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Effect of Cold Storage on Chemical, Physiochemical and Microbiological Attributes of African Catfish (*Clarias gariepinus*) from Two Different Egyptian Farms

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ABSTRACT

This research aimed to evaluate the effect of refrigerated storage at 4±1 °C for 12 days on the physiochemical and microbiological attributes of African Catfish. Samples were analyzed every 3 days of storage. Fish samples from the first farm had 76.75 and 6.56% while fish samples from the second farm had 66.79, 15.88 as moisture and fat content, respectively at zero time. Our findings obtained that TVN increased to 34.04 mg N/100g fish sample in the first farm and 27.11 mg N% in the second farm at the end of refrigerated storage. The fish samples of the second farm showed slow decrease in pH values, which was 6.72 at zero time and decreased to 6.50 at the end of storage. The rate of oxidation in fish samples of the second farm was higher which reached 0.98 mg MDA/Kg sample compared to the first farm which reached 0.69 MDA/Kg sample at the end of storage. The bound water of fish samples from the first farm was 73.14% at zero time and reached 54.13% at the end of storage period, while fish samples from the second farm started with 63.56% and reached 52.69% at the end of storage. Also, the initial TBC was 3.24 and 3.99 log CFU/g sample in fish samples from the first and second farm, respectively, which reached 9.32 and 8.08 log CFU/g at the end of storage. Our findings showed that catfish samples from the second farm had higher quality and more shelf life than the first one.

Keywords: Catfish, fish preservation, Physiochemical and microbiological attributes, refrigerated storage

INTRODUCTION

Catfish is one of the most tolerant species to severe aquaculture environmental conditions (Oluah and Magbenka, 2020, El-Mezayen *et al.*, 2024). Therefore, it is one of the most common cultivated fresh water fish worldwide (FAO, 2021). The production and cultivation of African catfish (*Clarias gariepinus*) is popularly increased worldwide, because of the desirable characteristics of this fish, e.g. resistance to inappropriate propagation conditions, rapid growth rate, ease of harvesting, nutritional requirements and cost effectiveness.

Global production of the catfish grew to reach 6.5 million metric tons (MT) in 2022, from which more than 20% is coming from the aquaculture sector. Therefore during 2022 catfish jumped to rank 2nd globally after it was 3rd during 2021 (FAO, 2024).

Egypt is the third largest producer of the catfish worldwide. Egyptian catfish production reported 8470 MT from aquaculture in 2021 (FAO, 2023).

However, despite of their low price and abundant supply, the marketing of these fish is still comparatively insufficient. African catfish (fresh, cooked or grilled) were classifies as from the less preferred and less consumed fish species (Oksuz *et al*., 2008).

The most crucial and essential factor in determining the ultimate product's quality is the fish's freshness. Fish has a shorter shelf life than chicken and red meat because it has a higher water activity, a higher final pH, and proportionately more free amino acids. As a result, it is more susceptible to deterioration. Fish spoilage happens simultaneously and independently, with the relative importance of each event depending on the species of fish (size, lipid content, maturation stage, etc.), the environment (feed availability, temperature, microbial load, etc.), the post-mortem handling technique, storage practices, and processing conditions (Isabel *et al.*, 2009).

Fish is an essential of the modern diet that is expanding quickly, thus it is becoming more and more crucial that these goods be fresh. To increase the microbiological safety and overall shelf life of fish, a variety of food preservation methods have been used, including as icing, freezing, chemical preservation, salting, and smoking. Currently, the most common methods used in tropical nations to prevent microbiological and biochemical degradation of freshly harvested fish during transportation and marketing are icing and mechanical refrigeration.

Therefore, the main goal of the present study was to assess the effect of cold storage on physicochemical and microbiological quality of the African catfish samples collected from Egyptian farms.

MATERIALS AND METHODS

Studied samples were collected from two different types of aquaculture farms during 2022, the first farm representing the new technology fish farming (Elserw Fish Research station, which belongs to the National Institute of Oceanography and Fisheries), while the second farm (Elsalam) representing the traditional fish farm which

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conducts fish farming for only catfish under bad conditions as described by El-Mezayen *et al.* (2024).

Preparation of fillets:

African catfish (*clarias gariepinus*) were collected from two local farms in Manzala Lake where located eastward of the Nile Delta between 31º 16′ N and 32º 12`E, where Port Said governorate located east of the lake and Damietta located to the west as described in El-Mezayen *et al.* (2024). Fish were transported in an ice-box to Food Science Department, Faculty of Agriculture, Damietta University. After being weighed, the catfish were slaughter, skinned, beheaded, washed in water and filleted. The catfish fillets were preserved under refrigerated conditions (4ºc ±1) up to 12 days. Physiochemical properties and microbiological examination of refrigerated catfish fillets were estimated every 3 days.

Chemical analysis of catfish fillets:

Proximate analysis of catfish fillets:

Proximate analysis of catfish fillets samples (moisture, total ash, crude protein, crude fat, and carbohydrates) were carried out accordily to the method described by AOAC (2016). The measurements were categorized based on the differences at zero time and after 12 days of storage.

Determination of total volatile nitrogen (TVN):

Proteins break down into simpler compounds (free amino acids, ammonia, trimethylamine, creatine, and purine bases) to create TVBN. Catfish fillets' total volatile nitrogen (TVN) was analyzed and calculated following the technique outlined by EOS (2006).

Determination of thiobarbituric acid (TBA):

Oxidation stability was assessed by measuring the Thiobarbituric acid (TBA) value of catfish fillets which determined using spectrophotometer at 538 nm according to the method described by EOS (2005). The TBA values were expressed as mg malonaldehyde (MDA)/kg fish sample.

Physiochemical analysis of catfish fillets:

Determination of pH value:

pH of the homogenized refrigerated catfish samples in distilled water (1: 5 W/V) was determined by using a glass electrode digital pH meter (Model JENWAY pH/ mv meter Model 3510 instruction Manual) at 25ºC for 30 min according to Fernández-López *et al.* (2020).

Water holding capacity (WHC):

The WHC of refrigerated catfish fillets samples were measured according to Egan *et al.* (1981). 0.3 g of fish meat was put above filter paper and pressed for 10 min using 1Kg weight. Two zones were formed on the filter paper and their surface areas measured. The outer zone resulted from water separated from the pressed fish tissue indicated the bound water.

$$\text{Bound water (\%)} = \{[(a \times m) - 8.4 \times (b\text{-}c)] / m\} \times 100$$

Where a: Moisture content/100.
m: weight of sample (mg).
b: the outer zone area (Cm^2).
c: the internal zone area (Cm^2).
1 Cm^2 of squeezed juice absorbed on filter paper was found to be about 8.4 mg water.

Microbiological examination of catfish samples

Fish samples preparation:

Samples of catfish fillets were weighed aseptically in a sterile environment. Samples of fish weighing 5 g were added to 45 ml of sterile water. To prepare a 1:10 dilution, the suspension was shaken by hand for five minutes. As needed, further dilutions were made and plated in triplicate.

Total viable bacterial count (TVBC):

Every three days during the catfish fillets were at refrigerated storage, the total viable bacterial count was determined. Using the poured plate method, samples of catfish fillets were prepared in serial dilutions and then aseptically transferred onto three sterile glass Petri dishes. Each plate was filled with around fifteen milliliters of nutrient agar medium that had been cooled to 45 - 50 ºC. It was then well mixed and allowed to solidify. For a whole day, the plates were incubated at 30°C. Following the incubation time, each plate's formed colonies were counted. Following the selection of the number of colonies, three duplicate plates with the same dilution were counted. The total number of colonies per gram of materials was then computed as follows:

Total bacterial count = average number of triplicate plates of the same dilution x reciprocal of the dilution used colony forming unit (CFU)/g sample (Anon, 1992).

Statistical analysis

The obtained results were analyzed using the analysis of variance (ANOVA) according to the statistical analysis system (Costa) version 6.303 and comparisons were done by Duncan's Multiple Range test (Steel *et al.*, 1997) at $P<0.05$ level of significance.

RESULTS AND DISCUSSION

Chemical composition of catfish

The approximate chemical composition of catfish samples were tabulated in Table 1. Tabulated data showed that there were inverse relationship between moisture and crude fat contents, where the first farm had high moisture content, which recorded 76.75% at zero time and 74.91% at 12 days of refrigerated storage compared to the other farm. Also, the second farm had high crude fat percentage, which was 15.88% at zero time and 13.02% at 12 days of refrigerated storage. On the other hand, the crude protein and ash content of both fish samples from the studied farms were in the same range. From our findings, it was also noticed that the cold storage period does not effect on catfish chemical composition. These results were in the same trend with Baryczka *et al.*, (2019) who illustrated that moisture content, total protein, crude fat and crude ash of chilled catfish fillets were 75.99%, 17.37%, 5.11% and 1.09%, respectively. The differences between the two studied farms may be due to the type of feed, weight and age of the fish.

Table 1 .Approximate chemical composition of catfish samples (% on wet weight basis) during refrigerated storage (4±1ºC) for 12 days

Components	Storage period (days)	Fish samples	
		Farm 1	Farm 2
Moisture	0	76.75±0.83	66.79±1.37
	12	74.91±0.76	68.26±1.66
Crude Protein	0	14.62±0.70	16.89±1.11
	12	16.91±0.08	17.06±1.18
Crude Fat	0	6.56±1.57	15.88±0.71
	12	6.43±0.73	13.02±1.50
Ash	0	0.83±0.11	0.74±0.08
	12	0.92±0.11	0.96±0.09

Total volatile nitrogen of catfish flesh during refrigerated storage (4±1°C) for 12 days:

Catfish fillets' total volatile nitrogen (TVN) was tabulated in Table 2. It is noted that as increasing of the

refrigerated storage period, the rate of formation of total volatile nitrogen increases. From obtained results, there were significant differences (P<0.05) between storage periods of the same farm, TVN content increased to 34.04 mg N% in the first farm and 27.11 mg N% in the second farm at the end of refrigerated storage which is within the range of recommended value of 30-35mg TVB-N/100g for fresh fish (Huss, 1988 and Connell, 1995). These results may be due to the farm difference and the long storage period at 4±1ºC. These results are consistent with the results of pH value and total bacterial count in Table 3 and Table 5, which showed that pH value and total bacterial count gradually increased during refrigerated storage period and the rate of increase in the first farm was higher than the second farm which led to a higher rate of accumulation of alkaline compounds such as ammonia, resulting in higher values of total volatile nitrogen. These

Table 2. Total volatile nitrogen (mg N/ 100 g sample) of catfish flesh during refrigerated storage (4±1ºC) for 12 days

Storage period (days)	Fish samples	
	Farm 1	Farm 2
0	26.85^{b}±2.61	21.16^{c}±0.45
3	27.84^{b}±2.27	22.54^{bc}±0.30
6	31.03^{ab}±0.91	24.50^{ab}±1.20
9	31.88^{ab}±0.92	25.87^{a}±1.04
12	34.04^{a}±0.40	27.11^{a}±1.13
LSD 0.05	5.23	2.85

Mean values ± standard error (n=3). Mean values in the same column with the same letter are not significantly different at *P* < 0.05.

pH value of catfish flesh samples changes during refrigerated storage (4±1°C) for 12 days:

Data tabulated in Table 3 showed the pH values of catfish flesh during refrigerated storage. From tabulated results, it could be noticed that there were no significant differences between storage periods of the first farm, where the initial pH value was 7.06 which gradually decreased to 6.74 at 6th day of refrigerated storage and then gradually increased. While there were significant differences between storage periods of the second farm which start 6.72 at the first day of storage and decreased to 6.47 at 6th day of storage and then gradually increased. From the previous results, it can be noted that there is a correlation between the slow decrease in pH values and the increase in total volatile nitrogen values (Table 2), as well as the increase in the total bacterial count values (Table 5) for fish in the first farm compared to the second farm. The decrease in pH may be due to the post-mortem glycolysis which causes the breakdown of glycogen and the accumulation of lactic acid; this is followed by partial hydrolysis of the protein by enzymes, causing the production of free amino acids that are decomposed by bacteria, producing nitrogenous bases, which cause the pH to gradually increase. These results were in the same trend with Pessu *et al.*, (2016) who obtained that pH value of *Claris gariepinus* ranged from 6.40 to 7.10 at refrigerated storage.

Table 3. pH value of catfish flesh during refrigerated storage (4±1ºC) for 12 days

Storage period (days)	Fish samples	
	Farm 1	Farm 2
0	7.06^{a}±0.02	6.72^{a}±0.08
3	6.75^{a}±0.13	6.64^{ab}±0.02
6	6.74^{a}±0.10	6.47^{c}±0.02
9	6.83^{a}±0.09	6.55^{bc}±0.05
12	6.74^{a}±0.11	6.50^{bc}±0.02
LSD 0.05	0.31	0.14

Mean values ± standard error (n=3). Mean values in the same column with the same letter are not significantly different at *P* < 0.05.

Our findings in Figure 1 showed the thiobarbaturic acid content of refrigerated catfish flesh samples which points to the high oxidative stability of lipids. From the presented results, it can be inferred that the rate of oxidation in the fish of the second farm sample was higher compared to the first farm, which was 0.54 mg MDA/Kg sample as the initial value of the first farm sample which gradually increased to 0.69 mg MDA/Kg sample at the end of the storage period (12 days). While the results of the second farm showed an increase in the TBA value at the beginning of storage (1.07 mg MDA/Kg sample), which fluctuated throughout refrigerated storage period until it reached 0.98 mg MDA/Kg sample at the end of the storage period.

These results may be due to the difference in the percentage of fat between the two farms, as fat content increased in the second farm as shown in Table 1, and the TBA rate increased compared to the fish of the first farm, This fluctuation in TBA values is also due to the instability of the products of fat oxidation, which are transformed from peroxides and hydro-peroxides to aldehydes and vice versa. Despite this, the results did not exceed the permissible limits 4.5 mg MDA/Kg sample according to (EOS, 2005).

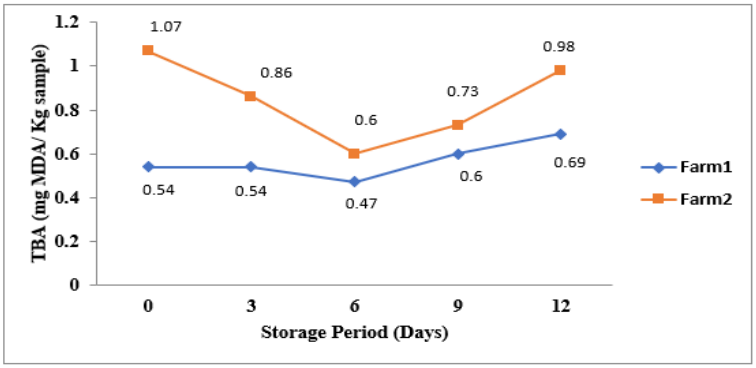

Figure 1. TBA value (mg malonaldehyde/kg sample) of catfish flesh samples during refrigerated storage (4±1ºC) for 12 days.

The results presented in Table 4 illustrated the rate of change in the bound water values of catfish muscles during the refrigerated storage period. The results showed that there were significant differences between the storage periods of the same farm, as the first farm started with a value of 73.14% and reached 54.13% at the end of the cold storage period, while the second farm started with a value of 63.56% and reached 52.69% at the end of cold storage period. This change

in BW values may be due to the difference in pH values in Table 3, as the pH values in the second farm were lower than the first farm, which leads to a decrease in the ability of the fish's muscles to hold water. Also, this decrease in the bound water values in the second farm may be due to the high fat content of this farm. Our findings are the same trend with Paleckaitis *et al.* (2018) who obtained that the water-holding capacity of African catfish fillets which ranged 61.25-64.25% in fish due to feed type difference.

These results were in agreement with Zhuang *et al*. (2019) who illustrated that microbes play an important role in breaking down proteins into peptides and amino acids by enzymes during fish storage, which in turn cause a change in the physicochemical properties of fish, such as their texture and water holding capacity.

Table 4. The bound water percentage (BW %) of catfish flesh during refrigerated storage (4±1ºC) for 12 days.

Storage period (days)	Fish samples	
	Farm 1	Farm 2
0	73.14^{a}±0.48	63.56^{a}±3.65
3	71.38^{a}±1.18	58.93^{ab}±1.23
6	63.88^{b}±3.62	55.11^{b}±1.26
9	59.20^{bc}±2.77	54.28^{b}±1.08
12	54.13^{c}±1.08	52.69^{b}±1.35
LSD 0.05	6.84	6.21

Mean values ± standard error (n=3). Mean values in the same column with the same letter are not significantly different at $P < 0.05$.

The total bacterial count is used as one of the criteria for judging the microbiological quality of fish. Table 5 shows the rate of increase in bacterial load during the cold storage period of fish from the two farms. The results showed a difference in the initial bacterial count for both farms, which is due to the difference in the source of the two farms, aquaculture water and the type of feed, which was 3.24 and 3.99 log CFU/g sample in the first and second farm, respectively and which gradually increased during the cold storage period, but at a different rate from the starting point, which reached 9.32 and 8.08 log CFU/g sample in the first and second farm fish samples, respectively. This difference in the rate of increase between the two farms may be due to the difference in the species of the growth bacteria, which may be due to the pollution rate and their type. These results were confirmed by (Zotta *et al*., 2019) who explained that storage temperature is the most influential factor on the rate of microbial growth, which affects the type of species that can grow.

Table 5. Total bacterial count TBC (Log CFU/g sample) of catfish flesh during refrigerated storage (4±1ºC) for 12 days

Storage period (days)	Fish samples	
	Farm 1	Farm 2
0	3.24^{d}±0.22	3.99^{d}±0.25
3	4.80^{ccd}±0.12	4.23^{d}±0.16
6	5.63^{bc}±0.38	4.81^{c}±0.09
9	7.51^{ab}±0.68	5.79^{b}±0.27
12	9.32^{a}±1.39	8.08^{a}±0.07
LSD 0.05	2.27	0.58

Mean values ± standard error (n=3). Mean values in the same column with the same letter are not significantly different at $P < 0.05$.

CONCLUSION

From previous results, it could be concluded that chemical, physiochemical and microbiological characteristics of African catfish stored at refrigerated conditions refers to significant differences between the two studied farms. Sure, these differences were affected by many variables, such as feeding, water attributes and pollution level. So, catfish samples from the second farm seem to have higher quality and more shelf life than the first one.

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تأثير التخزين المبرد على الخصائص الكيميائية والفيزيوكيميائية والميكروبيولوجية لسمك القراميط الإفريقي من مزرعتين مصريتين مختلفتين

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الملخص

يهدف هذا البحث إلى تقييم تأثير التخزين المبرد على الصفات الفيزيوكيميائية والميكروبيولوجية لسمك القراميط الافريقي عند درجة حرارة 4 ± 1°م لمدة 12 يوم. وقد تم تحليل العينات كل 3 أيام من التخزين. وقد أوضحت النتائج احتواء عينات الأسماك من المزرعة الأولى علي قيم 76.75 و 6.56 % لكلا من الرطوبة والدهن بينما احتوت عينات الأسماك من المزرعة الثانية على 66.79 و 15.88 %على التوالي عند بداية فترة التخزين. كما أوضحت النتائج زيادة قيمة النيتروجين الكلي المتطاير إلى 34.04 ملجم نيتروجين/ 100 جم عينة لأسماك المزرعة الأولى، و 27.11 ملجم نيتروجين/ 100 جم عينة لأسماك المزرعة الثانية في نهاية فترة التخزين المبرد. أظهرت أسماك المزرعة الثانية معدل انخفاض بطئ في قيم رقم الحموضة والتي كانت 6.72 في بداية التخزين والتي انخفضت إلى 6.50 في نهاية فترة التخزين. وكان معدل الأكسدة أعلى في عينات أسماك المزرعة الثانية حيث وصلت إلي 0.98 ملجم مالونالدهيد/ كجم عينة سمك مقارنة بأسماك المزرعة الأولى والتي وصلت إلى 0.69 ملجم مالونالدهيد/ كجم عينة سمك في نهاية فترة التخزين. بلغت نسبة الماء المرتبط في عينات أسماك المزرعة الأولى 73.14% في بداية التخزين والتي وصلت إلى 54.13% في نهاية فترة التخزين، بينما بدأت عينات أسماك المزرعة الثانية بقيمة 63.56% ووصلت إلى 52.69% في نهاية فترة التخزين. أيضًا بدأ العد الكلي للبكتريا بقيمة 3.24 و 3.99 Log CFU / جم عينة في عينات أسماك المزرعة الأولى والثانية على التوالي والتي وصلت إلى 9.32 و 8.08 Log CFU / جم عينة على التوالي في نهاية فترة التخزين. أظهرت النتائج أن عينات أسماك قراميط المزرعة الثانية كانت أعلى جودة وأطول فترة صلاحية من أسماك المزرعة الأولى.

الكلمات الدالة : سمك القراميط، حفظ الأسماك، الخصائص الفيزيوكيميائية والميكروبيولوجية و التخزين المبرد.