -20℃ in both types of fish, especially on the total protein and protein fractions.

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