

Evaluation of Chemical, Microbial, Sensory, and Shelf Life Characteristics of Full Farmed *Acipenser persicus* During Storage in Ice Powder

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Abstract

The present study was conducted to investigate the chemical, microbial, sensory, and physical characteristics and to determine the storage time of two-year-old farmed *A. persicus* in ice. In the present study, 20 numbers with an average weight and length of 3.50 kg and 70 cm, were kept in CSW (Chilled sea water) tanks, and their microbial, chemical, physical and sensory properties were investigated for 12 days. Microbial agents including the total bacteria counts and *Staphylococcus* and Enterobacteriaceae bacteria, physical such as pH and chemical such as peroxide value, thiobarbituric acid (TBA), free fatty acids, and total volatile nitrogen bases increased significantly during storage ($p < 0.05$). Contamination to coliform, *Escherichia coli*, and *Pseudomonas* bacteria was not observed during storage. But sensory characteristics including skin general appearance, texture, odor, the appearance of gills, eyes, and general acceptance decreased significantly during storage ($p < 0.05$). According to the obtained results, the chemical, microbial, sensory, and physical properties were of good quality during the 8 days of keeping two-year-old farmed *A. persicus* in ice. Therefore, the shelf life of this fish was determined to be 8 days.

Introduction

Fish is a highly perishable commodity and it is necessary to preserve its safety before consumption or processing through methods such as cooling, freezing, or other methods. Around 1000 BC, chilling and freezing were used by the Chinese. Greeks and Romans also used these methods to preserve food.

Fish has been consumed fresh for thousands of years, and producers have always faced the demand of consumers to invent methods to maintain freshness and supply fish fresh (Bozari, 2014). Fresh fish lose their freshness and quality due to various reasons such as autolysis and microbial spoilage. These changes start

immediately after catching fish and spoilage affects the sensory characteristics (The softness of the tissue under the influence of the increase in the number of microbes and the enzymes secreted by them), nutritional value, and safety of products through several reactions, which determines the shelf life of the product over time and is related to maintaining the temperature during storage, transportation, and supply of the product until it reaches the consumer (Özoğul, 2020). Due to the relatively short shelf life of fish, various methods have been developed to preserve fish. Traditionally, methods such as transfer to living forms in containers containing water, as well as the use of ice, are among the most common methods used to supply fish fresh. If the source

of water used to make ice is free from pollution and the water from melting ice completely covers the body of the fish, using ice to cool the fish is effective. The use of ice has various advantages, including the need for 385 kJ kg⁻¹ of energy to melt at zero °C, absorbing heat from the surrounding environment, very high cooling capacity, and maintaining temperature. In addition, ice has many economic advantages, such as the possibility of installing relatively small machines on fishing vessels, low production costs, easy transportation, and the ability to store in fishing vessels (Seifzadeh, 2022).

During the last century, the development of transportation through railway systems, etc., made it possible to transport fresh and chilled fish over long distances. Air transport of fish is another method that has led to the growth of the fresh fish trade over the past 15 years, and it has paved the way for the transfer of large volumes of fresh fish to the European Union, as well as the import of live fish and oysters to Japan. Considering the fish transported by the cold chain as a fresh product led to guaranteeing the development of global trade and the ability to use fresh fish on a large scale and increasing its economic value (Esteves and Diler, 2016). So that the development of cooling technologies, packaging, and distribution of live, fresh and cold fish increased due to consumer demand and accounted for about 10% of the global fish trade in 2014 (FAO, 2020).

Therefore, the fresh fish transportation system cannot be considered as a single chain, this network includes many chains that are made up of many variables. In general, the main supply chain of fresh fish includes catching or primary production, processing (mainly filleting), supply at the retail level and reaching the consumer, which is also added activities such as handling or storage. The increasing focus on fresh fish traceability over the past 15 years has shown that the chains of the fresh fish transport network often operate independently of each other, which cannot be continuously monitored (Seifzadeh, 2022). Over time, the amount of sturgeon fish caught decreased from 68 tons in 2012 to 17 tons in 2018. Also, the amount of cultivation of this species increased from 564 tons to 2839 tons during these years (Planning and Budget Office, 2019). And this point shows the tendency toward sturgeon breeding. *A. persicus* is one of the farmed species of Iran, due to the dispersion of sturgeon breeding places and the possibility of transferring them

to other sites the present study was carried out with the aim of investigating the chemical, microbial, sensory quality, and shelf life it's during storage in ice.

Materials and Methods

To carry out this study, two-year *A. persicus* with an average weight of 3.50 kg and 70 cm total 20 fish were prepared from the Shahid Beheshti Sturgeon Breeding and Breeding Center. After catching the fish, they were stored in CSW tanks using ice powder prepared from drinking water in proportion to twice the weight of the fish. In this way, the ice powder was placed in the layers of the fish and also covered the fish with a thickness of 5 cm. Every day, some ice was added to the samples to keep the temperature of the fish at 0-1°C. Sampling was done for ten days and with a time interval of 48 hours at certain times. The quality of the samples during the storage period was checked using microbial, chemical, and sensory tests, and was determined their shelf life (Zareh Gashti, 2001). For the microbial characteristics, studied of total bacterial counts and count of *Staphylococcus*, *Pseudomonas*, Enterobacteriaceae, coliform, and *Escherichia coli* by the culture method (Feldsine *et al.*, 2020) and for the chemical characteristics were investigated pH by the electrometric method, peroxide by the iodometric method, TBA by colorimetric method, free fatty acid by titration method, and total volatile nitrogen bases by distillation method (Swedish International Development Authority, 1986). Evaluation of sensory characteristics including smell, skin (general appearance), smell and appearance of gills, eyes, and general acceptance was done by 30 male and female evaluators in the age group of 30-40 years. In this method, zero, 1, 2, and 3 indicate good, average, poor, and reject quality, respectively (Gilbert, 2013). Also, the studied fishes became biometric (Farrag, 2022).

Nutritional values including protein, fat, moisture, and ash were determined by macrokegeldal, acid hydrolysis, dry oven, and gravimetric methods, respectively (FAO, 1986 and Latimer, 2016).

The results obtained from the microbial, chemical and sensory tests of experimental and control samples were compared with each other using SPSS version 25 software and a one-way analysis of variance and their changes during storage time using two-way analysis of variance. T-test was used to compare the experimental

Table 1. Changes in the chemical factors of farmed *A. persicus* during storage in ice for twelve days.

Index Sampling time (d)	pH	Free fatty acids (%)	TBA (mg kg ⁻¹)	Peroxide (meq kgoil ⁻¹)	TVB-N (mg 100g ⁻¹)
After of 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±0.24 ^d	1.63±0.35 ^d	12.87±1.51 ^f
4	6.39±1.26 ^c	5.46±1.59 ^e	0.76±0.19 ^{cd}	2.96±0.71 ^c	14.28±1.27 ^e
6	6.41±1.47 ^c	7.69±1.92 ^d	0.98±0.25 ^{bc}	4.98±0.68 ^a	16.87±1.32 ^d
8	6.78±1.58 ^{bc}	8.85±1.94 ^c	1.39±0.14 ^b	4.49±0.57 ^{ab}	17.98±1.49 ^c
10	6.89±1.52 ^{ab}	9.86±1.83 ^b	1.96±0.18 ^a	4.32±0.69 ^b	19.83±2.18 ^b
12	7.23±1.72 ^a	12.63±2.67 ^a	2.19±0.67 ^a	4.14±0.54 ^b	24.54±2.37 ^a

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

treatments with each other and also each of the experimental treatments with the control treatment.

Results

As can be seen in Table 1, the changes in TVB-N, TBA, and free fatty acid showed a significant increase during the storage time ($p < 0.05$). Changes in pH from the day after the catch to the eighth day are not significant ($p > 0.05$), nor are they significant during the tenth and twelfth days ($p > 0.05$). It is not significant on the eighth day compared to the tenth day ($p > 0.05$). The amount of peroxide increased from day 1 to day 8 and decreased from day 10 to day 12. This factor did not change significantly during the eighth, tenth, and twelfth days ($p > 0.05$). The chemical factors during 10 days of storage of cultured fish in ice were within the range accepted by the national standard.

According to Table 2 contamination to *Pseudomonas*, coliform, and *Escherichia coli* bacteria was not observed in the samples during storage in ice. Microbial agents showed a significant increase during storage from the day after catching to the tenth day of storage in ice ($p < 0.05$). These factors were within the range accepted by national standards during 10 days of storage on ice.

By increasing the points obtained, the quality of the fish decreases when stored in ice. The obtained scores show that from the day of catching to the 6th day

of storage in ice, the sensory characteristics of the fish were evaluated as good. On the 8th day, the quality is average, and by achieving a score of 2 -3 on the 10th day, the quality appears to be poor. By reaching a score of 3 on the 12th day, the fish's sensory characteristics are not acceptable (Table 3).

Based on Table 4, the humidity increased at the end of the storage period ($p > 0.05$). Other nutritional value factors were also changed, but their differences were not significant ($p > 0.05$).

Discussion and Conclusion

If the fish is stored in inappropriate conditions, the quality and nutritional value of the fillet and its fat will decrease rapidly. But freezing is one of the methods of preserving fish, which leads to an increase in the shelf life of fish through the delay in chemical and physical processes. As can be seen in Table 1, the amount of peroxide in fish increased during storage in ice that the storage time, the presence of unsaturated fatty acids, and their susceptibility to oxidation by the autocatalytic mechanism, which happens faster in dead tissues compared to living ones, are among the reasons for the increase of this factor. Also, peroxide is unstable and breaks down during storage and turns into TBA, which is one of the secondary products of fat oxidation. Therefore, the increase in this factor has also been observed during the storage time. Considering that the

Table 2. Microbial changes of the farmed *A. persicus* during storage in ice for twelve days (logCFU g⁻¹).

Index Sampling time (d)	Enterobacteriaceae	<i>Staphylococcus</i> bacteria	Total bacterial counts
After of 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 3. Sensory changes of farmed *A. persicus* during storage in ice for twelve days.

Index Sampling time (d)	Overall acceptance	Eye color	General appearance	Gills appearance	Odor of gills	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±0.08 ^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±0.02 ^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±0.09 ^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	0.53±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	1.52±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	2.56±0.68 ^b
12	3.21±0.86 ^a	3.74±0.79 ^a	3.25±0.84 ^a	3.18±0.37 ^a	3.71±0.94 ^a	3.19±0.45 ^a

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

Table 4. Nutritional value of cultured *A. persicus* during storage in ice (%).

Index Time storage (d)	Ash	Moisture	Fat	Protein
After of catch	1.88±1.57 ^a	79.05±1.96 ^a	2.37±1.37 ^a	16.78±1.72 ^a
12	2.13±1.43 ^a	81.06±1.78 ^a	1.16±1.56 ^a	15.65±1.83 ^a

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

maximum permissible amount of peroxide and TBA is 5 meq kgoil⁻¹ and 2 mg kg⁻¹, respectively, in this study, the values of these factors were acceptable on the 10th day of keeping the two-year *A. persicus*. Khodanazary and Pourashouri (2017) determined TBA in whole salted fish 4 and 20.5 mg kg⁻¹ after 9 and 12 days of storage in ice, which was increased compared to the results obtained from the present study. Mian *et al.* (2016) 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 kgoil⁻¹ after 16 days of storage, which the comparison with the results of the present study was more. Soheilnaghshi *et al.* (2015) investigated the quality of fat in silver carp (*Hypophthalmichthys molitrix*) during storage in ice and observed that the amount of peroxide was 8.81 and 12.15 meq kgoil⁻¹ on the 12th and 16th days, respectively. Also, the amount of TBA was determined as 3 mg of mg kg⁻¹ after 16 days of storage, which was increased compared to the results of the present study. Non-conformity is related to the fish species, the presence of unsaturated fatty acids, the initial quality of the fish, and the preservation of the quality of the fish during storage in ice. Sharifian *et al.* (2011) determined TBA 2.30 mg kg⁻¹ in salted fish during 15 days of storage in ice powder, which was consistent with the results of the present study. Mousavi *et al.* (2020) investigated the storage time of *Luciobarbus xanthopterus* in ice and found that TBA increased during the storage time of fish in ice, which was in line with the results of the present study. Aberoumand and Baesi (2017), in a study of the peroxide factor of *Siganus javus* kept in ice, reported that the peroxide was at its lowest value on the 10th day of storage, and the spoilage indices showed a change during storage in ice, which is consistent with the results of the present study.

A large number of volatile compounds such as ammonia, methylamine, dimethylamine, trimethylamine, etc., which indicate the metabolic activity of bacteria, are used to check the quality of fish meat and show spoilage. Paying attention Table 1 shows that the TVB-N reached 19.83 mg 100g⁻¹ after 10 days. Different sources have reported different values regarding the acceptable amount of TVB-N in fish, but some researchers have determined the acceptable amount of TVB-N in fish to be 20 mg 100g⁻¹, according to which this factor was acceptable until the 10th day of storage in ice. Mian *et al.* (2016) investigated the effect of ozonized ice on the chemical and microbial quality of Indian mackerel muscle during storage in ice and observed that the TVB-N reached 34.40 mg 100g⁻¹ after 16 days of storage, which was increased compared to the results of the present study. Soheilnaghshi *et al.* (2015) reported TVB-N as 26.35 and 33.75 mg 100g⁻¹ in silver carp after 12 and 16 days of storage in ice, respectively, which was increased compared to the results of the present study. Khodanazary and Pourashouri reported (2017) that TVB-N in whole salted fish reached 46.66 mg 100g⁻¹ after 12 days of storage in

ice, which was more compared to the results of the present study. Mousavi *et al.* (2020) investigated the shelf life of *Luciobarbus xanthopterus* in ice and found that the amount of TVB-N increased during 72 hours of keeping fish in ice, which was similar to the results of the present study. In general, the amount of TVB-N changes under the influence of species, gender, fishing place, season, and age of the fish. Therefore, the amount of this factor shows differences in different studies.

In addition to having nutritional value, unsaturated fatty acids can cause a bad taste in fish. Therefore, maintaining the quality of marine products due to the presence of these acids and their effects on sensory characteristics has always been a concern of the food industry. Considering that unsaturated fatty acids are naturally present in aquatic tissue, and considering that they are more sensitive to oxidation in the free state, their increase can be expected during the storage time, which happened in this study (Table 1). Khodanazary and Pourashouri (2017) reported free fatty acid of 15.8% after 12 days stored in ice, which was reduced compared to the results of the present study. The discrepancy could be due to differences in fish species and amounts of unsaturated fatty acids. Mousavi *et al.* (2020) investigated the shelf life of *Luciobarbus xanthopterus* in ice and found that free fatty acids increased during the storage of fish in ice, which was similar to the results of the present study.

According to Table 1, the pH in the farmed *A. persicus* did not show much change during storage in ice. In general, the pH of live fish muscle is close to 7, but after death, it changes from 6 to 7 according to the fishing season, fish species, and other factors. In the studied fish, the value of this index increased during the storage period, which can be attributed to the passage of storage time and the activity of autolytic enzymes and proteolytic bacteria spoiling fish. Also, with the increase of volatile nitrogenous bases during the storage period, such a trend was expected for pH. Viji *et al.* (2015) reported such a trend for pH, which compares with the results of the present study in the same direction. Khodanazary and Pourashouri (2017) determined the pH in whole salted fish to be 6.99 after 12 days of storage in ice, which was not consistent with the results of the present study. Mian *et al.* (2016) 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, which showed a decrease compared to the results of the present study. Mousavi *et al.* (2020) reported an increase in pH during storage of *Luciobarbus xanthopterus* in ice, which was consistent with the results of the present study. Storage in ice and rapid cooling leads to a reduction of physical changes in fish during storage. But the initial fish quality, storage conditions, increase in TVB-N during the storage period, and different microbial loads are the reasons for the change in this factor, and as seen in the studies, the pH shows many changes. Also, some researchers including

Khodanazary and Pourashouri (2017) reported that pH is not very important for evaluating the quality of whole fish in ice.

According to Table 2, the total bacterial counts and *Staphylococcus* bacteria increased in two-year *A. persicus* during storage in ice. The use of temperature reduction for keeping fish in markets around the world is of particular importance. Since the temperature drops to the melting point of ice (zero °C) during freezing, therefore, freezing leads to an increase in the shelf life of fish by reducing the action of enzymes and bacteria. Although ice has the ability to maintain the quality of fish during storage, it is one of the short-term preservation methods of fish. In addition, some researchers have also reported that ice is not a suitable method for the long-term storage of fish. In the present study, the two-year *A. persicus* had favorable microbial characteristics during 10 days of storage in ice. The water from melting ice is able to moisten the surface of the fish, which provides suitable conditions for the growth of bacteria, but it also leads to washing away the surface bacteria of the fish, therefore, the increase in the number of bacteria during storage in ice does not have a high speed and helps to maintain the microbial quality of fish during storage. Among other factors that are effective in maintaining the quality of fish during storage in ice, preventing the heating of the surface of the fish and speeding up the bacterial interactions as well as delaying the occurrence of spoilage, which was provided in the present study through the continuous addition of ice. Khodanazary and Pourashouri (2017) determined the total number of psychrophilic bacteria in whole salted fish to be $23.7 \log \text{cfu g}^{-1}$ after 9 days of storage in ice, which was inconsistent with the results of the present study. Mousavi *et al.* (2020) in the investigation of the storage time of *Luciobarbus xanthopterus* in ice, showed that psychrophilic bacteria did not grow during the storage period, which was consistent with the results of the present study. They also reported the number of mesophilic bacteria at zero time until 72 hours after storage of 1.53 to $72 \log \text{cfu g}^{-1}$ which was not consistent with the results of the present study. The difference in several studies is due to the diverse 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 3, the sensory characteristics of the cultured fish decreased during storage in ice. According to Table 3, gill smell and eye color are the best indicators for checking the quality of fish during storage in ice, and the importance of these characteristics was also confirmed in the present study. During storage in ice, the internal bacteria of the fish are slowly active and the reactions caused by their enzymes can cause changes in the appearance of the skin and color of the fish after a period of time has passed. Also, the increase in chemical characteristics, including TBA, are

considered as other effective factors in the sensory characteristics and smell of fish. Mousavi *et al.* (2020) stated that during storage of *Luciobarbus xanthopterus* in ice, that the sensory characteristics of fish were in a suitable and acceptable condition up to 24 hours after storage in ice. However, during the storage period, the acceptability gradually decreased and at 72 hours, most of the sensory indicators scored low, and the samples at this hour had the lowest sensory quality during the storage period, which was less reported compared to the results of the present study. These researchers did not report the use of ice as a suitable method for the long-term storage of *Luciobarbus xanthopterus*. Khodanazary and Pourashouri (2017) showed that the sensory characteristics of whole salted fish were of favorable quality when stored on ice for 6 days, and on the 9th day, it was not suitable for human consumption, which is not consistent with the results of the present study. In the present study, the cultured fish showed favorable sensory characteristics during 6 days of storage in ice, and then its quality was reduced, so that on the 8th day its quality was moderate, on the 10th day its preservation was poor, and on the 12th day it was not suitable for human consumption. Changes in the amount of fat, chemical factors such as peroxide and TBA, and the growth of psychrophilic bacteria and their effect on sensory characteristics are considered effective reasons for creating differences in these characteristics.

As can be seen in Table 4, changes were observed in the nutritional value, which includes the increase of moisture and ash and the decrease of fat and protein of fish when stored in ice. The increase in humidity is due to the absorption of water from the environment, which increases the moisture, and the presence of air provides conditions for better growth of microorganisms. Enzymes leached from proteolytic and lipolytic microorganisms lead to the breakdown of fish protein and fat and reduce their amounts. But on the basis that minerals are not degradable, they remain and no decrease was observed in their amounts. In addition to, Table 4 shows that *A. persicus* is rich in protein, fat, and ash and has a lot of nutritional value. Hung (2017) expressed 40-45% protein in different species of sturgeon, which is not the same as the results obtained from the present study. It is unclear whether there is a true difference in protein requirements between different species of sturgeon, or whether the differences in protein are mainly due to the different laboratory methods used in the studies. Also, other reasons such as different initial weights, dietary protein sources, other dietary components, differences in breeding conditions, habitat, and nutrition are effective in the occurrence of changes in nutritional value (Hung, 2017). Zareh gashti *et al.* (2006) reported the values of protein, fat, ash, and moisture in cultured *Huso. huso* as 17.29, 3.1, 1.09, and 79.4%, which are consistent with the results of the present study.

Storage in ice is considered as one of the methods of obtaining fresh and safe fish whose quality is acceptable, and it also leads to an increase in shelf life, which is one of the important points in the economic value and trade of fish. According to the obtained results and the achievement of shelf life in terms of chemical, microbial, sensory, and physical characteristics and considering that the physicochemical characteristics during the 10-day storage period of the two-year *A. persicus* in the ice were of favorable quality, however, considering the priority of sensory characteristics compared to physicochemical characteristics and the poor assessment of sensory factors on the tenth day of storage, therefore, the shelf life of the *A. persicus* in ice was determined to be 8 days.

Ethical Statement

Animal rights were respected during breeding, catching, slaughtering, and transportation.

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Author Contribution

Mina Seifzadeh: Writing, Zareh Gashti: Processing, Ali Raoufi: Collaboration in writing

Conflict of Interest

There is no conflict of interest in the implementation of this project.

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