Comparison of microbial and chemical changes in common carp (Cyprinus carpio) fillets reared in earthen basins, river cages and river fish during storage in refrigerator

Ahmed A. Hussein*, Itlal Hakim Almasoodi , Aqeel H. Atallah

Food Science Department, College of Agriculture, University of Karbala, Iraq
***Corresponding author e-mail: Ahmed.ameer@uokerbala.edu.iq**
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Abstract

The factors that greatly affect the quality of fish meat are type of nutrition and nutritional conditions, their effects are found in the muscle structure and stability of fish meat during the storage period. To conduct this study, fish were stored for 14 days at a temperature of 4 °C. During the refrigerated storage period of fish fillets, the approximate composition of fish fillets (moisture, protein, fat and ash) was measured, and the quality factors of fish were recorded. The results also showed that the highest amount of moisture and the lowest amount of fat were in fish cultured in earthen ponds, and the amount of protein did not change significantly between the different treatments, the amounts of TBA, FFA and TVN increased during the refrigerated storage period, and the pH changes were not regular between the different treatments. The highest amount of TBA and FFA was between the different treatments. It was also noted that the highest amount of moisture was in river fish. Comparison of the results of the different qualitative factors between the three treatments showed that treating fish in an earthen pond environment compared to the two treatments is more susceptible to spoilage during the refrigerated storage period. While the microbiological studies were conducted.

Keywords: Common carp, river cages, earthen basins, refrigerated storage.

Introduction

Common carp is a species that can be cultivated almost all over the world due to its rapid growth, ease of cultivation and high food productivity. It can be seen in most natural environments and is one of the most abundant species in Iraq [1]. Carp feeds on aquatic organisms such as insect larvae, worms and nematodes [2]. Due to the density of fish in the breeding ponds, natural food does not meet the needs of these fish, which is why hand feeding is used to feed them. The quality factors of carp meat include various aspects such as essential amino acids, the amount of protein, fats, vitamins, minerals and fatty acids and their composition [3]. These factors are affected by the chemical composition of the fish. The chemical composition of the fish varies from one species to another, and this difference can be observed even among fish of the same species [4]. The factors that can affect the chemical composition of fish are divided into two categories, internal and

external, which include the environment (season, salinity and temperature) and diet (food cycle and diet composition) [5]. Storing fish in the refrigerator causes a decrease in the speed of activities. Chemical, enzymatic and microscopic activity of living organisms, but keeping carp fish at 4°C in the refrigerator causes undesirable changes including hydrolysis of fats and oxidation that work slowly and reduce the quality of the fish [6].

Materials and Methods

Fish

This experiment was conducted at the College of Agriculture, University of Karbala in the summer of 2024, where common carp cultured in earthen ponds and river cages and river carp fish (live fish of the Euphrates River / Hindiya Dam City) were purchased with an average weight of 2 kg and an age of 1.5 years, where the cultured fish purchased from earthen ponds were fed with food rations (15% protein, 8% ash, 2% fat), and the cultured fish purchased from river cages were fed a diet containing (45% protein and 2% ash 15% fat) and stored in the refrigerator for 14 days at 4°C. Laboratory tests were conducted on the three treatments during different periods of dry refrigerated storage (times 0, 7 and 14) days with three repetitions.

Fish composition

The fish composition was based on AOAC [7], where the amount of crude fat was measured using the Soxhlet device, crude protein using the Caldahl method using the Dahl digestion and distillation device, ash using the oven at a temperature of 550 for 8 hours, and humidity was estimated using the Oon device at for 24 hours.

Measurement of pH

Two grams of the sample were added to 10 ml of distilled water and homogenized, then the samples were measured using a digital pH meter at room temperature.

TVN

The samples were measured by distillation and titration [8] and the extract resulting from distillation was titrated with normal sulfuric acid solution (0.1) and the concentration of volatile nitrogenous bases was determined on the basis of milligrams of nitrogen [9].

Measurement of moisture under pressure

A piece of carp meat 1 cm x 1 cm (separated and weighed) was placed between two sheets of paper, then an initial weight of 500 grams was placed on the sample for 5 minutes A, then another weight of 500 grams was placed on it again for 20 minutes B, and after pressing with these weights the sample was weighed again and the amount of moisture percentage under pressure was calculated according to the following relationship [10].

$$\frac{A - \mathrm{B}}{\mathrm{A}} \mathrm{X}100$$

TBA

It was measured colorimetrically and the absorbance was measured at a wavelength of 538 nm (using a spectrophotometer) [11].

FFA

The amount of free fatty acids was measured by the Woyewoda method [8].

Microbiological analysis:

Samples were prepared according to APHA [12]. The yeast counts were determined as per the procedure described in APHA [12]. The coliform count was determined as per the procedure described in APHA [12] using melted violet red bile agar. The average number of colonies was multiplied with dilution factor to obtain total count as colony forming unit (CFU) per gm of the sample. This count was then converted to coliform count of log CFU/g of sample.

Statistical analysis

Statistical analysis of all data for carp samples using SAS software.

Results and Discussion

Protein

From Table (1), we note that during the storage period of carp fillets in the refrigerator for a period of 14 days, no significant change was observed in the amount of protein during the storage period and between the three different treatments.

Table (1): The percentage of protein changes in carp fish during 14 days of storage in the refrigerator at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	7	14
River fish	19.78 ± 0.49 ^{A}	21.96 ± 1.27 ^{B}	22.98 ± 1.69 ^{C}
River cages	18.98 ± 0.71 ^{A}	20.36 ± 1.01 ^{A}	21.58 ± 1.73 ^{B}
Earth ponds	18.58 ± 0.69 ^{A}	19.56 ± 0.69 ^{A}	20.18 ± 0.69 ^{B}

Different letters refer to a significant ($p \leq 0.05$) differences between treatments

Moisture

The results of Table 2 showed that the moisture content of fish fillets during the refrigerator storage period did not change significantly.

Table (2): The percentage of moisture in carp fish during 14 days of refrigerator storage at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	7	14
River fish	74.44 ± 0.44 ^{A}	73.78 ± 0.27 ^{B}	74.84 ± 0.38 ^{C}
River cages	75.74 ± 0.47 ^{A}	73.92 ± 0.62 ^{B}	73.12 ± 0.37 ^{C}

Earth ponds	76.9 ± 0.68 ^{A}	77.02 ± 0.25 ^{A}	76.06 ± 0.74 ^{A}

Different letters refer to a significant (p≤0.05) differences between treatments

Fats

In Table 3 the results showed that the fat values in the three samples decreased during the storage period, and in treatment 3, treatment 1 had the highest amount of fat.

Table (3): The percentage of moisture in carp fish during 14 days of refrigerator storage at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	**7**	**14**
River fish	5.22 ± 0.35 ^{A}	4.82 ± 0.33 ^{A}	4.28 ± 0.54 ^{B}
River cages	7.32 ± 0.47 ^{A}	6.42 ± 0.42 ^{B}	5.72 ± 0.16 ^{C}
Earth ponds	2.52 ± 0.25 ^{A}	2.04 ± 0.24 ^{B}	1.52 ± 0.19 ^{C}

Different letters refer to a significant (p≤0.05) differences between treatments

Free Fatty Acid

The results of the chemical analyses in Table 4 showed that the free fatty acid index increases with the increase in the storage period of carp slices stored at a temperature of 4 °C. From the table, we note that the free fatty acids have the lowest values on the zero day, but on the fifteenth day they have the highest values. However, treatment 3 had a significant effect in increasing the free fatty acids.

Table (4): The percentage of FFA in carp fish during 14 days of storage in the refrigerator at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	**7**	**14**
River fish	3.32 ± 0.37 ^{A}	4.94 ± 0.80 ^{B}	5.92 ± 0.81 ^{C}
River cages	3.58 ± 0.28 ^{A}	4.42 ± 0.21 ^{B}	5.38 ± 0.19 ^{C}
Earth ponds	11.82 ± 0.49 ^{A}	22.88 ± 0.85 ^{B}	25.18 ± 0.63 ^{C}

Different letters refer to a significant (p≤0.05) differences between treatments

Ash

The results in Table 5 showed that the ash values of carp muscles witnessed a significant increase during 14 days of storage in the refrigerator among the three different treatments, and the highest amount of ash was observed in carp fish cultured in earthen ponds.

Table (5): Percentage of ash in carp fish during 14 days of storage in the refrigerator at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	7	14
River fish	4.66 ± 0.30 ^{A}	5.42 ± 0.26 ^{B}	5.76 ± 0.38 ^{C}
River cages	3.62 ± 0.24 ^{A}	4.24 ± 0.33 ^{B}	4.78 ± 0.44 ^{C}
Earth ponds	5.22 ± 0.37 ^{A}	5.64 ± 0.46 ^{A}	6.02 ± 0.72 ^{A}

Different letters refer to a significant ($p \leq 0.05$) differences between treatments

Water Holding Capacity

The results in Table 6 showed that the measured values of fish samples during 14 days of storage in the refrigerator witnessed a significant increase. The results indicate that with increasing storage period in the refrigerator, its ability to retain water decreased and as a result the amount of moisture increased.

Table (6): Percentage changes in WHC in carp fish during 14 days of storage in the refrigerator at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	7	14
River fish	22.76 ± 0.41 A	25.78 ± 0.54 B	27.64 ± 0.68 C
River cages	12.84 ± 0.39 A	14.82 ± 0.46 B	16.68 ± 0.54 C
Earth ponds	20.72 ± 0.48 A	26.72 ± 0.41 B	32.98 ± 0.36 C

Different letters refer to a significant ($p \leq 0.05$) differences between treatments

Total Volatile Nitrogen

The results in Table 7 showed that TVN values during storage in the refrigerator caused a total increase between the different treatments during 14 days, but the lowest value was observed in the second treatment.

Table (7): Percentage of TVN in carp fish during 14 days of storage in the refrigerator at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	7	14
River fish	21.6 ± 1.14 A	51.4 ± 2.70 B	64.6 ± 2.07 C
River cages	18.4 ± 1.04 A	35.2 ± 1.48 B	52.6 ± 1.51 C
Earth ponds	36.1 ± 0.8 A	57.2 ± 0.6 B	72.8 ± 1.8 C

Different letters refer to a significant (p≤0.05) differences between treatments

Thiobarbituric Acid

From Table 8 we notice that the amount of thiobarbituric acid is significant at the 5% probability level, the process of TBA changes in an increasing direction during the storage period for all treatments, but the lowest amount of TBA was in river fish, and the highest values among the different treatments were related to earthen pond fish.

Table (8): The percentage of TBA in carp fish during 14 days of storage in the refrigerator at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	7	14
River fish	0.356 ± 0.04 A	0.504 ± 0.04 B	0.584 ± 0.05 C
River cages	0.626 ± 0.05 A	0.728 ± 0.045 B	0.884 ± 0.042 C
Earth ponds	0.718 ± 0.05 A	0.902 ± 0.03 B	1.108 ± 0.03 C

Different letters refer to a significant (p≤0.05) differences between treatments

PH

Table 9 shows the pH of the three different treatments and there was no significant difference between the pH values.

Table (9): pH value in carp fish during 14 days of storage in the refrigerator at a temperature of 4 (M±SD).

Treatment	Storage period in days (Mean + S.D.)		
	0	7	14
River fish	6.06 ± 0.03 A	6.08 ± 0.04 A	6.15 ± 0.03 B
River cages	6.08 ± 0.05 A	6.22 ± 0.05 B	6.48 ± 0.04 C
Earth ponds	6.06 ± 0.04 A	6.09 ± 0.02 A	6.18 ± 0.02 B

Different letters refer to a significant (p≤0.05) differences between treatments

Microbiological study

The mean values ± standard error of total platelet count, number of refrigeration bacteria, number of yeasts and coliforms were expressed as log cfug1 and presented in Table 10. In fish meat samples, coliforms were detected on day 7. It was also observed that the increase in total platelet count values of fish was significant (p<0.05) on day 3 when compared to the initial count. Microbiological studies indicated that all parameters showed a significant increasing trend (p<0.05) throughout the storage period.

Table (10): The total bacterial count in carp fish during 14 days of storage in the refrigerator at a temperature of 4 (M±SD).

Treatment		Storage period in days (Mean + S.D.)		
		0	7	14
River fish	Total Plate Count (log cfug-1)	2.83 ± 0.421 A	4.01 ± 0.206 B	6.89 ± 0.157 C
	Total Psychrophilic Count (log cfug-1)	1.05 ± 0.062 A	1.71 ± 0.067 A	2.86 ± 0.119 B
	Yeast Count (log cfug-1)	N.D. A	0.686 ± 0.126 B	2.256 ± 0.149 C
	Coliform count (log cfug-1)	N.D. A	0.850 ± 0.112 B	1.134 ± 0.152 C
River cages	Total Plate Count (log cfug-1)	3.12 ± 0.238 A	1.29 ± 0.053 B	5.71 ± 0.513 C
	Total Psychrophilic Count (log cfug-1)	1.29 ± 0.053 A	1.81 ± 0.167 B	2.25 ± 0.105 C
	Yeast Count (log cfug-1)	N.D. A	0.981 ± 0.117 B	1.963 ±0.130 C
	Coliform count (log cfug-1)	N.D. A	3.32 ± 0.431 B	4.52 ± 0.732 C
Earth ponds	Total Plate Count (log cfug-1)	2.35 ± 0.132 A	6.16 ± 0.035 B	6.52 ± 0.762 C
	Total Psychrophilic Count (log cfug-1)	2.561 ± 0.104 A	2.86 ± 0.269 A	4.31 ± 0.423 B
	Yeast Count (log cfug-1)	N.D. A	2.121 ± 0.196 B	2.653 ± 0.092 C
	Coliform count (log cfug-1)	N.D. A	1.56 ± 0.039 B	2.55 ± 0.205 C

In this study, we note that the amount of protein in carp fish in different treatments did not show a significant difference with each other, and no significant change was observed in the amount of protein during the cold storage period. In the research of many researchers, the change in the amount of protein in different diets has no effect on the amount of protein in fish fillets [13]. As for the moisture content, the decrease in the moisture content of carp fish samples during the cold storage period leads to an increase in oxidative changes and thus a decrease in the quality of the product [14]. The decrease and change in the amount of moisture causes an increase in fat oxidation and a change in its quantity. In this study, the decrease in moisture during the cold storage period was not significant, so the change in moisture in this study did not have a significant effect on

proteins, fat oxidation and a decrease in the quality of fish. However, we note the high percentage of moisture in carp fish farmed in earthen ponds due to the decrease in the fat reserve in the muscle of this fish, as water and fat usually have an opposite ratio in the muscles. The approximate analysis of the amount of fat in carp fish fillets shows a decrease during the storage period, From the table, we note that the fat content in the fish fillets cultured in earthen ponds is much lower than the other two treatments. The decrease in the amount of fat in these fish is attributed to the low percentage of fat in their diet compared to the diet of the other two treatments, especially the treatment. On the other hand, the sufficient space for them to move, which burns fat, and in the river cage environment, this causes more fat to accumulate in these fish, due to the small size of the breeding environment and the lack of movement of the fish, which made them contain more fat than the other two fish. During the storage period, the amount of fat decreased in all three treatments, which is related to fat spoilage. The amount of free fatty acids produced depends on the storage temperature [15] and the oxidation of fats turns into ketones and aldehydes. Determining the amount of free fatty acids is a good indicator to express the effect of enzymes that analyze fish fats and other products [15], and tissue damage resulting from the results obtained show that during the 14-day refrigerated storage period, free fatty acids also increase and reach their maximum during the storage period (the storage period on day 14 expressing the process of fat oxidation and fat loss [16], among the three treatments, the highest amount of fatty acids was associated with carp cultured in earthen ponds, due to the lack of fats in the reserve such as triacylglycerol, and it is proven in low-fat fish that phospholipids are subject to greater hydrolysis, as is clear from the results, river carp fish fed on a special diet, and river carp fish fed naturally on the river contain almost the same amount of fat, as for ash, there are studies conducted by many researchers during the cold storage period of carp fish, and it was reported that the amount of ash in fish fillets is not affected by the diet [2] , and from the table, the highest amount of ash was observed in carp fish cultured in earthen ponds, and the results of the WHC indicate that carp fish fillets with an increase in storage period decreased their ability to retain water, and this is due to the greater damage to the protein system (increase in TVN) [1,17], and in terms of the amount of TVN in the treatments, it was used very widely as one of the indicators of fish meat spoilage [18], as this test indicates the decomposition of proteins Due to bacterial and enzymatic activities, which lead to the production of amines and reduce the nutritional value of fish [19], The three treatments during the cold storage period, the amount of TVN increased for all different treatments, but in river fish and fish cultured in earthen ponds more than in river cage fish that feed on their own food, which is probably due to the more active nature of river fish and fish cultured in earthen ponds (suitable space for fish movement) and the presence of more sarcoplasmic proteins in the muscles of these fish [20]. The TBA table shows that the increase in the amount of thiobarbituric acid during the storage period means a decrease in the quality of the product due to an increase in fat oxidation [21]. The lowest amount was observed in river fish, and the highest value in carp cultured in earthen ponds,

and these values increase during the cold storage period [22]. The amount of thiobarbituric acid is an important quality indicator in the acidification process of products [23]. The presence of such a compound in fish meat causes changes in sensory properties, including smell and taste. This indicator is mainly used to evaluate the degree of fat oxidation and shows the amount of by-products of fat oxidation, which Mainly include carbonyls or aldehydes that cause bad odor and taste in fish [24]. The pH decreases after the death of fish due to the production of lactic acid resulting from the decomposition of sugar [25]. However, after the decomposition of sugar, self-changes such as protein denaturation will provide very suitable conditions for the reproduction and growth of microbes, which leads to an increase in pH [26]. The pH of the fish after death depends on the type of fish, the season, and the type of fishing. Physiological conditions, stress, and activity level before death, or both, can have an effect on the speed and development of self-changes. As a result, the pH rate of the fish muscles after death will be affected [27,28]. In this research, fresh fish were used to conduct the experiment. Due to the reduction of microbial contamination of these fish, the pH did not increase significantly for the samples in this experiment. The results obtained from this research showed that the pH values for different treatments had a difference during the storage period, and the lowest values were for the treatment of fish cultured in earthen ponds. It was also noted that This variation was not significant between different treatments in pH and sometimes the pH was irregular during the cold storage period [29,22].

Fish meat is characterized by its high content of non-protein nitrogenous compounds. Natural fish enzymes cause analytical changes that increase the food sources of bacteria that contaminate fish. These compounds include amines, amino acids, and glucose. Bacteria represent these compounds and they are a source of their activity and reproduction, so their numbers increase, Das et al. [30] observed similar increasing trend of Total plate count and Total psychrophilic count while studying on chevon nuggets in frozen storage. However, both the Total Plate Count and Total Psychrophilic Count values of fish muscles had not exceeded the permissible limit up to 10th day of storage, i.e. log 106 cfu g1 of sample for Total plate count [31] and 4.6 log cfu g1 for Total psychrophilic count values as reported by Cremer and Chipley [32]. But when coliform counts and yeast counts were concerned, it might be advisable to the consumers that fresh hot chilled fish may be consumed preferably up to 7th day to a maximum of 10th day's refrigerated storage to ensure microbial safety.

Comparing and examining the results of this research between different treatments for carp fish did not show significant differences between the quality factors of fish fillets during the cold storage period in the refrigerator. In general, the treatment of fish farmed in earthen ponds showed greater damage compared to the other two treatments during the storage period. The comparison between river fish fillets and fish farmed in river cages did not show that one treatment was better than the other during the cold storage period. In this field, more studies and research should be conducted on different types of fish, changing environments and diverse nutrition so that we can issue a more accurate judgment by comparing the results of different studies.

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