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Research Article

Evaluation of Freshness of Farmed Two-Year-Old *Acipenser persicus* during Storage in Ice

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Chilled fish is considered a fresh product and helps to ensure the development of world trade. So, the present study aimed to investigate the freshness of two-year-old farmed *A. persicus* by chemical, microbial, sensory, and physical experiments, and determine its shelf life during ice storage. 20 fish samples were maintained in chilled seawater tanks for 12 days. The ratio of ice powder to fish is of 2 : 1. The fish were separated by layers of ice with a thickness of about 5 cm. The temperature was maintained throughout the storage period at 0-1°C. The fish had an average weight and length of 3.50 kg and 70 cm, respectively. Total bacterial counts, *Staphylococcus*, and Enterobacteriaceae were acceptable (6.78, 2.96, and 3.99 log CFU·g^{-1}, respectively) for 10 days. Coliform, *Escherichia coli*, *Pseudomonas aeruginosa, Vibrio parahaemolyticus*, and *Aeromonas hydrophila* were not detected during storage. pH (6.89), peroxide value (4.32 meq O_2 kg oil^{-1}), thiobarbituric acid (0.96 mg·kg^{-1}), free fatty acids (10.86 g 100 g^{-1}), and total volatile nitrogen bases (19.83 mg 100 g^{-1}) were normal during 10 days. Texture (2.56), odor (2.50), the appearance of gills (2.59), eyes (2.67), belly cavity (2.58), skin (2.49), and outer surface (2.90) of *A. persicus* displayed a decrease throughout storage ($p < 0.05$). Sensory characteristics were acceptable for 10 days. Ash was 1.88–2.13% during storage ($p > 0.05$). Unlike moisture (79.05–81.06%), fat and protein showed a decrease of 1.16–2.33 and 15.65–16.74%, respectively, during storage ($p < 0.05$). According to the results of the present study, to maintain the economic value of sturgeon and for the expansion of their business, and to preserve the freshness of sturgeon during handling and the effect of freshness on the consumer's opinion, it is recommended that fisheries use ice for sturgeon transport for 10 days.

1. Introduction

In 2020, live, fresh, or chilled aquatic food continued to account for the largest share of fisheries and aquaculture production utilized for direct human consumption (44%), and it often represents the most preferred and highly priced form of fisheries and aquaculture products [1]. Ice can be an effective method of chilling and preserving fish [2]. The use of ice has various advantages, including the energy requirement of 385 kJ·kg^{-1} to melt at 0°C, heat absorption from the environment, very high cooling capacity, and temperature maintenance. The use of ice also possesses several economic benefits, including its compatibility with relatively small machinery installed in fishing vessels, low production costs, ease of transportation, and its ability to be stored on fishing vessels [3].

Sturgeons are among the most valuable aquatic species in the world and six (*Huso huso*, *A. gueldenstaedtii*, *A. persicus*, *A. stellatus*, *A. nudiventris*, and *A. ruthenus*) of the 27 sturgeon species are found in the Caspian Sea and its drainage basin. Sturgeons are one of the world's oldest fish families and are thought to have been around for 250 million years, and have been caught for centuries for their flesh and eggs, which when processed and salted and marketed as caviar, are the world's most expensive delicacy. Sturgeons are fish species belonging to the Acipenseridae family

originally distributed throughout the Northern Hemisphere. They inhabited mainly the large river systems of the Ponto-Caspian region and the Black, Azov, and Caspian seas [4]. The basic techniques and practices of artificial reproduction of sturgeon were then developed in the 1940s and 50s by Stroganov [5]. Only in recent decades, specific farming techniques for sturgeons have been established, and under the strong pressure of market demand for caviar, the sturgeon farming industry has increased, so that, since the end of the 20th century, it has been the fastest-growing aquaculture sector [4]. So far, more than 116 active and licensed farms have been built in Iran, which have a total production capacity of 6332 t of meat and 95 t of caviar [6]. Nowadays, sturgeon farming exceeds fisheries, with China as the worldwide leader (104.280 t of sturgeon biomass production in 2020), followed by Russia (4.836 t), Armenia (4.200 t), and Iran (2.640 t) [7].

Sturgeons and especially the commercial species of the Caspian Sea have undergone a dramatic decline. This was caused mainly by legal and illegal overfishing, habitat deterioration, and river fragmentation including damming [8], and pollution [9], their caviar, and the failure to manage the caviar trade [8]. These species are now protected in all range states with legal fisheries for limited quantities in only some countries while being listed in Annex I and II of the Convention on International Trade in Endangered Species regulations [10]. Despite the increased protection efforts, their status continuously decreased [11].

However, the history of the mass reproduction of fish in Iran has more than 40 years of history. But more than two decades have not passed since the life of sturgeon breeding in breeding environments. Tasmanian breeding started in 1990 at Shahid Beheshti Fish Breeding Complex for the first time [6]. Sturgeon farming started to partially substitute the production from fisheries. Although some market sectors were still demanding products from wild sources, gradually the sturgeon from farmed sources gained similar acceptance as the product from wild origin was no longer available. Trends in global sturgeon production and trade have repeatedly been presented since the 1990s [11]. Consequently, sturgeon aquaculture has developed to cope with the increasing demand and to reduce the pressure on wild sturgeon [9].

The production of meat from sturgeon has grown commercially in many countries. Gradually, the mandatory protection of the wild population shifted the production of sturgeon toward aquaculture [9]. The farmed sturgeon species in Iran are Siberian sturgeon (*A. baerii*), *A. persicus, A. ruthenus*, Stellate sturgeon (*A. stellatus*), and Beluga (*H. huso*) [6]. The cultivation of these species increased from 2146 to 3144 t during 2017–2022. Sturgeon are bred in 20 provinces of Iran, but in 2021, Mazandaran (2064 t) and Guilan (597 t) accounted for the largest amount of breeding of this fish [12]. Meat of these species are known to have different market values. The chemical quality of meat from different sturgeon species of different ages and weights has been investigated during past years. Researchers found that the meat of farmed sturgeons contained variable amounts of lipid, protein, and fatty acids including omega-3 fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid. Sturgeons often have good nutritional value and health-promoting potential [4]. Considering the benefits of ice and the economic value of sturgeon, no research has been done on the freshness and shelf life of these fish in ice. Theodore's present study was conducted to examine the freshness and shelf life of *A. persicus* during ice storage.

2. Materials and Methods

To conduct the present study, a total of 20 two-year-old *A. persicus* fish were provided from the Shahid Beheshti Sturgeon Breeding Center. The fish were transferred to chilled seawater tanks that were supplied with ice powder formulated using drinking water, at a ratio of twice the weight of the fish, for appropriate storage conditions. Subsequently, a layer of ice powder was incorporated between the fish with a thickness of 5 cm. The ratio of ice to fish was maintained at 2 : 1 to ensure optimal conditions for the preservation of the fish. Every day, some ice was added to the samples to keep the temperature of the fish at 0-1°C. For twelve days, samples were collected at predetermined intervals of 48 h. The evaluation of sample quality during the storage duration was conducted via an array of analytical techniques such as heavy metals, microbial, chemical, physical, and sensory examinations. The period of shelf life for the samples was determined based on the outcome of these tests [13].

2.1. Determination of Heavy Metals. Arsenic, lead, cadmium, mercury, bismuth, antimony, tin, molybdenum, copper, chromium, cobalt, and nickel were determined by the acid chemical digestion. 50 ml of 6 M hydrochloric acid was added to the 20 g of ash. The flask was placed in a water bath. 0.1 M nitric acid (30 ml) was added to it. The sample was laid in a laboratory water bath (Memmert, Germany) for 15 min. The sample was covered by aluminum foil and was put at 25°C for 2 h. The contents of the flask were stirred using a glass rod. The sample was filtered and followed by cooling and transferring to another flask, was added deionized 2-fold distilled water to it. The sample was homogenized with shaking. The light absorption of heavy metals was measured by using an optical atomic absorption spectrometer with a wavelength of 390–410 nm and a graphite furnace (Japan/ZA3700). 0.15–1.30 ppm and 80–107% are the measuring range and the recycling percentage of the spectrometer. 0.02–0.01 $mg \cdot kg^{-1}$ was determined as the limit of value quantitation [14, 15].

2.2. Microbial Examination. In this regard, a sample of 25 g was utilized to analyze the total bacterial counts, *Staphylococcus* spp., *P. aeruginosa*, *A. hydrophila*, and Enterobacteriaceae counts, while a sample of 50 g was employed to study the counts of coliform, *E. coli,* and *V. parahaemolyticus*.

The samples were homogenized with 9-fold physiological serum. Total bacterial counts were qualified using the pour plate method. While *Staphylococcus*, *Vibrio*,

Aeromonas, and *Pseudomonas* were cultured by the surface method, Enterobacteriaceae, coliform, and *E. coli* were cultured by the double-layer plate technique. Total bacterial counts were cultured by the pour plate method. Total bacterial counts and counts of *Staphylococcus, Pseudomonas*, *Enterobacteriaceae*, coliform, *E. coli*, *V. parahaemolyticus*, and *A. hydrophila* were cultured using Plate Count Agar, Mannitol Salt Agar, Cetrimide Agar, Violet Red Bile Dextrose Agar, Violet Red Bile Agar, Sorbitol MacConkey Agar, Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS), and Ampicillin Dextrin Agar, respectively. Ryan's Ampicillin Agar was also used for *A. hydrophila*. A 10^{-1} dilution was used to culture *P. aeruginosa,* coliform, *E. coli*, *Aeromonas,* and *Vibrio* whereas total bacterial counts were cultured by 10^{-2} and 10^{-3} dilutions. These bacteria were incubated in the air. Furthermore, Enterobacteriaceae and *Staphylococcus* were cultured by 10^{-1}-10^{-3} dilutions. For culturing procedures, 1 ml of each dilution was cultured on the respective specific media, followed by incubation of the plates in an incubator at a temperature of 37°C for 48 h [16–24].

Vibrio and *Aeromonas* samples were enriched before cultivation. To enrich the *Vibrio*, two suspensions were prepared from the sample. To prepare the first suspension, 25 g of the sample was mixed with 225 ml of Polymyxin Salted Broth enrichment medium (10^{-1} dilution). To prepare the second suspension, 25 g of the sample was mixed with 225 ml of saline-alkaline peptone water. Two suspensions were kept for 7-8 h in an incubator at 35–37°C. A loop from suspensions was transferred to TCBS and Tryptic soy agar-magnesium sulfate-3% NaCl culture mediums [20].

An alkaline peptone water with pH 8.7 was used for *Aeromonas*. 225 ml of this medium was added to 25 g of sample. This suspension was placed in an incubator (Memmert, Germany) at 37°C for 18–24 h. Plates with 20–200 colonies of growth incorporated by iodine-potassium iodide solution. Colonies with a clear halo on a purple-red background were selected for monitoring [23].

2.3. Chemical Examination. In terms of investigating the chemical characteristics, the peroxide value, thiobarbituric acid reactive substances value (TBARS), free fatty acids value (FFA), and total volatile base-nitrogen value (TVB-N) were determined using iodometric titration, colorimetric, titration, and distillation methods, respectively [25].

2.4. TVB-N. The sample (100 g) was blended with 300 ml trichloroacetic acid. It was centrifuged (C7000 model of Centoriun UK company) to obtain a clear extract. 5 ml of the extract was transferred into the Glass Markham apparatus. 5 ml of 2 N NaOH was added to it. Steam was collected into 15 ml standard 0.01 N HCl containing 0.1 ml Rosolic acid indicator. Titration was conducted using standard 0.01 N NaOH until the color changed to pink [25].

2.5. TBARS Value. 10 g of the minced sample was macerated with 50 ml water for 2 min and then was transferred to a distillation flask, using 47.5 ml water for washing. 2.5 ml of 4 N HCl (pH should be 1.5), antifoam, and a few glass beads were added to it. 50 ml of distillate was collected in 10 min from the time boiling commences. 5 ml of the distillate was pipetted into a glass-stoppered tube. 5 ml of the TBA reagent was added to it. Then it was shaken and heated in boiling water for 35 min. A blank was prepared similarly, using 5 ml water, for 35 min. The sample and blank tubes cooled and was measured the absorbance of the sample against the blank at 538 nm using 1 cm cells [25].

2.6. FFA. 20 ml of fat filtrate was transferred into a 250 ml conical flask. 20 ml of neutralized ethanol was added to it. Titration was conducted using 0.02 N sodium hydroxide solution. Phenolphthalein was an indicator. The sample was shaken vigorously during the titration [25].

2.7. Peroxide Value. The filtrate (20 ml) was transferred into a 250 ml glass-stoppered Erlenmeyer flask. 15 ml of glacial acetic acid and 0.5 ml of saturated potassium iodide were added to it. This solution shacked for 1 min. Then, 30 ml of distilled water was added to it. Titration was conducted with 0.01 N sodium thiosulphate. The titration continued until the yellow color had almost disappeared. 0.5 ml of starch solution was added as an indicator. The thiosulphate was added dropwise until the blue color disappeared. The blank was 0.5 ml of the 0.01 N sodium thiosulphate solution [25].

2.8. Physical Examination (pH). The pH was measured by the electrometric method. First, the pH meter (AZ Taiwan) was calibrated with pH values of 4 and 6.88. 5 g of the ground sample was homogenized with 50 ml of distilled water. Then, the pH was obtained by placing the electrodes inside this solution [25].

2.9. Sensory Examination. The sensory evaluation was descriptive and conducted by the scoring method utilizing a 7-level method, where sensory attributes such as the gill's appearance, the outer surface, texture, skin, belly cavity, and eyes were evaluated by a trained panel including 30 evaluators (15 male and 15 female participants aged 30–40 years). Sensory tests were performed at 7 stages. The first stage was conducted after catch, and the other stage was performed during the storage period in ice (2, 4, 6, 8, 10, and 12 days). These tests were performed in triplicates at each sampling time. The samples were assessed by a scoring range of 0-1, 1-2, 2-<3, and 3, for good, average, poor, and reject quality, respectively (Table 1) [26].

2.10. Biometric Assay. The piscine subjects were subjected to biometric assessment. The measurement of weight was accomplished using a scale (AND Germany) with a precision of 1 g. However, subjects weighing less than 100 g were measured with a more accurate scale, having a precision of 0.1 g. On the other hand, the length measurements were performed using a caliper or ruler with an accuracy of 1 mm, while in the case of subjects weighing less than 100 g, a more accurate caliper having an accuracy of 0.01 mm was utilized [27].

Table 1: Sensory characteristics for evaluation of whole sturgeon during storage in ice.

Odor of gills	Appearance of gills	Outer surface	Texture	Skin (damage)	Belly cavity	Eyes
Fresh, characteristic, neutral (0-1) Slightly sour and slightly stale (1–<3) Definite spoilage and putrid (3)	Color: bright red or pink (0-1), beached (1–<3), discolored (3) Mucus: clear (0-1), opaque (1–<3), discolored (3)	bright (0-1), dull (1–<3), bleached (3)	Smooth, gritty, flesh, firm (0–<3), soft (3)	none (0-1), punctures (1–<3), abrasions (3)	Guts: intact, abdomen is relatively bulging and the anus is colorless and indented (0–<3), the muscles of the anus become inflexible and loose, and the color becomes red. The abdomen becomes depressed and tears with the least pressure, and the smell of putrefaction comes out (3), belly walls: bright, clean (0–<3), discolored (3), blood: bright, red (0–<3), brown (3)	Shape: convex (0-<1), flat (1–<3), concave (3) Brightness: clear (0-1), cloudy (1–3) Color: normal (0–<3), cloudy (3)

2.11. Nutritional Value. Nutritional assessments including protein, fat, moisture, and ash were determined by *Macro* Kjeldahl, acid hydrolysis, dry oven, and gravimetric methods, respectively [28]. These tests were performed in 7 stages. The first stage was conducted after of catch, and the other stage was performed during the storage period in ice (2, 4, 6, 8, 10, and 12 days).

2.12. Protein. Macro Kjeldahl (Gerhardt-Behr, Germany) was used for protein measurement. For the digestion of the samples, 2 g of the sample plus 8 g of catalyst and 25 ml of concentrated sulfuric acid were transferred to the digestion balloon. In the next step, the clear and greenish liquid was distilled along with two-thirds of the volume of the distilled water flask and some boiling stones. The vapors were collected in an Erlenmeyer flask containing 50 ml of 2% boric acid with 3-4 drops of bromocrosol as a reagent, and titration was performed with 0.1 N sulfuric acid [28].

2.13. Fat. Fat was measured by the acid hydrolysis method. To determine fat, 50 ml of 4 N hydrochloric acid was added to 5 g of homogenized fish sample. After being placed for 1 h in a bain-marie at a temperature of 80°C, it was smoothed using Whatman paper. In the next step, the filter paper was transferred to the Soxhlet extractor (Behr-AK5, Germany). After connecting the balloon with a certain weight, two-thirds of its volume was filled with solvent (hexane) and hydrolysis took place for 6–8 h [28].

2.14. Ash. Ash was measured by the gravimetric determination method. To measure ash, a clean cruise was placed in a furnace (Fine tech, Korea) with a temperature of 600°C for 1 h. The cooled cruise was transferred to a desiccator and cooled to room temperature. Then, they were weighed quickly and with 10 g of wet sample placed for 12–18 h at a temperature of 550°C in an electric oven (Fine Tech, Korea). After this period and cooling, the cruise was weighed and the ash content was calculated [28].

2.15. Moisture. Moisture was determined by the dry oven method. To determine the moisture content of the sample, 10 g of fish were placed in a Petri dish with a certain weight and placed in an oven (Memmert, Germany) at a temperature of 100°C. It was placed in a desiccator for 1 h [28].

2.16. Data Analysis. Results were analyzed using the SPSS version 25 software. Additionally, the changes undergone during the storage period were studied by conducting a two-way analysis of variance, utilizing a significance level of 5%.

3. Results

According to the results, the average weight and length of the fish were found to be 3.50 ± 1.78 kg and 70 ± 1.34 cm, respectively.

In Table 2, it can be observed that significant increases ($p < 0.05$) were recorded in the levels of TVB-N, TBARS, and FFA, during the storage period. However, no significant changes were noted in the pH levels from the day of catch to day eight ($p > 0.05$) and during 10 days until 12 days ($p > 0.05$), or on the eighth day compared to the tenth day ($p > 0.05$). The level of peroxide increased from day one to day eight and decreased from day ten to the 12^{th} day. However, it was not significant during the eighth, tenth, and twelfth days ($p > 0.05$). It was observed that the obtained chemical factors within 10 days of storage for cultured fish maintained in ice were within the acceptable range (according to the results).

Results presented in Table 3 indicated that no contamination by *P. aeruginosa*, *A. hydrophila, V. parahaemolyticus,* coliform, and *E. coli* bacteria was detected in the samples during the storage period within the ice ($<10\ \text{CFU}\cdot\text{g}^{-1}$). The microbial population showed a significant increase ($p < 0.05$) between the day after catching and the tenth day of storage in ice. Despite this increase, the microbial agents were still within acceptable limits during 10 days of ice storage.

With an increase in the score of sensory characteristics such as odor and appearance of gills, texture, skin damage, and those of the outer surface, belly cavity, and eyes, fish declined when stored in an ice storage environment (3 at the end of the storage period). The observational results highlight that, during a period spanning from the day of initial capture up to six days of storage in ice, the fish's sensory characteristics were deemed to be of good quality. However, by the 8th day of storage, the quality had become average, while reaching a score of 2-3 on the 10th day indicated that the quality was poor. Finally, a score of 3 recorded on the 12th day demonstrated that the fish had lost its acceptable sensory qualities, as illustrated in Table 4.

Table 5 revealed that the moisture content increased at the end of the storage period. However, it is important to note that these differences were not statistically significant ($p > 0.05$), while other nutritional value factors exhibited some changes with nonsignificance ($p > 0.05$).

As can be seen in Table 6, the amounts of heavy metals in the edible tissue of *A. persicus* at the beginning and end of the storage time in ice were within the acceptable range according to the FDA.

4. Discussion

Inadequate storage conditions can lead to a rapid decrease in the quality as well as the nutritional value of fish fillets and their fats. Nevertheless, chilling fish is one of the preservation methods that can effectively extend the shelf life of fish by delaying the spoilage processes in the form of chemical, microbial, physical, and sensory changes [29].

As indicated in Table 2, the levels of peroxide value and TBARS significantly increased in fish when stored in ice. Such changes in the fish's fat content are crucial in indicating a decline in the overall quality of the fish. The increase in peroxide value and TBARS, as shown in Table 2, is attributed to the formation of FFA as a result of fat hydrolysis. Unsaturated fatty acids are susceptible to oxidation, and when separated from triglycerides, they are 3-4 times more

Table 2: Chemical changes of farmed *A. persicus* during storage in ice for twelve days.

Index Sampling time (d)	pH	FFA (g $100\,g^{-1}$)	TBARS (mg kg^{-1})	Peroxide (meq O_2 kg oil^{-1})	TVB-N (mg $100\,g^{-1}$)
After catch	6.17 ± 1.25^{c}	2.07 ± 1.16^{g}	0.08 ± 0.17^{e}	0.11 ± 0.12^{e}	11.48 ± 1.16^{g}
2	6.26 ± 1.14^{c}	3.34 ± 1.38^{f}	$0.49 \pm 0.24d^{e}$	1.63 ± 0.35^{d}	12.87 ± 1.51^{f}
4	6.39 ± 1.26^{c}	5.46 ± 1.59^{e}	0.66 ± 0.19^{cd}	2.96 ± 0.71^{c}	14.28 ± 1.27^{e}
6	6.41 ± 1.47^{c}	7.69 ± 1.92^{d}	0.78 ± 0.25^{bc}	4.98 ± 0.68^{a}	16.87 ± 1.32^{d}
8	6.78 ± 1.58^{bc}	9.85 ± 1.94^{c}	0.89 ± 0.14^{b}	4.49 ± 0.57^{ab}	17.98 ± 1.49^{c}
10	6.89 ± 1.52^{ab}	10.86 ± 1.83^{b}	0.96 ± 0.18^{a}	4.32 ± 0.69^{b}	19.83 ± 2.18^{b}
12	7.23 ± 1.72^{a}	12.63 ± 2.67^{a}	1.39 ± 0.67^{a}	4.14 ± 0.54^{b}	25.54 ± 2.37^{a}

The same letters in the same column indicate no significant difference ($p > 0.05$).

Table 3: Microbial changes of farmed *A. persicus* during storage in ice for twelve days (log $CFU{\cdot}g^{-1}$).

Characteristics Sampling time (d)	Enterobacteriaceae	*Staphylococcus*	Total bacterial counts
After catch	1.72 ± 0.46^{e}	1.11 ± 0.63^{f}	2.11 ± 0.82^{g}
2	2.19 ± 0.73^{e}	1.49 ± 0.76^{ef}	2.79 ± 0.19^{f}
4	2.71 ± 0.64^{d}	1.76 ± 0.59^{de}	3.71 ± 0.18^{e}
6	3.18 ± 0.95^{cd}	1.98 ± 0.81^{d}	4.68 ± 0.56^{d}
8	3.47 ± 0.94^{c}	2.48 ± 0.91^{c}	5.47 ± 0.62^{c}
10	3.99 ± 0.47^{b}	2.96 ± 0.99^{b}	6.78 ± 0.28^{b}
12	5.23 ± 0.99^{a}	4.53 ± 1.24^{a}	7.93 ± 0.78^{a}

The same letters in the same column indicate no significant difference ($p > 0.05$).

Table 4: Sensory qualities of farmed *A. persicus* during storage in ice for twelve days.

Characteristics Sampling time (d)	Belly cavity	Eyes	Outer surface	Gills appearance	Odor of gills	Skin	Texture
After of catch	0.00 ± 0.02^{e}	0.00 ± 0.05^{e}	0.00 ± 0.01^{e}	0.00 ± 0.03^{e}	0.00 ± 0.06^{e}	0.00 ± 0.09^{e}	0.00 ± 0.08^{e}
2	0.00 ± 0.04^{e}	0.00 ± 0.03^{e}	0.00 ± 0.06^{e}	0.00 ± 0.01^{e}	0.00 ± 0.05^{e}	0.00 ± 0.07^{e}	0.00 ± 0.02^{e}
4	0.00 ± 0.05^{e}	0.00 ± 0.09^{e}	0.00 ± 0.04^{e}	0.00 ± 0.02^{e}	0.00 ± 0.07^{e}	0.00 ± 0.01^{e}	0.00 ± 0.09^{e}
6	0.73 ± 0.41^{d}	0.76 ± 0.44^{d}	0.49 ± 0.59^{d}	0.73 ± 0.19^{d}	0.67 ± 0.21^{d}	0.57 ± 0.24^{d}	0.53 ± 0.47^{d}
8	1.62 ± 0.71^{c}	1.56 ± 0.54^{c}	1.74 ± 0.75^{c}	1.42 ± 0.25^{c}	1.69 ± 0.39^{c}	1.63 ± 0.37^{c}	1.52 ± 0.61^{c}
10	2.58 ± 0.88^{b}	2.67 ± 0.75^{b}	2.39 ± 0.51^{b}	2.59 ± 0.49^{b}	2.50 ± 0.98^{b}	2.49 ± 0.99^{b}	2.56 ± 0.68^{b}
12	3.00 ± 0.86^{a}	3.00 ± 0.79^{a}	3.00 ± 0.84^{a}	3.00 ± 0.37^{a}	3.00 ± 0.94^{a}	3.00 ± 0.97^{a}	3.00 ± 0.45^{a}

The same letters in the same column indicate no significant difference ($p > 0.05$).

susceptible to oxidation than when bound to them. The oxidation process for unsaturated fatty acids occurs via an autocatalytic mechanism that is relatively faster in dead tissue than in living tissue. Moreover, the presence of molecules within the muscles that intensify oxidation creates a suitable environment for fat damage and an increase in peroxide levels during storage. The storage of fish in ice can allow oxygen to penetrate the tissue, thereby facilitating the oxidation of unsaturated fats, and leading to the production of peroxide. The activity of tissue lipase enzyme, as well as the presence of *Staphylococcus* bacteria, are additional factors that can contribute to an increase in peroxide content [30, 31]. It is worth noting that peroxide is an unstable substance and breaks down during storage, eventually transforming into TBARS, which is a secondary by-product of fat oxidation. Therefore, the increase in this factor has also been observed during the storage time. As the maximum permissible levels of peroxide and TBARS are 1 $mg{\cdot}kg^{-1}$ and 5 meq O_2 kg oil^{-1} [30], respectively, in this study, TBARS and peroxide values were 0.08–1.39 $mg{\cdot}kg^{-1}$ and 0.11–4.14 meq O_2 kg oil^{-1} in thought storage period that seemed to be acceptable on the 10th day of keeping. Khodanazary and Pourashouri determined TBARS in whole salted fish by 4 and 20.5 $mg{\cdot}kg^{-1}$ after 9 and 12 days of storage in ice, indicating an increase compared to the results of the present study [32]. Mian et al. investigated the effect of ozonized ice on the chemical quality of Indian mackerel (*Rastrelliger kanagurta*) muscle during storage in ice and observed that peroxide reached 7.52 meq·O_2 kg oil^{-1} after 16 days of storage [33], indicating a higher value compared to the findings of the present study. Furthermore, Soheilnaghshi et al. investigated the quality of fat in silver carp (*Molitrix hypophthalmichthys*) during storage in ice and observed that the peroxide level was 8.81 and 12.15 meq O_2

Table 5: Nutritional value changes of farmed *A. persicus* during storage in ice for twelve days (%).

Index Time storage (d)	Ash	Moisture	Fat	Protein
After catch	1.88 ± 1.57^{a}	79.05 ± 1.96^{e}	2.33 ± 1.37^{a}	16.74 ± 1.72^{a}
2	1.89 ± 1.59^{a}	79.26 ± 1.36^{de}	2.23 ± 1.38^{a}	16.62 ± 1.70^{a}
4	1.91 ± 1.47^{a}	79.59 ± 1.58^{d}	2.06 ± 1.49^{ab}	16.44 ± 1.64^{a}
6	1.93 ± 1.34^{a}	80.20 ± 1.67^{c}	1.69 ± 1.53^{bc}	16.18 ± 1.62^{ab}
8	1.98 ± 1.23^{a}	80.43 ± 1.39^{bc}	1.54 ± 1.14^{c}	16.05 ± 1.59^{b}
10	2.05 ± 1.17^{a}	80.75 ± 1.42^{ab}	1.37 ± 1.12^{cd}	15.83 ± 1.39^{bc}
12	2.13 ± 1.13^{a}	81.06 ± 1.78^{a}	1.16 ± 1.09^{d}	15.65 ± 1.83^{c}

The same letters in the same column indicate no significant difference ($p > 0.05$).

Table 6: The results of heavy metals of farmed *A. persicus* during storage in ice for twelve days (ppm).

Sampling time (day) Index	1	12	FDA range
Arsenic	0.08 ± 0.02^{A}	0.09 ± 0.04^{A}	3
Chrome	0.06 ± 0.05^{A}	0.07 ± 0.04^{A}	50
Cobalt	0.05 ± 0.03^{A}	0.08 ± 0.05^{A}	5
Mercury	0.13 ± 0.07^{A}	0.15 ± 0.09^{A}	1
Bismuth	0.14 ± 0.06^{A}	0.14 ± 0.08^{A}	ND
Tin	0.06 ± 0.02^{A}	0.08 ± 0.05^{A}	ND
Antimony	0.13 ± 0.05^{A}	0.14 ± 0.04^{A}	ND
Copper	0.08 ± 0.06^{A}	0.09 ± 0.05^{A}	ND
Molybdenum	0.02 ± 0.01^{A}	0.02 ± 0.03^{A}	ND
Cadmium	0.06 ± 0.04^{A}	0.07 ± 0.06^{A}	0.5
Lead	0.03 ± 0.01^{A}	0.03 ± 0.02^{A}	10
Nickel	0.10 ± 0.08^{A}	0.10 ± 0.07^{A}	0.2

ND: not determined. The same letters in the same column indicate no significant difference ($p > 0.05$).

kg oil^{-1} on the 12th and 16th days [34], respectively. Also, the TBARS content was determined as 3 mg·kg^{-1} after 16 days of storage, which was higher than the values obtained in the present study. The issue of nonconformity in fish storage and preservation can be attributed to various factors, including the fish species, the presence of unsaturated fatty acids, the initial quality of the fish, and the methods employed in preserving the quality of the fish during storage in ice. Sharifian et al. determined TBARS content by 2.30 mg·kg^{-1} in *Otolithes ruber* fish for 15 days which was stored in ice powder [35]. These results were consistent with the present study's findings. Similarly, Mousavi et al. investigated the storage time of *Luciobarbus xanthopterus* in ice and found that TBARS increased over the storage period [36], which was in line with the results of the present study. Aberoumand and Baesi, in a study of the peroxide measuring of *Siganus javus* kept in ice discovered that the peroxide value attained the lowest value on the tenth day of storage [37]. Additionally, the spoilage indices were observed to be altered throughout the preservation period in ice, which corresponds to the outcomes attained in the present study.

According to the results presented in Table 2, it is evident that the TVB-N levels in the farmed *A. persicus* exhibited significant changes throughout its preservation in ice. Numerous volatile compounds, such as ammonia, methylamine, dimethylamine, and trimethylamine, among others, are commonly used to assess the quality of fish meat, as they are indicative of bacterial metabolic activity and subsequent spoilage [38, 39]. Table 1 revealed that the TVB-N reached 19.83 mg 100 g^{-1} after 10 days of preservation. Varied sources have reported differing acceptable levels of TVB-N in fish. Nevertheless, according to some researchers, the permissible amount of TVB-N in fish is established at 25 mg 100 g^{-1} [40], suggesting that the levels observed in the present study were within acceptable limits until the tenth day of ice preservation. Mian et al. investigated the effect of ozonized ice on the quality of Indian mackerel muscle during storage in ice and observed that the TVB-N reached 34.40 mg 100 g^{-1} after 16 days of storage [33], which was higher than values obtained in the present study. Soheilnaghshi et al. reported TVB-N by 26.35 and 33.75 mg 100 g^{-1} in silver carp after 12 and 16 days of preservation in ice, respectively [34], which in comparison to the current study, was found to be increased. Moreover, Khodanazary and Pourashouri reported that TVB-N in whole salted fish reached 46.66 mg 100 g^{-1} after 12 days of storage in ice [32], which was higher than our results. In a related study conducted by Mousavi et al. on the preservation of *L. xanthopterus* in ice, it was discovered that the TVB-N levels increased over time, up to 72 h of storage period [36], which is consistent with the outcomes obtained in the present study. It is noted that the TVB-N levels tend to vary depending on the species, gender, fishing location, season, and age of the fish being evaluated. Thus, differing outcomes concerning this factor are evident in various studies [41].

The hydrolysis of glycerides, glycolipids, and phospholipids by lipase enzymes results in the conversion of these compounds into FFA, which subsequently undergo oxidation to produce aldehydes and ketones and impart an unpleasant taste to fish. Hence, the quantification of FFA serves as a reliable means of assessing the impact of lipolysis enzymes on fish fat and other meat products. While existing reports may not directly attribute FFA to a decline in product quality, it is noteworthy that an increase in FFA concentration has been correlated with heightened lipid oxidation, altered protein structure leading to textural changes, and the emergence of an unpleasant taste. Consequently, an increased FFA level can reduce product quality. Therefore, the preservation of the quality of marine products has always been a concern of the food industry due to the presence of these acids and their effects on sensory properties [39, 42]. Table 2 illustrates that the concentration of FFA in farmed *A. persicus* underwent significant

alterations during storage under the ice. Considering that aquatic tissues are typically rich in unsaturated fatty acids, which are more prone to oxidization and the resulting production of FFA, the observed increase in FFA levels during storage was expected, which happened during storage of 10 days (2.07–10.86 g $100\,g^{-1}$). Notably, the allowable limit of free fatty acid in fish is stipulated at 12 g $100\,g^{-1}$ [30]. Accordingly, the presence of FFA in the stored fish samples within the studied timeframe of 10 days seemed to be acceptable. Khodanazary and Pourashouri reported free fatty acid of 15.8% after 12 days of storage in ice [32], which increased compared to the results of the present study (12.63 g $100\,g^{-1}$). The discrepancy could be due to differences in fish species and amounts of unsaturated fatty acids. Mousavi et al. investigated the shelf life of *L. xanthopterus* in ice preservation and found that FFA content increased during the storage of fish in ice [36], which was similar to the results of our study.

According to Table 2, the pH in the farmed *A. persicus* significantly differed during storage in ice (6.17–7.23). In living fish muscles, the pH is usually close to 7. However, after fishing and the completion of life processes, the pH of fish muscles shifts to a range of 6-7 [43], depending on factors such as the fishing season and the species of fish and so on. Within the context of the studied fish, the pH values of stored samples exhibited an increasing trend over the storage period, with values reaching up to 7.23 by the 12th day of storage. These values exceeded the permissible limits, which was unacceptable. This result can be attributed to the expiration of the storage period and the activity of autolytic enzymes and proteolytic bacteria that spoil the fish. Another reason for the observed pH could be the increase in volatile nitrogen bases during the storage period. The findings of the present study regarding the increase in pH values during storage are consistent with those reported by Viji et al., reflecting a similar trend in the change of pH over time [44]. Similar to our findings, Khodanazary and Pourashouri determined the pH of whole salted fish by 6.99 after 12 days of storage in ice [32]. Mian et al. investigated the effect of ozonized ice on the chemical quality of Indian mackerel muscle during storage in ice and observed that the pH value after 16 days of storage was 6.39 [33], which was lower than the values obtained in the present study. Moreover, Mousavi et al. documented an increase in pH during the storage of *L. xanthopterus* in ice [36], which was consistent with our findings. Generally, storing fish in ice and rapid chilling techniques serve to reduce the physical changes that occur during storage. Nevertheless, the alteration in pH values during storage can be influenced by several factors, including initial fish quality, the conditions of storage, the increase in TVB-N during the storage period, and variations in microbial loads. Whereas earlier studies noted that the pH has many changes, some researchers including Khodanazary and Pourashouri reported that pH is not very important for evaluating the quality of whole fish in ice [32].

Microbial spoilage is the main reason for the deterioration in the quality of fresh fish. In this process, microbial activity plays an important role in their shelf life. An aerobic plate count was carried out because of its usefulness as an indicator of spoilage and the shelf life of the product [45]. *V. parahaemolyticus* is a halophilic organism and human pathogen. This bacterium is of widespread occurrence in inshore marine waters, sediments, and marine animals, and it is usually present in significant numbers only on seafood taken from tropical or subtropical waters or from temperate-zone waters in summertime [20]. Enterobacteriaceae is a hygiene indicator organism. Additionally, the food contaminated by this bacterium poses a microbiological risk for consumers [46]. *P. aeruginosa* is a food-poisoning bacterium. It is a common environmental microorganism and can be found in soil, water, and sewage. It can multiply in water environments and also on the surface of suitable organic materials in contact with water such as fish storage in ice [47]. *Staphylococcus* is the natural flora of human skin and is an indicator of hand contamination, but coliform and *E. coli* are indicators of water contamination with sewage. Therefore, they are easily transferred to the product during the processing, and hence they were investigated in the present study [3]. The *A. hydrophila* group includes *A. hydrophila*, *A. cavie*, *A. veronii biovar,* and *A. serbia* species. *A. hydrophila* is a widespread representative of *Aeromonas* found in water, surface water, freshwater, groundwater, chlorinated drinking water, nonchlorinated drinking water, bottled mineral water, and foods such as fish. The microorganism has the potential to be a foodborne pathogen. This group can grow at 37°C and is important in terms of public health and human health. They are found in the intestinal tract of humans and animals, soil, sewage-contaminated waters, and so on [48, 49]. Considering that rivers and groundwater are used to breed sturgeon, the presence of this bacterium in the studied fish is not far from expected. According to Table 3, the total bacterial counts, Enterobacteriaceae, and *Staphylococcus* bacteria significantly increased in two-year *A. persicus* during storage in ice. But *Aeromonas, V. parahaemolyticus,* and *P. aeruginosa* were not observed in the present study. *Aeromonas* can grow at a pH of 2.5–9.8 and a temperature of 10–45°C. Therefore in this study, temperature was not suitable for the growth of this bacterium. In several studies, *Aeromonas* bacterium was identified as a fish pathogen [48–51], but no published report of food poisoning caused by this bacterium through fish consumption was found. Temperature of 3–13°C and pH 5.8–7.5 are suitable conditions for the growth of *Vibrio* bacteria. Because fresh or groundwater was used to feed the pool (without salt), this bacterium was not observed in the studied species [52]. The use of temperature reduction methods, such as ice storage, is widely employed to preserve the fish quality and maintain product safety in markets worldwide. Since the temperature drops to the melting point of ice (zero °C) during storage; therefore, ice leads to an increase in the shelf life of fish by reducing the action of enzymes and bacteria. While ice is effective at preserving the quality of fish during storage, it is considered a short-term preservation method. Studies have shown that ice is not a suitable option for long-term storage of fish [53, 54]. The acceptable values for total bacterial counts, *Staphylococcus* bacteria and Enterobacteriaceae were

7, 3, and 4log CFU·g^{-1}, respectively [55]. Furthermore, the values for the same bacteria were 2.11–6.78, 1.11–2.96, and 1.71–3.99 log CFU·g^{-1}, respectively, during 10 days of storage in ice. Therefore, in the present study, the two-year-old *A. persicus* had favorable microbial characteristics during 10 days of storage in ice. Melting ice water can moisten the surface of fish, which can create favorable conditions for bacterial growth. However, the same phenomenon also washes away surface bacteria. Therefore, the rate of bacterial growth during storage in ice is not significant, ultimately contributing to the preservation of fish microbial quality during storage [31]. Several factors contribute to maintaining fish quality during storage in ice, including prevention of surface heating, acceleration of bacterial interactions, and delay of spoilage. In the present study, these factors were successfully addressed by continuously adding ice to the storage environment. Khodanazary and Pourashouri determined the number of psychrophilic bacteria in whole salted fish at 7.33 log CFU·g^{-1} after 9 days of ice preservation [32], which was not inconsistent with the results of our study. Mousavi et al. studied the shelf life of *L. xanthopterus* in ice and found that psychrophilic bacteria did not grow during the storage period [36], which was in agreement with the results of the present study. They also reported the number of mesophilic bacteria from 1.53 to 72 log CFU·g^{-1} at zero time until 72 h after storage, which differs from the findings of the present study. Safari et al. identified *V. vulnificus* (biotype 2) from cultured beluga and *H. huso* in Iran [56]. Xiao et al. determined *V. metschnikovii* in freshwater-cultured hybrid sturgeon [52]. Results of these studies were not inconsistent with the results of our study. These differences in several studies can be attributed to the diversity in the size of ice particles used for cooling, the amount and proper contact of ice and fish, storage method, size, shape, and thickness of fish, the rate of microbial spoilage of fish, microbial flora, ambient temperature, water condition, and access on oxygen.

As shown in Table 4, the sensory characteristics of the cultured fish significantly decreased during storage in ice. Accordingly, gill smell (0–2.50) and eye color (0–2.67) increased during 10 days of ice storage. Sensory properties reached a score of 3 at the end of the storage period (12th day), which is not acceptable. The aforementioned factors serve as strong indicators for evaluating the quality of fish during storage in ice, and their importance was further confirmed by the findings of the present study. The activity of internal bacteria within the fish is slowed during ice storage; however, reactions caused by their enzymes can still bring about changes in the appearance, texture, and color of the fish over time. Furthermore, the increase in chemical characteristics, including TBARS, is considered another effective factor in the sensory characteristics and smell of fish [32]. Mousavi et al. found that when *L. xanthopterus* was stored in ice [36], the sensory characteristics of the fish maintained satisfactory and acceptable quality during ice storage for up to 24 h. However, during the storage period, the acceptability score gradually decreased. Subsequently, most of the sensory indicators scored low over 72 h. In other words, the samples at this hour had the lowest sensory quality during the storage period, which was a lower value compared to the results of the present study. These researchers did not document the ice preservation technique as a suitable method for the long-term storage of *L. xanthopterus*. Khodanazary and Pourashouri found that the sensory attributes of whole salted fish were of satisfactory quality when stored on ice for up to six days, but on the ninth day, the samples were deemed unsuitable for human consumption [18]. However, these results are not aligned with those obtained from the present study. According to the current study, cultured fish exhibited appreciable sensory properties for up to 6 days of ice storage. However, the quality of the fish gradually deteriorated, with the 8th day showing moderate quality, the 10th day demonstrating poor quality, and by the 12th day, the fish was unsuitable for human consumption due to marked sensory degradation. Changes in the fat content, chemical factors such as peroxide and TBARS, and the growth of psychrophilic bacteria and their effect on sensory characteristics are considered effective factors for differences observed in these characteristics.

Table 5 displays notable changes in the nutritional profile of the samples. The moisture content showed a significant increase, rising from 79.05% to 81.06%. Moreover, although the change was not statistically significant, an upward trend was observed in the ash content which increased from 1.88% to 2.13%. Furthermore, a significant decrease was recorded in fat content from 2.33 to 1.16% and in protein content from 16.74 to 15.65%. The increased moisture can be attributed to the absorption of water from the environment. Furthermore, the presence of air in the storage environment can promote the growth and proliferation of microorganisms [57].

Enzymes secreted by proteolytic and lipolytic microorganisms can exacerbate the breakdown of fish protein and fat, leading to consequent reductions in their amounts. However, minerals are not degradable and, as a result, their amounts remained consistent over time, with no significant reductions being observed [57]. Table 4 presented data indicating that *A. persicus* possesses considerable levels of protein, fat, and ash, highlighting its high nutritional value. Hung stated that the protein content of various sturgeon species is 40–45% [58], which is contrary to the results of the present study. It is unclear whether there is a difference in protein requirements among the different sturgeon species or whether the differences in protein requirements are mainly due to the different laboratory methods used in the studies. Additionally, factors such as initial weight, the origin of dietary protein sources, as well as variations in other dietary components, breeding conditions, habitat, and nutrition, may significantly impact the fluctuations of nutritional value [58]. Zareh et al. reported the protein, fat, ash, and moisture contents in cultured *H. huso* were 17.29, 3.10, 1.09, and 79.4% [59], respectively, which are consistent with the results of the present study.

Fish consumption has been considerably increased in Iran. On the other hand, the increase in aquatic ecosystem pollution can cause the accumulation of heavy metals in fish. Therefore, measuring the amount of heavy metals in fish is of great importance for consumer health [60]. According to the

Food and Drug Administration, the amounts of heavy metals in the *A. persicus* were in the acceptable range. Although the proximity of fish breeding ponds to areas agricultural or rural residential areas and the infiltration of agricultural effluents containing chemical fertilizers to groundwater or entrance to river the amounts of heavy metals in farmed *A. persicus* were acceptable. However, environmental conditions are effective in the absorption and accumulation of heavy metals by *A. persicus*. Colloidal, particulate, and dissolved are forms of heavy metals that exist in rivers whose absorption to fish depends on chemistry agents including pH, hardness, temperature of river, and other factors. Nutrition, fat content, and the main organ of accumulation of heavy metals in fish are also important. In water, these metals undergo changes, which affect their behavior and bioavailability. Dissolution ability, river flow, heavy metals concentration, redox potential, regeneration of the river, sedimentation, surface absorption, and complex formation with the other compounds are part of these changes. In addition, the skin of farmed *A. persicus* contains mucous and one of its functions is to prevent the binding of heavy metals to its skin [61]. Hedayatifard et al. conducted a study on the acute accumulation of heavy metals (Cd, Pb, Hg, and Cu) in the fillet, of stellate sturgeons in the Southwest and Southeast coasts of the Caspian Sea [62]. Their results showed that all measured heavy metals were in the standard range. Onara et al. investigated bioaccumulation of heavy metals including Cd, Cu, Zn, Pb, Mn, Fe, and Ni in the muscle of sturgeon species (*A. stellatus, A. gueldenstaedtii*, and *H. huso*) of the North-Western Black Sea and Lower Danube River [63]. In the stellate sturgeon, muscle surpassed the admitted limits for human consumption (Cd: 0.05; Zn: 50; Cu: 5.0; Pb: 0.3 (mg/kg wet weight)). Accumulation of heavy metals in sturgeon tissues varied with sample origin and also physiological and biological peculiarities of the fish.

5. Conclusion

The application of ice storage is recognized as a reliable means of obtaining fresh and uncontaminated *A. persicus*, while also enhancing its shelf life, which is a fundamental factor in determining the economic value of *A. persicus*. Heavy metals were not observed in *A. persicus*. Based on findings of chemical, microbial, sensory, and physical results, physicochemical attributes of the two-year-old *A. persicus* were satisfactory in ice storage for 10 days. However, given the higher priority of sensory characteristics over physicochemical features and the unfavorable rating of sensory factors on the 12th day of storage, the shelf life of the mentioned *A. persicus* stored in ice was 10 days. Overall, it can be concluded that it is possible to provide fresh sturgeon with ice keeping. Therefore, according to the economic value of sturgeon and the expansion of their business, to preserve the freshness of this fish during handling and the effect of freshness on the consumer's opinion, it is recommended that fisheries use ice to transport it. However, because the present study was conducted on the two-year-old cultured *A. persicus*, for the handling of sturgeon through storage in ice during trade, it is necessary to investigate the present study on all of species of sturgeon at different ages. It should also be checked on wild species.

Data Availability

This article has no additional information to share.

Ethical Approval

I was not involved in fish farming, catching, and slaughtering. But based on the fact that the fish were taken from the official and legal center for the breeding of sturgeons, animal rights were respected during breeding, catching, and slaughtering.

Conflicts of Interest

The authors declare that they have no conflicts of interest in the implementation of this project.

Authors' Contributions

Mina Seifzadeh processed the experiment and wrote the manuscript. Ali Raoufi collaborated in the writing of the manuscript.

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