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Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the Black Sea, during chilled storage

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Abstract

Freshness of wild turbot (*Scophtalmus maximus*) stored in ice was assessed by chemical, sensory and microbiological methods. The limit for sensory acceptability of wild turbot stored in ice was ~12–15 days. The quality of turbot decreased on day 15 (B) and they were no longer acceptable on day 19 (C). The TVB-N level showed fluctuations during storage, indicating that TVB-N could not be a good indicator of turbot quality. The release of FFA increased from an initial value of 6.33 (expressed as % of oleic acid) to a final value of 20.6 during the storage period. The initial PV value was 5.60 meq/kg for turbot stored in ice and it started to increase to 21.6 meq/kg on day 12 and then started to decrease to 13.6 meq/kg at the end of storage period. The level of TMA in wild turbot increased sharply from an initial value of 9.36 mg/kg to a final value of 38.9 mg/kg. Linear regressions (r^2) obtained from K, K_i, G, P, H and F_r were 0.92, 0.89, 0.99, 0.89, 0.96 and 0.89, respectively, for the wild turbot stored in ice. Turbot maintained high (E) and good quality (A) during the first 12 days of chilled storage when the average K, K_i and P values were ~78–85%, and H, F_r and G values were ~45%, 15% and 149%, respectively. Eight biogenic amines were investigated, namely, histamine, putrescine, cadaverine, spermidine, spermine, tryptamine, tyramine, and 2-phenylethylamine, three amines (histamine, tyramine, and tryptamine) were not detected in any of the fish samples during the storage period. As storage time progressed, putrescine and cadaverine became the dominant amines, reaching 22.7, and 16.9 mg/kg, respectively, at 19 days of storage in ice. Total viable counts of whole gutted turbot increased from the initial value of 3.3 log cfu g^{-1} (day 0) to 7.87 log cfu g^{-1} (day 19) over the period of storage. If 10^6 microorganisms/g are considered to be the TVC limit of acceptability, the shelf life of turbot was approximately ~13–14 days.

Keywords: Wild turbot; Freshness indicators; Fish quality; Shelf life

1. Introduction

Fish is considered as a valuable source of protein in the human diet. The significance of long chain polyunsaturated fatty acids has gained attention because of their prevention of human cardiovascular diseases (Shahidi & Botta, 1994). Fish are the main contributors of *n*3 PUFA for human diet According to the American Heart Association (Kraus et al., 2000), fish consumption frequency should be at least twice a week to present cardioprotective effects. Consumption of both freshwater and seawater fish is, therefore, encouraged.

Turbot (*Scophthalmus maximus*, also known as *Psetta maxima*) is found naturally in the Black Sea and is an economically important fish species along the northern coast of Turkey. Although it is considered an expensive and luxurious dish, the market demand for fresh turbot is quite high. Therefore, the study of biochemical and sensory attributes of wild turbot is of interest to both retailers and consumers.

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The quality of fish decreases after death due to chemical reactions (changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine) and microbiological spoilage. As a result of these events, sensory quality of fish deteriorates (Chang, Chang, Shiau, & Pan, 1998; Kyrana & Lougovois, 2002; Özogul, Polat, & Özogul, 2004; Özogul, Özyurt, Özogul, Kuley, & Polat, 2005; Rodríguez, Besteiro, & Pascual, 1999; Surette, Gill, & LeBlanc, 1988; Yamanaka, Shiomi, & Kikuchi, 1989). There are studies on the effects of storage, in slurry ice, on the microbial, chemical and sensory quality of farmed turbot (Rodríguez, Barros-Velázquez, Piñeiro, Gallardo, & Aubourg (2006)) and biochemical changes in the quality of farmed turbot (Aubourg, Piñeiro, Gallardo, & Barros-Velazquez, 2005). However, there is limited information on the shelf life and freshness quality of wild turbot, *S. maximus,* from the Black Sea. Therefore, the objectives of this study were to investigate the shelf life and biochemical and sensory attributes of wild turbot stored in ice.

2. Materials and methods

2.1. Sample preparation and storage of turbots

Turbot (*S. maximus*) were caught by bottom trawling. They were immedialtely iced in a box. Turbots were 2 days post-capture on arrival at the laboratory in ice. The average weights and lengths of turbot were 525.93 ± 49.31 g and 31.75 ± 1.81 cm, respectively. The turbots were gutted and washed. After that, they were stored in ice at a fish-to-ice ratio of 2:1 (w/w). All boxes were then stored in ice for up to 19 days. Sensory and chemical analyses were performed on days 0, 5, 9, 12, 15, 19, whereas peroxide value (PV) and free fatty acids (FFA) were analysed on days 1, 6, 10, 14, 17, 20, after extraction of fat. Data were obtained using three fish which were minced for each sampling.

2.2. Proximate analysis

The fish samples were analysed in triplicate for proximate composition: lipid content by the Bligh and Dyer (1959) method, moisture content by the AOAC (1990) method, total crude protein by the Kjeldahl method (AOAC, 1984) and ash content by the AOAC (1990) method.

2.3. Analytical methods

The TVB-N content was determined according to the method of Antonocopoulus (1973) and expressed as mg TVB-N per 100 g muscle. The value of TBA was determined according to Tarladgis, Watts, and Yonathan (1960) in turbot fillets, to evaluate the oxidation stability during storage, and the results expressed as TBA value, miligrammes of malondialdehyde per kg of flesh. Free fatty acid analysis (FFA), expressed as % of oleic acid, was determined by AOAS (1994). Peroxide value (PV), expressed in miliequivalents of peroxide oxygen per kilogramme of fat, was determined according to AOAS (1994). The pH of turbot fillets was determined using a pH meter (315i, Germany). The sample was homogenised in distilled water in the ratio 1:10 (w/v).

ATP and its degradation products were analysed using a rapid HPLC method (Özogul, Taylor, Quantick, & Özogul, 2000). The K, K_i,G, P, H and F_r values were calculated by the procedures described by Burns, Ke, and Irvine (1985), Gill, Thompson, Gould, and Sherwood (1987), Karube, Matsuoka, Suzuki, Watanabe, and Toyama (1984), Luong, Male, Masson, and Nguyen (1992), Saito, Arai, and Matsuyoshi (1959), Shahidi, Chong, and Dunajski (1994), respectively.

Biogenic amines were analysed using an HPLC method (Özogul, Taylor, Quantick, & Özogul, 2002). Benzoyl chloride, as a derivatization reagent, was used and the derivatization procedure was based on that of Redmond and Tseng (1979).

2.4. Apparatus

High-performance liquid chromatography (HPLC) used a Shimadzu LC-10VP (Shimadzu, Kyoto, Japan) apparatus equipped with a UV/VIS detector (Spectra-Physics SP 8450, Analytical Inc., UK) and a low gradient pump (Shimadzu LC-10ATVP) with four channel mixer (Shimadzu FVC-10ALVP). For biogenic amine analysis, the column was reverse-phase, C18, nucleosil, 250×4.6 mm, particle diameter 5 μm (Mecherey-Nagel, Duren, Germany) For nucleotide determination, the column was a Sphereclone ODS 2 C18, 150×4.60 mm, particle diameter 5 μm micrometer. (Phenomenex, Macclesfield, Cheshire, UK).

2.5. Sensory analysis

For sensory analysis, triplicate samples were taken at regular intervals. Sensory analysis was assessed using traditional guidelines regarding fresh and chilled fish (Council Regulation, 1990) with minor modifications for gutted fish. Each assessment was carried out by a minimum of 6 trained panellists. Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and unacceptable quality (C).

The measurement of freshness of cooked fish (odour, flavour and texture) was assessed according to Torry Scheme (Howgate, 1982). A scale from 10 to 3 was used, 10 denoting absolutely fresh and 3 completely putrid or spoiled. To prepare the cooked fish sample, fish from each of the storage conditions were filleted and cooked in a microwave oven for 2 min at medium temperature. The cooked samples were served hot to panellists.

2.6. Microbiological analysis

Samples from each of three different turbot (triplicate) were taken to estimate total viable counts (TVC). Ten

grammes of fish muscle were mixed with 90 ml of Ringer solution and then stomached for 3 min. Further decimal dilutions were made up to 10^{-8} and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar plates in triplicate. They were then incubated for 2 days at 30 °C.

2.7. Statistical analysis

For data analysis, standard deviation and ANOVA were used. Significance of differences was defined at $P < 0.05$.

3. Results and discussion

3.1. Sensory assessment

Table 1 shows the results of the sensory analysis of the wild turbot stored in ice. Turbot maintained high (E) and good quality (A) during the first 12 days of chilled storage. The quality of turbot decreased on day 9 (A), and they were no longer acceptable on day 19 (C). In the case of the wild turbot, the progressive loss of freshness was observed, relating to formation of mucus, loss of pigmentation, odour of gills, belly cavity and flesh, which were the limiting factors for acceptability of the wild turbot. Skin aspects and consistency were still acceptable up to day 19 of storage. The limit for acceptability of turbot stored in ice was ~12–15 days. Aubourg et al. (2005) and Rodríguez et al. (2006) studied farmed turbot and found high and good quality (categories E and A, respectively) during the first 14 days of chilled storage and up to day 22 for turbot stored in slurry ice or up to day 14 for turbot in flake ice, respectively.

A sensory quality test was carried out in parallel to establish the rejection point of the cooked wild turbot fillets. The fillets were cooked at medium setting for 2 min using microwave voltage (600 W). Fig. 2 shows sensory evaluation scores of turbot fillets. The sensory score for flavour of the cooked fillets decreased with storage time. The fresh flavour characteristics of the species were strong between 0 and 8 days, slowly decreasing in intensity to the flavourless stage by 12 days. Off-flavour was detected after 15 days due to bacterial metabolites. As spoilage progressed, the off-flavour increased in intensity until the fish were no longer edible by 19 days. The rejection point for the cooked fillets was below 6 at 19 days.

Table 1
The sensory evaluation of the wild turbot stored in ice

Attribute	Days of storage					
	0	5	9	12	15	19
Skin aspect	E	E	A	A	B	B
External odour	E	A	A	A	B	C
Gills	A	A	A	A	B	C
Consistency	A	A	A	A	B	B
Flesh odour	E	E	A	A	B	C
Belly cavity	E	E	A	A	B	C

E, highest quality; A, good quality; B, fair quality and C, unacceptable.

3.2. Chemical assessment

The proximate composition of the wild turbot on day 0 is shown in Table 2. The fat content of turbot was found to be slightly higher (1.30%) than that of farmed turbot (% 0.8–1.2) (Aubourg et al., 2005). Moisture contents of were similar.

TVB-N concentrations of the wild turbot stored in ice are shown in Fig. 1. At the begining of storage, the TVB-N value was 12.1 mg/100 g flesh for turbot stored in ice. The TVB-N values showed a decreasing pattern up to 9.99 mg TVB-N/100 g of flesh by day 8 and then started to increase up to 31.1 mg TVB-N/100 g at the end of storage. The level of TVB-N in freshly caught fish is generally between 5 and 20 mg N/100 g muscle. However, levels of 30–35 mg N/100 g muscle are considered the limit of acceptability for ice-stored cold water fish (Connell, 1995; Huss, 1988). In the present study, the TVB-N level showed fluctiations during storage, indicating that TVB-N could not be a good indicator of turbot quality.

Similar results were obtained for farmed turbot (Rodríguez et al. (2006)), for farmed sea bass (Papadopoulos, Chouliara, Badeka, Savvaidid, & Kontominas, 2003), and for gilthead sea bream (Tejada & Huidobro, 2002). However, TVB-N content has been shown to be an indicator of freshness in a variety of fish, such Atlantic cod (Botta, Lauder, & Jewer, 1984), sardine (Ababouch et al., 1996; Özogul et al., 2004), European eel (Özogul et al., 2005).

Table 2
Proximate analysis (%) of the wild turbot, *Scophthalmus maximus*

	Protein	Fat	Moisture	Ash
Turbot	17.6 ± 0.31	1.30 ± 0.12	80.13 ± 0.18	0.97 ± 0.01

Data are expressed as means ± standard deviation ($n = 3$).

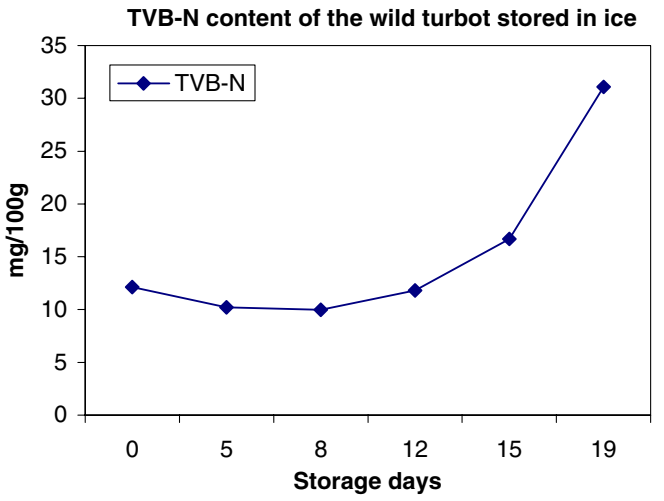

Fig. 1. TVB-N content of the wild turbot, *Scophthalmus maximus,* stored in ice.

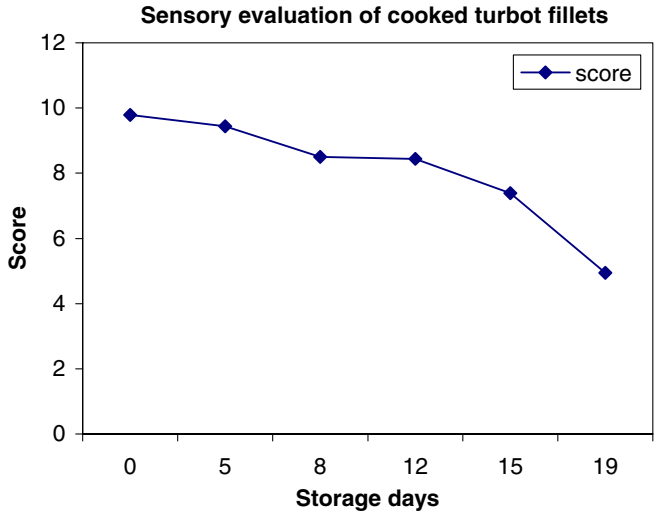

Fig. 2. The sensory evaluation of cooked turbot by odour, flavour and texture.

Mean pH measurements over the period of storage in ice are shown in Table 3. The increase in pH was observed on day 5. However, the pH value decreased on days 8 and 12, and after that increased toward the end of storage. Similar results were obtained for farmed turbot in ice (Rodríguez et al. (2006)), for farmed sea bass in ice (Papadopoulos et al., 2003) and farmed trout (Rodríguez et al., 1999). Unlike the results obtained in this study, pH values were found to increase steadily for other species (Özogul et al., 2005; El Marakchi, Bennour, Bouchriti, Hamama, & Tagafatit, 1990). Post-mortem pH varies from 6.0 to 7.1, depending on season, species and other factors (Simeonidou, Govaris, & Vareltzis, 1998).

The release of FFA increased from the initial value of 6.33 (expressed as % of oleic acid) to the final value of 20.6 during the storage period. Since the release of FFA content increased with time, as found in this study, it is reported that there is a relationship between FFA release and loss of freshness (Barassi, Pècora, Roldán, & Trucco, 1987; Özogul et al., 2005). Lipid hydrolysis occurred at a slower rate for the farmed turbot (Aubourg et al., 2005; Rodríguez et al., 2006) for sardine (Pacheco-Aguilar, Lugo-Sánchez, & Robles-Burgueño, 2000) and European eel (Özogul et al., 2005) compared to the results obtained from this study.

Table 3
Changes in pH, free fatty acids (FFA), peroxide value (PV), and thiobarbituric acid (TBA) value, in wild turbot over the period of iced storage

Days in ice	pH	FFA (% of oleic acid)	PV (meq/kg)	TBA (mg MA/kg)
0	6.71 ± 0.02	6.33 ± 0.28	5.60 ± 0.36	0.27 ± 0.01
5	6.96 ± 0.06	8.19 ± 0.03	11.6 ± 0.32	0.46 ± 0.01
8	6.93 ± 0.05	8.33 ± 2.14	15.2 ± 1.68	0.75 ± 0.02
12	6.94 ± 0.06	10.1 ± 0.09	21.6 ± 0.79	0.48 ± 0.02
15	7.14 ± 0.10	13.3 ± 0.61	20.7 ± 1.87	0.91 ± 0.05
19	7.53 ± 0.09	20.6 ± 2.32	13.6 ± 0.78	0.48 ± 0.05

Peroxide formation in the wild turbot in ice was observed to be very fast compared to that of farmed turbot (Aubourg et al., 2005). The initial PV value was 5.60 meq/kg for the wild turbot stored in ice (Table 3). However, the initial PV values were found to be 0.8–1.2 for herring (Smith, Hardy, McDonald, & Temhleton, 1980), <1 for the farmed turbot (Aubourg et al., 2005) and 27.6 for fresh sardine (Cho, Endo, Fujimoto, & Kaneda, 1989). In comparision with the initial value of PV (5.60 meq/kg), a considerable increase was observed on day 12 (21.6 meq/kg) and then it started to decrease to 13.6 meq/kg at the end of the storage period.

The TBA index is widely used as an indicator of degree of lipid oxidation. Nishimoto, Suwetja, and Miki (1985) reported, for mackerel, 4 and 27 mg of malonaldehyde (MA)/kg muscle, for good and low quality fish, respectively. The TBA values in this study were found to be lower (Table 3) than those reported for mackerel (Nishimoto et al., 1985; Ryder, Buisson, Scott, & Fletcher, 1984) but higher than those for farmed sea bass (Papadopoulos et al., 2003) and European eel (Özogul et al., 2005). Aubourg (1993) reported that TBA values may not give actual rate of lipid oxidation since malonaldehyde can interact with other components of fish such as nucleosides, nucleic acid, proteins, amino acids of phospholipids and other aldehydes which are end products of lipid oxidation. This interaction can vary with fish species.

The freshness indicators (K, K_i, G, P, H and F_r) of the wild turbot stored in ice were determined from the concentrations of nucleotides and are shown in Fig. 3. K and related values increased linearly (except F_r value) with storage time in turbot, as found for European eel (Özogul et al., 2005) and for sea bream (Alasalvar, Taylor, & Shahidi, 2002). Linear regressions (r^2) obtained from K, K_i, G,

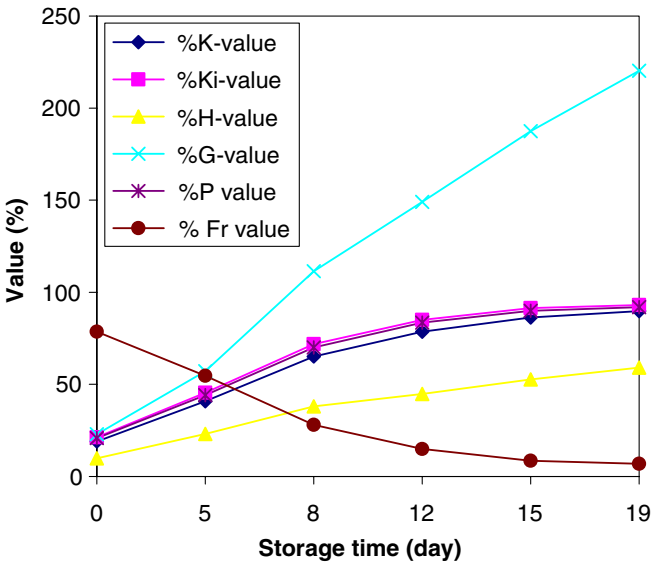

Fig. 3. K, K_i, G, P, H and F_r value changes of the wild turbot stored in ice. The r^2 values of linear regressions are 0.92 (K), 0.89 (K_i, P and F_r), 0.99 (G), and 0.96 (H) with time.

P, H and F_r were 0.92, 0.89, 0.99, 0.89, 0.96 and 0.89, respectively, for the wild turbot stored in ice. Aubourg et al. (2005) also found 0.96 for the K value of farmed turbot in chilled storage. In this study, the lowest value was obtained from K_i, P and F_r values for turbots in ice. Turbot maintained high (E) and good quality (A) during the first 12 days of chilled storage, when the average K, K_i and P values were ~78–85%, and H, F_r and G values were ~45%, 15% and 149%, respectively. The quality of turbot decreased on day 15 (B) and they were no longer acceptable on day 19 (C).

When turbots stored in ice were considered at the limit of acceptability (B) by assessors after ~15 days, the average K, K_i and P values were ~86–92%, and H and G values were ~53% and 188%, respectively. On the other hand, F_r value was ~9%. The highest value in this study was obtained for the G value (~220%) at the end of the storage period. Significant differences ($P < 0.05$) were found between K and related values (H, G and F_r values) over the storage period, except the K_i and P values ($P > 0.05$). K values increased with storage time, reaching 90% from the initial value of 19% in turbots stored in ice for 19 days. Aubourg et al. (2005) reported that the K value of farmed turbot increased from the initial value of ~6% to ~75% after 19 days. The K value provided a useful indicator for freshness in turbots stored in ice. Similar results were found with farmed turbot, sardine, herring and European eel stored in ice (Aubourg et al., 2005; Özogul et al., 2004; Özogul et al., 2005).

The concentrations of the biogenic amines and TMA present in the muscle of turbot stored in ice are given in Table 4. Eight biogenic amines were investigated, namely, histamine, putrescine, cadaverine, spermidine, spermine, tryptamine, tyramine, and 2-phenylethylamine, three amines (histamine, tyramine and tryptamine) were not detected in any of the fish samples during the storage period. As storage time progressed, putrescine and cadaverine became the dominant amines, reaching 22.7, and 16.9 mg/kg, respectively, at 19 days of storage in ice. Valle, Malle, and Bouquelet (1996) found that, when the fish were inedible, putrescine and cadaverine contents of herring stored at 0 °C were 1.01 and 2.3 mg/100 g, respectively, whereas plaice and whiting contained 1.57 and 5.8 mg/100 g putrescine and 9.1 and 9.2 mg/100 g cadaverine, respectively. However, in this study, when turbot in ice (on day 19) were rejected by the sensory panel, the level of putrescine was 22.7 mg/kg, and cadaverine level was and 16.9 mg/kg. Although spermidine and spermine contents of turbot showed fluctuations, they increased to 6.87 and 4.61 mg/kg, respectively, at the end of storage.

Unlike farmed turbot (Rodríguez et al. (2006)), the level of TMA in wild turbot increased sharply from the initial value of 9.36 mg/kg to the final value of 38.9 mg/kg, which is higher than those found for European eel (Özogul et al., 2005), sardine (Özogul et al., 2004). Fish (mainly marine fish) contain trimethylamine oxide (TMAO) and the quantity depends on fish species and the environment. TMA is associated with the fishy odour of spoilage and is part of the spoilage pattern of many fish. Seawater fish contain 1–100 mg TMAO in every 100 g muscular tissue, whereas freshwater fish generally contain only 5–20 mg/100 g (Stansby & Olcott, 1963, Chap. 26).

3.2.1. Microbiological assessment

Microbial counts on the wild turbot kept in ice are shown in Fig. 4. Initial total viable count of whole gutted turbot was 3.3 log cfu g^{-1} (day 0) and the growth of microorganisms increased to 7.87 log cfu g^{-1} (day 19) over the period of storage. On day 15 of storage, TVC was 6.54

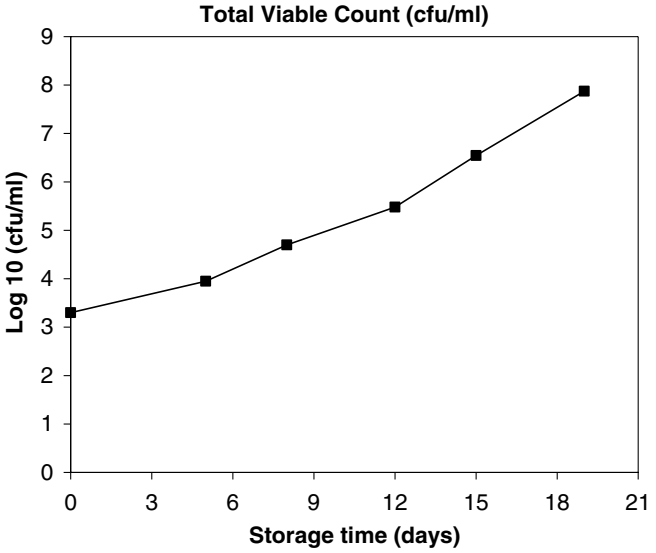

Fig. 4. Changes in TVC of the wild turbot stored in ice.

Table 4
The formation of biogenic amines and TMA (mg/100 g) in turbot kept in ice

Storage days	HIS	PUT	CAD	SPD	SPN	TYR	TRPT	2-PHENY	TMA
0	–	6.21 ± 4.85	3.93 ± 1.14	1.37 ± 1.01	0.81 ± 0.44	–	–	–	9 ± 1.82
5	–	7.67 ± 0.82	6.21 ± 2.23	3.48 ± 1.25	1.01 ± 0.58	–	–	–	14.6 ± 1.00
8	–	12.2 ± 1.66	8.93 ± 1..33	2.45 ± 0.40	2.84 ± 1.03	–	–	–	19.6 ± 1.75
12	–	17.9 ± 4.23	11.4 ± 3.01	3.19 ± 1.23	1.08 ± 0.46	–	–	–	25.8 ± 3.08
15	–	18.8 ± 3.29	13.6 ± 2.79	4.60 ± 2.21	3.72 ± 2.11	–	–	–	31.7 ± 4.24
19	–	22.7 ± 3.95	16.9 ± 2.54	6.87 ± 1.68	4.61 ± 1.94	–	–	–	38.9 ± 2.59

HIS, histamine; PUT, putrescine; CAD, cadaverine; SPD, spermidine; SPN, spermine; TYR, tyramine; TRYP, tryptamine; 2-PHENY, 2-phenylethylamine; TMA, trimethylamine; –, not detected; ±, standard deviation.

cfu g^{-1}. If 10^6 microorganisms/g are considered the TVC limit of acceptability, the shelf life of turbot was approximately 13–14 days, indicating that sensory analysis of turbots correlated well with microbiological analysis. Similar results were reported by Rodríguez et al. (2006) for farmed turbot, by Özogul et al. (2004, 2005) for sardine and for European eel, by Randell, Hattula, and Ahvenainen (1997) for rainbow trout, and by Papadopoulos et al. (2003) for farmed sea bass.

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