Effect of Cooling and Chilling on Chemical Composition and Quality Attributes of Nile Lebeo (*Labeo niloticus*) and Sharp Tooth Catfish (*Clarias gariepinus*) Fishes from Nasser Lake

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Abstract:

This study aimed to investigate the effect of cooling and chilling on the physicochemical and quality attributes of two fish species: Nile Lebeo (*Labeo niloticus*) and Sharp Tooth Catfish (*Clarias gariepinus*) that inhabit in Nasser Lake. Fish samples were preserved by cooling at 4°C and chilling by crushed ice in a ratio of 1:2 (fish: ice) for four days and freeze storing at -18°C for (12) weeks. Samples were taken every two weeks for analysis. The chemical and physical change such as proximate chemical composition, protein fractions, fatty acid composition, amino acid composition, and thiobarbituric acid reactive substances (TBA) were examined in fresh, after cooling and chilling (zero time) and during freeze storing of fish samples. The obtained results revealed that the moisture, crude protein, myofibrillar, and (TBA) contents were significantly increased (p≤0.05) during storage, while the crude lipids, ash and sarcoplasmic contents were decreased for both of fish samples. However, fatty acids, amino acids composition and pH showed highly significant (p≤0.05) increasing trend in both the samples. Generally; cooling process of fish at 4ºC caused comparatively lower changes than chilling process.

Keywords: Nile Lebeo, Sharp Tooth Catfish, Cooling, Chilling, Protein Fractions.

1- Introduction

Nasser Lake is the most significant fishery source in upper Egypt, due to its relative affordability as a source of fresh protein, fish is one of the main traditional components of an Egyptian citizen's meal economic uses of fish include food production, the fishing industry, aquaculture, and fish farming, while recreational uses include keeping fish [1].

Fish can be a very nutrient-dense component of a man's diet since it is abundant in the majority of the vitamins he needs, has a good variety of minerals, and has proteins that include all the necessary amino acids in the proper amounts. Fish and fishery products contain water, proteins and other nitrogenous substances, lipids, carbohydrates, minerals, and vitamins, just as many other animal products [2]. However, depending on age, sex, environment, season, and species, fish's chemical make up changes drastically from one fish to another [3]. Compared to other meats, fish are more perishable due to their high moisture content, high levels of free amino acids, and volatile nitrogenous bases [4].

Fish called cyprinids one of the most popular Cyprinidae fish in Egypt is (*Labeo niloticus*), sometimes known as Lebeis. In the past, *Labeo niloticus* was crucial to the River Nile's fish[5]. *Labeo niloticus* is a common market fish species that is economically classified into various traditional classes based on consumer and fisher preferences [6].

The African Catfish; (*Clarias gariepinus*) is a fish food rich in protein and characterized by a rapid growth rate, excellent meat quality, a source of energy in human food, and its lipids are a good source for polyunsaturated fatty acids, especially what is known as omega 3 fatty acids [7].

Cooling has traditionally been the most widely utilised method for short-term preservation of perishable food in order to retain quality and extend the shelf life of fish and shellfish maintaining the 'Cold Chain' from harvest to consumption is crucial for maintaining freshness and quality [8]. Chilled fish are particularly perishable food commodities that lose quality
quickly after harvesting. Chilled fish spoilage is a complex process caused by a combination of autolysis, spontaneous chemical reactions, bacterial attack, and leaching by ice-melt water [9]. As traditional chilling on ice represents 20–30% of the total weight of each box of fish this prolongs shelf life of food [10].

The most important factor in preventing fish deterioration is by controlling the temperature, using of low temperature by the common method is to use a cooling machine or use ice would result in preventing the growth of spoilage bacteria and slowing biochemical processes [11].

Muscle proteins can be divided into three major groups based on their solubility: sarcoplasmic proteins (water-soluble), myofibrillar proteins (salt-soluble), and stromal proteins (insoluble). Based on above dividing, myofibrillar proteins consider the main factor in processing and storage, fish quality and consumer approval are influenced by the fish’s texture, a highly economic characteristic [12],[13]. Fatty acid content changes have been demonstrated to be a reliable indicator of the freshness and deterioration of fish throughout storage [14]. Who found that between 31 and 65 percent of saturated fatty acids (SFA) and 16 and 33 percent of monounsaturated fatty acids (MUFA), with palmitic, stearic, and oleic acids being the main fatty acids. The species under investigation were also good sources of polyunsaturated fatty acids (PUFAs). The ratio of omegas 3 to 6 was (0.9 to 3.6) [15]. Except for essential amino acids, all amino acids are produced by the liver's transaminase enzyme and transamination processes, in which vitamin B₆ functions as a coenzyme. Since humans and other mammals are unable to synthesise essential amino acids, must be obtained through diet. In addition to being a good source of important amino acids including lysine, cystine, methionine, threonine, and tryptophan, fish is also considered to be a good source of protein [16]. This study examined the effects of cooling, chilling and frozen storage time on the chemical, physical changes on quality of two fish species namely Nile Lebeo (Labeo niloticus) and Sharp Tooth Catfish (Clarias gariepinus).

2- Materials and Methods

2.1. Preparation of Samples:

Fish samples, Nile Lebeo (Labeo niloticus) and Sharp Tooth Catfish (Clarias gariepinus) were caughted from Nasser Lake, Aswan, Egypt during February 2023. About 60 Kg of each sample were used in this study. Then washed by running water and divided into two groups:
1- Group one (Cooling method) was put in refrigerator at 4ºC for 4 days.
2- Group two (Chilling method) was put in ice box and mixed with crushed ice, ratio 2:1, two layer of ice and layer of fish for 4 days.

Both of groups were stored at (-18ºC) for (12) weeks.

2.2. Chemical Analysis:

2.2.1. Chemical analysis (moisture, crude lipid, and ash) were determined according to [17].

2.2.1.1. Moisture content was determined by heating samples at 105ºC for 3 hours in a hot air oven according to [17].

2.2.1.2. Crude lipid was determined using soxhlet apparatus (petroleum ether) for 16 hours according to [17].

2.2.1.3. Ash was determined in samples using Muffle Furnace at 550ºC for 3 hours according to [17].

2.2.2. Determination of total protein:

The total protein content of final protein isolates was estimated by the biuret method. According to [18],[19]. Sample (3g) was homogenized with 30 ml of sodium dodecyl sulfate (SDS 10%) for Nile Lebeo, while Catfish, (SDS 5%) heated in the boiling water (85ºC) for 1 hour and cooled in an ice-water bath. protein concentration was determined by the biuret assay method.

2.2.3. Determination of sarcoplasmic protein:

Sarcoplasmic protein was determined according to [20]. Samples (3.0 g) were homogenized with (30 ml) phosphate buffer (pH 7-7.4) for 2 min the homogenate by homogenizer was centrifuged at 6000 rpm for 25 min at 4°C for (Nile Lebeo) while Catfish at (6000 rpm for 50 min at 4°C), the sarcoplasmic protein concentration was determined by the biuret assay method.

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2.2.4. Determination of myofibrillar protein:

Myofibrillar proteins were determined according to [21]. Following, the pellet recovered was resuspended in (30 ml of 15% NaCl solution for Nile Lebeo while Catfish (30 ml of 10% volumes of NaCl solution). The supernatant was collected, and the myofibrillar protein concentration was determined by the biuret assay method.

2.2.5. Determination of denatured protein:

Denatured protein was determined according to [22]. The denatured protein was extracted by 30 ml 0.1 N NaOH solution centrifuged at 6000 rpm for 25 min at 4°C. After centrifugation, the supernatant was determined by the biuret assay method.

2.2.6. Determination of connective tissue (Stroma protein):

Connective tissues were calculated by difference as the following equation:

\[ \text{Connective tissues} = \text{Total protein} - (\text{myofibrillar protein} + \text{sarcoplasmic protein} + \text{denatured protein}) \]

2.3. Physical Analysis:

2.3.1. pH determination using a digital pH meter (JENWAY,3510) according to [23]. The samples were analyzed for titratable acidity according to [24].

2.3.2. Drip loss was determined according to [25].

2.3.3. Cooking loss was determined according to [26].

2.3.4. Determination of thiobarbituric acid (TBA) according to [27].

Fish sample (10g) was homogenized with 25 ml of 10% trichloroacetic acid (TCA) and 25 ml of distilled water for 1 min. The suspension was then filtered and 4 ml of the filtrate was added to 1 ml of TBA reagent (0.06 M). The mixture was immersed heated in boiling water bath at 100°C for 30 min to develop a pink color and cooled with a running water for 10 min, the absorbance of the solution was read at 532 nm by using spectrophotometer (model: T60UV, PG instrument. U. K). The constant 7.8 was used to calculate the TBA number. The TBA value was expressed as mg malonaldehyde / kg sample.

2.4. Determination of fatty acids composition:

Fatty acids composition was determined by GC-MS according to [28]. The methyl esters of fatty acids separated using HP 6890 GC (at Agriculture Research Center, Cairo, Egypt.). 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.

2.5. Determination of Amino acids composition:

Amino acids composition was determined in triplicates after hydrolysis of the fillet samples, about 0.2 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 120°C for 24hrs and then filtered. Finally 1 mL of the filtrate was dried and resuspended in 0.1 M HCL and injected into HPLC at Agriculture Research Center, Cairo, Egypt) [29].

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 [30].
3. Results and discussions

3.1. Change in Chemical properties for fish meat:

The moisture content of Nile Lebeo and Catfish was revealed in table (1) generally increased significantly at (P≤0.05). Across all treatments. The groups that were chilling had the higher moisture content in the end of storage period in both kinds (80.57%) (83.8%), followed by the groups that were cooling (80.06%), (80.43%), caused the increased somewhat during the chilling storage period, most likely due to moisture absorption from the chilly atmosphere. Results are in agreement with[11], also these results are disagreement with [31].

The total protein in muscles of two species are presented in tables (1), Nile Lebeo had a higher level of total protein than Catfish (18.88%) and (13.49 %) respectively, these results are in agreement with[32] and [33] but disagreement with [34]. In general by end of storage period total protein increased for Nile Lebeo and Catfish at cooling (19.2-15.36 % respectively) and chilling (20.07 – 14.91% respectively). These results are in agreement with [35] and [36]. There were seemed to be significant at (P ≤0.05). At the end of storage, at all treatments protein increased in cooling and chilling for the two species.

Data in table (1) showed that lower lipid values than fresh samples due to lipid oxidation during storage in fish (Nile Lebeo and Catfish). There were very minor differences between the fresh and all treatments during storage period. Similar results are obtained by [33]. The lipids value decrease during storage period in two species of fish Nile Lebeo and Catfish. In cooling groups, lipid content was decreased (1.20 %), (3.98 %), followed by groups chilling (1.45%), (1.59 %) in the two studied species, respectively. These results are in agreement with[37] and [38].

In table (1) ash content decreased and that variances can be influenced by variances in the size of the used fish and the meat-to-bone ratio of the fish habitat affects the content of mineral elements in the bodies of live fish. [39]found that the level of ash significantly at (P≤0.05) decreased in both species during storage. Also found ash content at Nile Lebeo and Catfish in fresh (5.66 %), (5.33 %) these results are in agreement with [33]. The level of ash decreased in both species during storage period at all treatments. At least (3.21%), (4.87%), (4.13 %) these results were in agreement with [40]. In Catfish after cooling in week (2) and chilling in week (12) ash content was increased (6.66%), (5.58 %) respectively; these results are in agreement with[39].

3.2. Change in protein fractions for fish meat:

Data in table (2) showed that myofibrillar protein (M.P) content of Nile Lebeo and Catfish were significant differences at (p≤0.05). The content of myofibrillar protein was unstable during storage for all treatments of the both species (Nile Lebeo and Catfish). Myofibrillar protein at cooling treatments, decreased and it increases gradually till reach of end period (7.99 %), (7.20 %), respectively and these results are in agreement with [41]. The extractability of myofibrillar proteins in chilled samples of both species Nile Lebeo and Catfish were respectively (7.98 %), (5.08 %), and slightly increased at the end of storage period. These results are in agreement with[42], [43], [44] and [45].

Data in table (2) showed that sarcoplastic protein (S.P), at Nile Lebeo and Catfish in fresh was (5.06 %), (3.93 %), respectively. Which revealed that there is a significant at (p≤0.05)? there are slight changes observed, which decreased sarcoplastic protein solubility which is easily dissolved in water, at Nile Lebeo all treatments at least (4.09 %), (4.38%), notice increase in control all treatments and week (2,4) at cooling, similar results with [46]. The cooling and chilling at Catfish unstable during storage at all treatments, at least decrease at cooling (3.07%) these results are in agreement with [47]. And increase at chilling (4.23 %) these results are in agreement with[45].

Decrease of (S.P) of fish during storage period followed by the denaturated protein (D.P) had significant at (P ≤0.05). Increased as shown in table (2). Denaturated protein in Nile Lebeo is higher than Catfish at fresh, it were (4.03%) .(3.23 %) after 12 weeks of storage denaturated protein increased during storage period at all treatments (cooling and chilling) of the two species, Nile Lebeo (5.76 - 5.74%). And Catfish (4.03 - 4.75%). These results are in agreement with [48].
In table (2) the obtained data revealed the connective tissue protein at fresh Nile Lebeo and Catfish, it was (1.66%), (1.49 %), respectively. In general, the connective tissue protein (C.T.P) often significant at (P ≤0.05). Decreased at all treatments of the two species Nile Lebeo and catfish in two species at cooling decreased during storage period at least (1.34%), (1.05 %), however Nile Lebeo at chilling increase to (1.95 %). While Catfish at chilling decreased to (1%) except week (8) increase (2.06%). These results are in agreement with [47].

3.3. Change in physical properties for fish meat:

In table (3) the obtained data showed the change in pH during cooling and chilling method in the both Nile Lebeo and Catfish during storage period. The values of pH at fresh samples in Nile Lebeo and Catfish, 7.03 , 6.8; respectively were approximately neutral. These results are in agreement with [36] while disagreement with [49]. At the end of storage, pH values were significantly at (p≤0.05). Increased continuously during storage period at cooling (7–6.9) and at chilling (7.2-7). The increase of pH value especially at late periods of storage due to an increase in volatile bases from the decomposition of nitrogenous compounds. These results are in agreement with [50] and [51].

From the data in table (3) found that changes in acidity values of the two types of fishes. In general, after storage period the acidity were significantly increased at (p≤0.05). Effects of cooling and chilling method on acidity in Nile Lebeo, Catfish during storage period, the samples at fresh (0.53%, 0.25%). The acidity values were increased in Nile Lebeo at all treatments (0.70%) while decreased at cooling treatment in week 6, 12 (0.51%, 0.46%), respectively. While for Catfish increased at cooling and chilling(1.85-0.48%) but at chilling decreased only control (0.20%). These results are in agreement with [49] but disagreement with [37].

As in table (3) could be noticed that the TBA values were low in fresh fish at Nile Lebeo and Catfish ranging between 1.03 and 0.56 mg malonaldehyde /1000 g and the TBA values were markedly increased with progressing in storage time, that indicates the fat oxidation with the formation of malonaldehyde. These results are in agreement with [51]. The TBA values significantly at (P≤0.05). Increased in cooling and chilling, also notice increased cooling more than chilling in both species during storage period reached the end TBA values at cooling for both species (4.1- 0.62 ). The increase of the TBA values during all treatments respectively highest values at Nile Lebeo in week 4 (9.14–9), for Catfish in week 6 (0.86- 2.48) mg malonaldehyde /1000g. These results are in agreement with[52] and [53].

In table (4) showed the drip loss growth tended to continuously increase significant at (p≤0.05). With storage current result found that all the treatments led to increase the drip loss in Nile Lebeo and Catfish also found that the decreased value on both species by the end period of cooling treatment was recorded (13.75- 5.5%) and chilling (17.08- 16.5 %). After (12) weeks during storage the values of drip loss recorded its maximum percentage at all treatments, the values of drip loss for both species Nile Lebeo and Catfish recorded highest values respectively (20.8, 24.98, 17.47, 21.79 %). Similar results were found by [54] and disagreement with [55]. These result may be due to decrease the ability of muscle proteins to bind and hold water [56].

Data in table (4) showed that change in cooking loss of the both Nile Lebeo and Catfish during storage period, from this table. At fresh fishes content cooking loss (22.5 and 25 %) for Nile Lebeo and catfish were significantly differed at (P≤0.05). The content of cooking loss in two kinds of fishes at all treatments during storage period was decreased. Similar results were reported by [57]. Cooking loss were highest during the first of storage in control at chilling for Nile Lebeo (23- 24.76%) and Catfish (26%). Slightly decreased with end storage time for two kinds of fishes at cooling treatment (13.67- 15.83 %) and at chilling treatment (18.67 - 18.5 %), respectively. These results are in agreement with [58].
Table (2): Change in Protein Fractions (%) of Nile Lepto and Cattle Samples:

<table>
<thead>
<tr>
<th>Storage Period/Week</th>
<th>Lepto</th>
<th>Cattle</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12%</td>
<td>10%</td>
<td>11%</td>
</tr>
<tr>
<td>2</td>
<td>8%</td>
<td>7%</td>
<td>8%</td>
</tr>
<tr>
<td>3</td>
<td>4%</td>
<td>5%</td>
<td>4.5%</td>
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<tr>
<td>4</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>5</td>
<td>1%</td>
<td>1%</td>
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<tr>
<td>6</td>
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<td>7</td>
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<td>9</td>
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<tr>
<td>10</td>
<td>6%</td>
<td>6%</td>
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</tbody>
</table>

Means with different letters (a, b, c) in the same row and the same parameters are different significantly at P<0.05, while those with similar letters are not.
Table (4): Change in physical properties (% of Nile Tebra and Cairoin samples)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nile Tebra</th>
<th>Nile Cairoin</th>
<th>Storage Period/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leafe</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Leafe/Tea</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Drip loss</td>
<td>0</td>
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</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nile Tebra</th>
<th>Nile Cairoin</th>
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</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Leafe</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Leafe/Tea</td>
<td>0</td>
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</tr>
<tr>
<td>Drip loss</td>
<td>0</td>
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</tbody>
</table>

Table (5): Change in physical properties (% of Nile Tebra and Cairoin samples)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nile Tebra</th>
<th>Nile Cairoin</th>
<th>Storage Period/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leafe</td>
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<tr>
<td>Leafe/Tea</td>
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<td>Drip loss</td>
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Table (6): Change in physical properties (% of Nile Tebra and Cairoin samples)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nile Tebra</th>
<th>Nile Cairoin</th>
<th>Storage Period/Week</th>
</tr>
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<tbody>
<tr>
<td>Fresh</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leafe</td>
<td>0</td>
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<tr>
<td>Leafe/Tea</td>
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<tr>
<td>Drip loss</td>
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</tbody>
</table>

Table (7): Change in physical properties (% of Nile Tebra and Cairoin samples)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nile Tebra</th>
<th>Nile Cairoin</th>
<th>Storage Period/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leafe</td>
<td>0</td>
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<tr>
<td>Leafe/Tea</td>
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<tr>
<td>Drip loss</td>
<td>0</td>
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</tr>
</tbody>
</table>
3.4. Change in fatty acids:
Fatty acids profiles (mg/g of total fatty acids) of fresh, control and end the storage after (12) weeks samples are shown in table (5). Twenty five fatty acids were identified in the samples. Total fatty acids, determined to be 97.53 mg/g in fresh Nile Lebeo and 97.36 mg/g in the fresh Catfish these results also are in agreement with [59]. The content of saturated fatty acids (SFA) was lower than the unsaturated fatty acids (UFA) in fresh Nile Lebeo (39.15 - 58.38 mg/g) and catfish (43.3 - 54.06 mg/g), these results are in agreement with [60] and [61]. After (12) weeks at the end of storage samples are increased slightly, similar to the increase in control samples and the increase in cooling treatment higher than chilling treatment for both fishes (99.96, 98.37/ 99.97, 97.51 mg/g) and unsaturated fatty acids higher than saturated fatty acids in two species fishes all treatment respectively at cooling (64.45 - 53.52) and chilling (57.3 - 52.99) mg/g. These results are in agreement with [62]. Palmitic acid (C16:0) was the most abundant saturated fatty acid in the both species, forming (24.14 – 26.38 mg/g). Myristic (C14:0) recorded (4.62 – 3.28), heptadecanoic (C17:0) recorded (3.86 – 1.29), stearic acid(C18:0) recorded (4.05 – 6.73) acids which was the second major SFA in both the two fish species. Palmitic acid was found to be the most abundant saturated fatty acid in muscle tissues of the Nile fish species these results are in agreement with [63]. Oleic acid (C18:1) recorded (11.68-17.44), Palmitoleic acid (C16:1), was (20.95 -10.78), eicosapentaenoic acid (C20:5) ranged (7.6-3.39), docosahexaenoic acid (C22:6) ranged (5.58-6.37) which was the major USFA in both the two fish species, these result are in agreement with [64].

3.5. Change in amino acids:
The amino acids (AA) composition of Nile Lebeo and Catfish calculated in (mg/g) are shown in table (6). Nonessential and essential Amino acids, determined to be (64.36-35.55) mg/g, respectively in fresh Nile Lebeo and (65.77-34.16) mg/g, respectively in the fresh Catfish. These results are in agreement with [65]. Glutamic, aspartic, glycine acids were considered a major nonessential amino acids in the two kinds of fish in Nile Lebeo and Catfish recording (15-15.73), (11.66-12.36), (10.32-11.61) mg/g, respectively. Also leucine, Valine threonine were considered a major essential amino acids in Nile Lebeo and Catfish which ranged (11.45-11.93), (5.14 -5.32), (5.05-5.20) mg/g. These results are in agreement with [66]. After (12) weeks, at end of storage, Nile Lebeo nonessential amino acids in cooling treatment was higher than chilling treatment (67.16-63.11 mg/g), respectively. While, Catfish after (12) weeks of storage was higher content of nonessential amino acids in chilling treatment than cooling treatment as (67.06 - 64.73 mg/g ), respectively. These results are in agreement with [67].
Table 5: Change in fatty acids (mg/g of Nile Lebeo and Cachalot samples:...
The image contains a table with data. The table appears to compare different values across various conditions or treatments. The table is not fully legible due to the quality of the image, but it seems to be related to amino acid changes or some other form of measurement.

The table includes columns for different conditions, such as 'Control', 'Fresh', 'Nile Leedo', 'Caspian', and '12 Weeks'. The rows seem to represent different samples or treatments, with values listed under each condition.

The table is titled 'Table 6 (g) Change in amino acids (mg/g) of Nile Leedo and Caspian samples.'
4. Conclusions:

The effect of cooling and chilling methods on the nutritional quality of the Nile Lebeo and Catfish fish were investigated during storage period for (12) weeks. During both storage conditions degrading changes in chemical were occured, changes in physical properties and protein fractions in chilling treatment were more than cooling treatment, that also affected on sarcoplasmic, stroma proteins which decreased in both species, while the percentage of denaturated, myofibril proteins, and amino acids increased. Also, fatty acids and TBA are very affected by cooling treatment they which are increased gradually. Generally; cooling process of fish at 4ºC caused comparatively lower changes than chilling process. It is recommended to eat fresh and stored cooled fish muscle to preserving the better quality of fish product.

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