PAPER

QUALITY CHANGES OF SPOTLESS SHAD DURING STORAGE AT DIFFERENT CONDITIONS

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ABSTRACT

This study investigates the effect of using ice in combination with refrigeration on the sensory, physico-chemical and microbiological attributes of spotless shad during storage. Spotless shad kept in ice under refrigerated conditions had better sensory, physico-chemical and microbiological quality as compared with control groups. The shelf life of samples kept at ambient temperature without ice was 2 days. Using ice and refrigeration only extended the shelf-life for 3 days and 4 days, respectively, while ice application with refrigeration further increased the shelf-life by 10 days. Physico-chemical and microbiological results usually supported sensory values. Histamine values were below EU (European Union) and FDA permitted levels for the shelf-life of fish.

Keywords: spotless shad, biogenic amines, quality changes, sensory values, shelf life

1. INTRODUCTION

Spotless shad, *Alosa immaculata,* Bennett, 1835 is the largest representative species of the *Clupeidae* family, it has a pelagic life and is classified under diadromous fish species (FAO, 2014). This shad species is the most abundant in Eastern Europe both in inland and marine waters (NAVODARU *et al*., 2003). It is a commercially important species in Turkey (TUİK, 2013) and some other European countries (FAO, 2014). The fishing period of shad was recorded to take place between March and October for the Black Sea. Various catching methods and materials are used for shad (ERGÜDEN *et al*., 2007) and the total catch value was 1,541 tons for the year 2013 in Turkey (TUİK, 2013). Some other European countries also reported a total catch of about 1,029 tons (FAO, 2014). However, this species is commonly caught by local fishermen and therefore, most of their catch is likely not to be recorded.

Fish spoilage relates to both seafood quality and safety that further affects the market value of the product as well as its nutritional value. Spoilage is mainly caused by bacterial activity, other than sensory quality loss (such as its appearance, flavour, odour and texture), some compounds, such as Biogenic Amines (BAs), can be formed by bacterial decarboxylation of the precursor amino acids, thus, leading to food safety issues. Most reports have focused on histamine, which is reported to cause scombrotoxin poisoning, closely linked to the consumption of fish kept in abused time/temperature conditions and species belonging to different families, including *Clupeidae*, due to high content of free histidine in their muscle (LEHANE and OLLEY, 2000; EU DIRECTIVE, 2005). Putrescine and cadaverine are also known to enhance the toxicity effect of histamine. Other than food safety issues, several BAs are known to be associated with fish decomposition and the formation of cancerous nitrosamines (LEHANE and OLLEY, 2000; PONS-SÁNCHEZ-CASCADO *et al*., 2006).

Although, previous studies demonstrated the advantage of using chilled storage conditions for various fish species and the different shelf lives were observed (VARLIK and HEPERKAN, 1990; VECIANA-NOGUÉS *et al*., 1990; CARACHE *et al*., 2002; KÖSE and ERDEM, 2004; CHOTIMARKORN, 2014; KORAL and KÖSE, 2012), and limited studies are available for the quality changes of spotless shad stored at chilled temperatures. So far, DUYAR *et al*. (2012) investigated the shelf life of this species at refrigerated temperature and obtained 6 days of shelf life. In our previous study, we studied physico-chemical and sensory quality of shad, obtained in a market in the months of July and August and kept in refrigerated storage in open air for 3 days. We gathered that the quality of shad varies depending on the initial quality of fresh fish (KÖSE *et al*., 2000). However, storage quality and development of biogenic amines under various chilled temperature conditions of this species are still unknown. Previous literature, including our past studies, showed that keeping fish in ice during refrigerated storage improves the storage quality, while extending the shelf life. Similarly, this type of application retarded BAs development (KORAL and KÖSE, 2012).

Shad is known to contain high fat, up to 26%, which affects its storage quality more than less fatty fish (BORAN and KARAÇAM, 2011). Moreover, this species is highly susceptible to the formation of histamine and other biogenic amines, which result in seafood health risk for the consumers. Therefore, we aimed to investigate the effect of different ice application under refrigerated temperature of this species on its shelf life and biogenic amine development.

2. MATERIALS AND METHODS

2.1. Materials and experimental design

Fresh, spotless shad samples used in this study were caught in the South-Eastern Black Sea in the month of January 2013. The samples that were previously kept in ice and placed in EPS (expanded polystyrene) boxes for 3hours after the catch, were collected directly from a boat at the port of Trabzon, Turkey. The fishes were then kept in EPS boxes containing ice and were transferred to the laboratory within 1hour. The fishes which weighed 30kg (125 fishes) in total were immediately washed with tap water and then, the fishes were randomly divided into 4 groups. Each group was kept in plastic containers. Ice application on fish was carried out layer by layer. The containers were covered with plastic wrap (cling film). The sample groups were;

(i) Control: Fish stored at ambient temperature without ice (C): The container temperature: 17.0°C ± 3.0°C, Fish average temperature: 16.0°C ± 4.0°C. The average length and weight of the fishes weighing a total of 5.40 kg (24 fishes) are 29.59 cm ± 1.98 cm, 225.59 g ±37.38 g, respectively.

(ii) Fish stored in ice, ratio is at 1:1 (w: w), and at ambient temperature (IAT) 17.0°C ± 3.0°C: Fish temperature fluctuated between 4.0°C and 14.0°C with daily ice replacement (Fish temperature dropped to 4°C in 2 hours after ice addition). The average length and weight of the fishes weighing a total of 7.2 kg (30 fishes) are 30.19 cm ± 2.30 cm, 238.98 ± 53.17 g, respectively.

(iii) Refrigerated (in a refrigerator: Arçelik, 8810NF, Turkey) fishes without ice (RT): Storage in cold air at 4.8°C ± 0.3°C. Fish temperature 3.8°C ± 0.4°C. The average length and weight of the fishes weighing a total of 7.2 kg (30 fishes) are 29.88 ± 2.15 cm, 241.2 ± 59.94 g, respectively.

(iv) Iced storage in refrigerator (IRT) at 4°C ±1°C: Fish and ice ratio, (1:1 w:w). Fish temperature, 0.5°C ± 0.3°C. The average length and weight of the fishes weighing 10kg (40 fishes) are 30.58 cm ± 1.76 cm, 246.25g ± 40.8 g, respectively. Ice was replaced daily.

Sampling was carried out daily by randomly choosing spotless shad, until the products were spoilt based on sensory results. The ice was the flake iced type obtained freshly from an ice maker (Hoshizaki, FM-80EE, Amsterdam, Holland). The internal temperatures of the fishes in each group were measured on a daily basis using a digital thermometer (Taylor, 9842N Waterproof Digital Thermometer, USA) by injecting the upper dorsal part of the fish.

3. METHODS

3.1 Sensorial evaluation

Sensory analysis was carried out using eight trained panellists who judged the freshness of the samples using a 10-point scale (ARCHER, 2010). The panellists were comprised of 6 males and 2 females (5 academicians and 3 administrative staff). The subjects were qualified after passing the screening tests stated by BOTTA (1995). The panellists are 20 in number and they were chosen from previously trained people who are regular shad consumers based on the criteria given by BOTTA (1995).

Table 1 shows the sensory score sheets used to evaluate spotless shad samples. This structured category scale is based on the traditional freshness, quality grading system for the whole iced and refrigerated spotless shad. According to scale, 4 is the limit for acceptable/unacceptable, <4: unacceptable. The results were presented as the means of data obtained from 8 panellists.

Table 1: Sensory evaluation criteria of shad samples.

Score	Appearance					Odour	Texture
	Eyes	Gills	Skin	Flesh	Appearance of Belly Walls		
10	Slightly convex, clear, black pupil.	Dark red, purple, clear slime	Bright colours, iridescent, few loose scales.	Glossy, rosy hue, fresh bright blood on fillet.	No belly burst	Oily, marine, fresh blood, sulphide, weak odour.	Firm, stiff, smooth
9	Flat, slightly convex, clear black pupil.	Dark red, pink, slightly faded.	Bright, slight iridescence, few loose scales.	Slight translucency, rosy hue, bright blood on fillet.	No belly burst.	Oily, marine, fresh fruit, sulphide.	Loss of stiffness, still firm, smooth.
8	Flat, slightly convex, slightly cloudy	Dark red, pink, slight brown, slight reddening on gill covers	Loss of brightness, some loose scales.	Slight translucency, slight discoloration of belly flaps.	No belly burst or very slight belly burst	Oily, musty, leathery, slight sulphide.	Loss of stiffness, slight softening, smooth.
7		Red, pink, slight bleaching, slight reddening on gill covers.	Dull, slight bronzing, some loose scales.	Slightly opaque, slightly brown, slight discoloration of belly flaps.	Slight belly burst.	Oily, musty, sulphide, slightly sour.	Limp, slightly soft, slightly gritty.
6	Flat, slightly cloudy	Red, pink, brown, slight bleaching, reddening on gill covers.	Dull, slight bronzing, some dirty scales.	Opaque, dull, brown, reddening on belly flaps.		Musty, stale fruit, stale grass, malty, sour.	
5	Flat, slightly sunken, slightly cloudy, grey pupil.	Pink, brown slime, bleached, reddening on gill covers.	Dull, bronzing, dirty scales.		Definite belly burst.	Stale fruit, stale grass, sour, malty, slightly rancid.	
4	Flat, slightly sunken, cloudy, bloodshot, discoloured.			Opaque, brown, discoloured belly flaps and tail.		Sweaty, sour sink, stale meat, H_2S	Limp, soft, gritty.
3	Flat, slightly sunken, wrinkled, discoloured, blood shot.	Pink, bleached, brown slime, bronzing on gill covers.	Dull, bleached, brown slime.	Opaque, general discoloration.		Sweaty, cheesy, sour sink, byre-like, rotten fruit.	

Triplicate samples (3 randomly selected fish) were taken per day at other intervals from each group to evaluate their quality criteria at the sensory laboratory section. Fresh spotless shad was also included in the evaluation, starting from the 2nd day, to support the judgement of the assessors as a reference sample (although, the panellists were blind to its code). Different samples were simultaneously presented in plates coded. They were expected to give the highest score for the blinded reference sample (freshly caught spotless shad). After the sensory analysis, the fish were further used for physico-chemical analysis.

3.2. Physico-chemical analysis

Total Volatile Basic Nitrogen (TVB-N) was determined using the method of Lucke and Geidel as described by INAL (1992). Thiobarbituric acid value (TBA) was estimated according to SMITH *et al*. (1992). The method of BOLAND and PAIGE (1971) was used for analyzing Trimethylamine Nitrogen (TMA-N). The pH measurements were taken with a digital pH meter (Jenco 6230N, CA, USA) by placing the electrode into the homogenized samples (KÖSE *et al*., 2012). Biogenic amines were analyzed using a High Performance Liquid Chromatography (HPLC) method according to KÖSE *et al*. (2012) and KORAL and KÖSE (2012). HPLC equipment was Shimadzu Prominence LC-20 AT series (Japan) with auto sampler (sıl20ac, Shimadzu, Japan), a Diode Array Detector (SPD-M20A, Shimadzu, Japan) and Intertsil column (GL Sciences, ODS-3, 5μm, 4.6×250mm). All physico-chemical analysis was carried out in triplicate sampling, and results were represented as means ±SD.

3.3. Bacterial analysis

Total aerobic Viable Bacteria (TVB) were counted using plate count agar according to KÖSE and ERDEM (2004). Mesophilic and psychrophilic microorganisms were counted after incubation at 4°C for 8 days for psychrophilic microorganisms and at 37°C for 48 hours for mesophilic microorganisms. Histamine-forming Bacteria (HFB) were determined in accordance with the method used by KÖSE (1993). Total mesophilic and psychrophilic HFB were determined using the same condition applied for TVB with the exception of using Niven's medium, instead of plate count agar. Triplicate samples were used for each type of analysis, while each analysis was performed in duplicate. Counts of bacteria were expressed as log cfu/g. The results were presented as means of all counts ±SD.

3.4. Statistical analysis

All statistical analysis was performed using JMP software (Version 8.02, 2009, SAS Institute. Inc., Cary, NC, USA) (SOKAL and ROHLF, 1987). Analysis of variance (ANOVA) was used to compare the results within the groups as well as during storage period. When significant differences were found, comparisons among data were carried out using Turkey test. A significant level of 95% ($p<0.05$) was used throughout the analysis.

4. RESULTS AND DISCUSSION

Sensory analysis is important in any food quality evaluation program because the ultimate criterion for judgment is the human response (XU *et al*., 2014). In this study, sensory evaluation was based on appearance, texture and odour and the results are presented in Table 2. Sensory scores decreased significantly in both control and experimented samples with increasing storage ($p<0.05$). Significant changes occurred between the control group and others, starting from the 1st day of storage ($p<0.05$) for the relating sensory attributes.

Table 2: Sensory values of shad groups stored under various conditions.

Day of storage	Sample type	Appearance	Odour	Texture	Over all mean
0	**Fresh**	9.66 ± 0.21	9.80 ± 0.10	9.62 ± 0.36	9.69 ± 0.09
1	**C**	$8.28\pm0.19^{a}_{A}$	$7.28\pm0.15^{a}_{A}$	$8.30\pm0.16^{a}_{A}$	$7.95\pm0.58^{a}_{A}$
	IAT	$9.84\pm0.09^{b}_{A}$	$9.28\pm0.29^{b}_{A}$	$9.30\pm0.23^{b}_{A}$	$9.47\pm0.32^{b}_{A}$
	RT	$9.68\pm0.23^{b}_{A}$	$9.40\pm0.37^{b}_{A}$	$9.76\pm0.11^{b}_{A}$	$9.61\pm0.19^{b}_{A}$
	IRT	$9.78\pm0.13^{b}_{A}$	$9.80\pm0.10^{b}_{A}$	$9.94\pm0.09^{b}_{A}$	$9.84\pm0.13^{b}_{A}$
2	**C**	$6.04\pm0.11^{a}_{B}$	$6.12\pm0.16^{a}_{B}$	$7.22\pm0.22^{a}_{B}$	$6.46\pm0.66^{a}_{B}$
	IAT	$8.18\pm0.13^{b}_{B}$	$8.16\pm0.11^{b}_{B}$	$9.10\pm0.22^{b}_{A}$	$8.48\pm0.54^{b}_{B}$
	RT	$9.12\pm0.13^{c}_{B}$	$9.12\pm0.23^{c}_{A}$	$9.50\pm0.10^{c}_{A}$	$9.24\pm0.45^{c}_{B}$
	IRT	$9.48\pm0.22^{c}_{A}$	$9.64\pm0.45^{c}_{A}$	$9.74\pm0.09^{c}_{A}$	$9.65\pm0.10^{c}_{A}$
3	**C**	$4.08\pm0.08^{a}_{C}$	$3.22\pm0.13^{a}_{C}$	$4.14\pm0.11^{a}_{C}$	$3.81\pm0.51^{a}_{C}$
	IAT	$7.18\pm0.08^{b}_{C}$	$7.16\pm0.17^{b}_{C}$	$8.16\pm0.18^{b}_{B}$	$7.50\pm0.57^{b}_{C}$
	RT	$9.06\pm0.15^{c}_{C}$	$8.70\pm0.16^{c}_{C}$	$8.88\pm0.25^{c}_{B}$	$8.71\pm0.45^{c}_{C}$
	IRT	$8.74\pm0.15^{c}_{B}$	$8.62\pm0.09^{c}_{B}$	$9.10\pm0.11^{c}_{B}$	$8.77\pm0.16^{c}_{B}$
4	**C**	$3.04\pm0.05^{a}_{D}$	$2.02\pm0.11^{a}_{D}$	$3.04\pm0.11^{a}_{D}$	$2.70\pm0.59^{a}_{D}$
	IAT	$6.12\pm0.13^{b}_{D}$	$6.10\pm0.10^{b}_{D}$	$7.22\pm0.13^{b}_{C}$	$6.48\pm0.64^{b}_{D}$
	RT	$8.18\pm0.11^{c}_{D}$	$8.24\pm0.19^{c}_{D}$	$8.56\pm0.19^{c}_{B}$	$8.32\pm0.49^{c}_{D}$
	IRT	$8.44\pm0.29^{c}_{B}$	$8.40\pm0.14^{c}_{B}$	$8.70\pm0.07^{c}_{C}$	$8.50\pm0.08^{c}_{B}$
5	**C**	$1.06\pm0.09^{a}_{E}$	$0.54\pm0.11^{a}_{E}$	$0.88\pm0.15^{a}_{E}$	$0.83\pm0.26^{a}_{E}$
	IAT	$5.04\pm0.15^{b}_{E}$	$5.26\pm0.11^{b}_{E}$	$6.30\pm0.21^{b}_{D}$	$5.53\pm0.67^{b}_{E}$
	RT	$7.14\pm0.11^{c}_{E}$	$7.28\pm0.13^{c}_{E}$	$7.26\pm0.11^{c}_{C}$	$7.23\pm0.08^{c}_{E}$
	IRT	$8.26\pm0.15^{d}_{B}$	$8.12\pm0.08^{c}_{C}$	$8.38\pm0.19^{c}_{D}$	$8.12\pm0.17^{d}_{C}$
6	**IAT**	$3.34\pm0.42^{a}_{F}$	$4.36\pm0.21^{a}_{F}$	$4.12\pm0.13^{a}_{E}$	$3.94\pm0.53^{a}_{F}$
	RT	$6.08\pm0.15^{b}_{F}$	$6.40\pm0.19^{b}_{F}$	$6.16\pm0.15^{b}_{D}$	$6.21\pm0.17^{b}_{F}$
	IRT	$8.04\pm0.32^{c}_{B}$	$7.92\pm0.29^{c}_{C}$	$7.80\pm0.08^{c}_{E}$	$7.82\pm0.12^{c}_{D}$
7	**IAT**	$2.98\pm0.08^{a}_{G}$	$3.22\pm0.16^{a}_{G}$	$3.16\pm0.09^{a}_{F}$	$3.12\pm0.12^{a}_{G}$
	RT	$5.14\pm0.22^{b}_{G}$	$5.00\pm0.19^{b}_{G}$	$4.90\pm0.07^{b}_{E}$	$5.02\pm0.11^{b}_{G}$
	IRT	$7.16\pm0.17^{c}_{D}$	$7.14\pm0.19^{c}_{D}$	$7.04\pm0.09^{c}_{F}$	$7.11\pm0.06^{c}_{E}$
8	**IAT**	$1.12\pm0.15^{a}_{H}$	$1.00\pm0.07^{a}_{H}$	$1.02\pm0.11^{a}_{G}$	$1.05\pm0.06^{a}_{H}$
	RT	$3.02\pm0.23^{b}_{H}$	$3.02\pm0.25^{b}_{H}$	$3.20\pm0.33^{b}_{F}$	$3.45\pm0.13^{b}_{H}$
	IRT	$6.78\pm0.33^{c}_{E}$	$7.04\pm0.15^{c}_{D}$	$6.84\pm0.23^{c}_{G}$	$6.89\pm0.14^{c}_{F}$
9	**RT**	$0.76\pm0.18^{a}_{I}$	$1.06\pm0.23^{a}_{I}$	$0.72\pm0.40^{a}_{H}$	$0.85\pm0.19^{a}_{I}$
	IRT	$6.34\pm0.15^{b}_{F}$	$6.50\pm0.19^{b}_{E}$	$6.58\pm0.16^{b}_{H}$	$6.47\pm0.13^{b}_{G}$
10	**IRT**	$5.98\pm0.31_{F}$	$6.20\pm0.31_{EF}$	$6.18\pm0.16_{I}$	$6.12\pm0.16_{H}$
11	**IRT**	$5.44\pm0.29_{FG}$	$5.92\pm0.29_{F}$	$5.56\pm0.10_{i}$	$5.81\pm0.10_{I}$
12	**IRT**	$4.90\pm0.23_{G}$	$4.96\pm0.27_{G}$	$5.04\pm0.11_{J}$	$4.97\pm0.07_{i}$
13	**IRT**	$3.94\pm0.21_{H}$	$3.92\pm0.08_{H}$	$3.78\pm0.27_{K}$	$3.88\pm0.09_{J}$

'4' is the limit for acceptability/unacceptability of the samples.
Different superscript small letters (a,b,c) represents statistical differences among groups ($p<0.05$). Different superscript capital letters (A,B,C,D,..) represents statistical differences amongst different days within the same group during storage ($p<0.05$).C: Control, IAT: Fish stored in ice at ambient temperature; RT: Refrigerated fish without ice; IRT: Fish stored in ice at refrigerator temperature.

The changes were also found to be significant between samples stored in ice at ambient temperature and the samples stored at refrigerated temperatures with and without ice, starting from the 2nd day of storage ($p<0.05$). The sensory scores of both groups kept at refrigerated temperatures were similar up to the 4th day, thereafter, the variations occurred. According to the overall sensory data, the control group sample got spoilt on the 3rd day, whereas using ice extended its shelf life for 3 more days. Refrigerated temperature without ice also supported sensory values having a shelf life of 7 days, although, a dryness on the surface of the fish occurred. The samples kept in ice at refrigerated temperature received the highest sensory scores compared to other samples with a shelf life of 12 days. This may be attributed to the stable as well as cold temperature of the fish under this condition.
In our previous study with fresh Atlantic bonito, we demonstrated that filleting can improve the sensory quality of fish for about 6 days if kept in ice and under refrigerated temperature (KORAL and KÖSE, 2012). We also demonstrated that sensory quality of shad can vary at refrigerated temperature depending on the initial quality of fish (KÖSE *et al*., 2000). DUYAR *et al*. (2012) investigated the quality changes of shad (*Alosa tanaica*) at refrigerated storage packed in stretch film without ice. According to sensory values, they obtained one day higher shelf life than present study for the shad samples kept at refrigerated temperature without ice. The difference can be explained in the protecting effect of using stretch film, since we obtained dryness on the surface of fish at open air in the refrigerator. XU *et al*. (2014) determined the effect of electrolyzed oxidizing water and chitosan on the microbiological, physico-chemical, and sensory attributes of American shad (*Alosa sapidissima*) during refrigerated storage (4±1°C) kept in air-proof polyethylene pouches. They showed that the shelf life of fish can further be improved by 9-10 days during refrigerated storage with this treatment.
The pH can be used as a spoilage indicator of fish and fish products (XU *et al*., 2014). The pH of fresh fish after death is usually reported as close to neutral and varies between 6.0 and 7.1 during post-mortem storage depending on the fish species, diet, season, type of muscle, and other factors (HUSS, 1988; XU *et al*., 2014). The pH of fresh shad was recorded as 6.8 and it has increased significantly throughout storage period for all groups ($p<0.05$) (Table 3). The pH values of control group were significantly higher than the experimental groups and reached 7.18 at the time of spoilage. Similar result was obtained on the 5th day for the group stored in ice at ambient temperature, while higher values were recorded for fish samples stored at refrigerated temperatures on the day when the fish was organoleptically unfit for consumption. Although, increase in pH values correlates well with the spoilage during storage, the values do not support sensory values. Although, different authors obtained lower pH values for fresh shad species, they likewise obtained similar trend for pH values during spoilage (KÖSE *et al*., 2000; DUYAR *et al*., 2012; XU *et al*., 2014).
TVB-N has been used as a quality indicator of fish products (HUSS 1988; CONNELL 1990). It includes ammonia; primary, secondary, and tertiary amines; and products of protein breakdown. An increase in TVB-N is attributed to an increase in the activity of endogenous enzymes and bacterial growth (XU *et al*., 2014). The European Union sets varying TVB-N limits as 25-35 mg/100 g for unprocessed fishery products that is regarded as unfit for human consumption, where organoleptic assessment has raised doubts as to their freshness (EU DIRECTIVE 2005 and 2008). The changes in the TVB-N values during storage are shown in Table 4. The values increased significantly with the increasing storage time for all groups ($p<0.05$). This increase may be attributed to the production of ammonia. Significant differences were also observed between control group and the experimental groups beginning from the 1st day of storage ($p<0.05$). TVB-N value of the control group was close to the suggested limit on the 3rd day of storage, when the fish

became organoleptically unfit for consumption. The levels reached 82.6 mg/100g on the 4th day. However, TVB-N values did not support sensory results obtained for the experimental groups and the levels reached unacceptable limit on the 6th and 8th day of storage for samples stored in refrigerator and ice at ambient temperature, respectively. TVB-N values of the samples stored in ice at refrigerated temperature were found within the acceptable quality throughout the storage. This result confirms our earlier findings with fresh bonito on the beneficiary effect of using ice during refrigerated storage (KORAL and KÖSE, 2012). Previous studies also demonstrated that filleting and addition of electrolyzed, oxidized water and chitosan can retard the development of TVB-N in fish (KÖSE and KORAL, 2012; XU *et al*., 2014). However, higher TVB-N values were found by KÖSE *et al*. (2000) with shad species kept at refrigerated temperature and packed in stretch film without ice. The higher values can be explained by the sampling season, being carried out in summer in previous study, while sampling was done in winter in the current study, since the water temperature can affect the initial microbial load and variations in bacterial species, leading to differences in TVB-N formation. DUYAR *et al*. (2012) also obtained higher TVB-N values for *A. Tanaica,* which was kept in refrigerated temperature without ice application. However, they did not state the sampling time and the initial conditions of fish before storage. Therefore, we assumed that the initial conditions of fish may be the reason for differences in the results.

Trimethylamine is a pungent, volatile amine often associated with the typical "fishy" odour of spoiling seafood. Its presence in spoiling fish is due to the bacterial reduction of trimethylamine oxide, which is naturally present in the living tissue of many marine fish species. Although, TMA is believed to be generated by the action of spoilage bacteria, its correlation with bacterial numbers is often not very good (HUSS 1995). A suggested, acceptable level is reported as 12 mg/100 g (GOULAS and KONTOMINOS 2005). Initial TMA-N values was 0.28 mg/100g and it significantly increased throughout the storage period for all experimental groups (Table 4). Significant variations were observed between control and other groups, beginning from the 1st day of storage, and also within the groups ($p<0.05$). The lowest TMA-N values were found in the group stored in ice at refrigerated temperature, while the highest levels were received by the control group. The values were within the acceptable levels for this parameter throughout the storage period. Therefore, TMA values did not support the sensory results for this species. The results were in agreement with our previous findings for fresh bonito stored under refrigerated conditions (KORAL and KÖSE, 2012).

TBA is a by-product of lipid oxidation. Therefore, it is a common method for assessing lipid oxidation in fish products (XU *et al*., 2014). The TBA value for a good quality chilled fish is reported to be between 5 and 8 mg malonaldehyde/kg, while the levels of 8 mg malonaldehyde/kg fish flesh are generally regarded as the limit of acceptability for most fish species (SCHORMÜLLER, 1969). The values of TBA increased significantly depending on time, and significant variations were also observed between the control group and the other groups ($p<0.05$), beginning from the 1st day of storage (Table 4). Significant differences were observed within the experimental groups beginning from the 2nd day of storage. The control group reached an unacceptable limit on the 4th day, while fish stored at refrigerated temperature without ice got spoilt on the 5th day. The sample kept in ice and stored at ambient temperature had a slightly favourable condition and TBA value reached an unacceptable level on the 7th day. Fish kept in ice and in refrigerated conditions had the lowest values that were within the acceptable quality throughout the storage period. These results suggest that storage of fish in ice should also be applied in refrigerated/cold storage conditions to avoid lipid oxidation. Cold storage without ice application is not suitable to retard oxidation. TBA values were only in support of control group in terms of its overall acceptability of sensory values. Lower values were recorded from previous studies for bonito and Indian shad during refrigerated storage (KÖSE *et al*., 2000; KÖSE and KORAL, 2012; MASSA *et al*., 2012; XU *et al*., 2014). The differences in TBA values might be attributed to the fat content in different fish species. The results of DUYAR *et al*. (2012) supported our findings for TBA results.

The results of the total mesophilic and psychrophilic viable bacteria and HFB are shown in Table 5. Initial counts of mesophilic and psychrophilic total viable bacteria, and the total mesophilic and psychrophilic HFB of fresh fish were observed as 1.95, 2.18, 2.15 and 2.45 log cfu/g, respectively. Significant differences were found amongst the groups along with the increasing levels throughout the storage with some exceptions (p<0.05). Storage in ice and under refrigerated conditions significantly decreased the total mesophilic viable bacteria and HFB load of the samples in the first day of storage, then significant increase occurred in the counts during further storage (p<0.05). The lowest mesophilic counts were obtained for samples containing ice at refrigerated temperatures within a variation of 1.30-3.18 log cfu/g.

Table 3: The changes in pH values of spotless shad groups stored under various conditions.

Day of storage	C	IAT	RT	IRT
Fresh	6.80±0.08	6.80±0.08	6.80±0.08	6.80±0.08
1	$7.02±0.02^{a}_{A}$	$6.88±0.03^{b}_{A}$	$6.87±0.04^{b}_{A}$	$6.86±0.05^{b}_{A}$
2	$7.14±0.03^{a}_{B}$	$6.90±0.06^{b}_{A}$	$6.97±0.02^{b}_{B}$	$6.88±0.03^{b}_{A}$
3	$7.18±0.09^{a}_{C}$	$6.94±0.04^{b}_{A}$	$7.07±0.04^{c}_{C}$	$6.90±0.03^{b}_{A}$
4	$7.37±0.02^{a}_{D}$	$7.02±0.03^{b}_{B}$	$7.17±0.02^{c}_{D}$	$6.94±0.04^{d}_{A}$
5	$7.64±0.02^{a}_{E}$	$7.08±0.06^{b}_{B}$	$7.21±0.03^{c}_{E}$	$6.98±0.04^{d}_{A}$
6	*	$7.12±0.03^{a}_{BC}$	$7.26±0.03^{b}_{E}$	$6.99±0.04^{c}_{A}$
7	*	$7.18±0.04^{a}_{C}$	$7.38±0.02^{b}_{F}$	$7.00±0.02^{c}_{A}$
8	*	$7.24±0.08^{a}_{C}$	$7.50±0.09^{b}_{G}$	$7.05±0.01^{c}_{B}$
9	*	*	$7.40±0.07^{a}_{D}$	$7.09±0.02^{b}_{C}$
10	*	*	*	$7.18±0.03_{D}$
11	*	*	*	$7.23±0.04_{D}$
12	*	*	*	$7.26±0.05_{D}$
13	*	*	*	$7.34±0.06_{E}$

*: Not Analyzed, Different superscript small letters (a,b,c) represents statistical differences among groups (p<0.05). Different superscript capital letters (A,B,C,D,…) represents statistical differences amongst different days within the same group during storage (p<0.05).C: Control, IAT: Fish stored in ice at ambient temperature; RT: Refrigerated fish without ice; IRT: Fish stored in ice at refrigerator temperature.

Table 4: The changes in the levels of chemical quality parameters of spotless shad groups stored at various conditions.

Day of storage	Sample type	Chemical Criteria		
		TVB-N (mg/100 g)	TMA (mg/100 g)	TBA (mg MDA/ kg)
0	Fresh	9.11±0.30	0.28±0.13	0.46±0.28
1	C	14.71±0.28$^{a}_{A}$	1.36±0.06$^{a}_{A}$	0.83±0.10$^{a}_{A}$
	IAT	11.21±0.28$^{b}_{A}$	1.08±0.18$^{b}_{A}$	0.57±0.05$^{b}_{A}$
	RT	12.61±0.15$^{b}_{A}$	0.53±0.04$^{c}_{A}$	0.63±0.08$^{b}_{A}$
	IRT	10.51±0.16$^{c}_{A}$	0.39±0.08$^{d}_{A}$	0.47±0.09$^{b}_{A}$
2	C	21.01±0.16$^{a}_{B}$	2.58±0.10$^{a}_{B}$	1.68±0.06$^{a}_{B}$
	IAT	14.85±0.34$^{b}_{B}$	1.78±0.05$^{b}_{B}$	1.09±0.08$^{b}_{B}$
	RT	14.48±0.26$^{b}_{B}$	1.01±0.08$^{c}_{B}$	1.47±0.09$^{c}_{B}$
	IRT	11.91±0.15$^{c}_{A}$	0.65±0.13$^{d}_{B}$	0.63±0.02$^{d}_{B}$
3	C	28.72±0.18$^{a}_{C}$	2.94±0.08$^{a}_{C}$	4.62±0.23$^{a}_{C}$
	IAT	14.71±0.22$^{b}_{B}$	2.08±0.16$^{b}_{C}$	2.06±0.17$^{b}_{C}$
	RT	17.02±0.20$^{c}_{C}$	1.33±0.19$^{c}_{C}$	3.55±0.05$^{c}_{C}$
	IRT	13.31±0.12$^{b}_{B}$	0.77±0.08$^{d}_{B}$	0.90±0.14$^{d}_{C}$
4	C	82.65±0.21$^{a}_{D}$	3.86±0.18$^{a}_{D}$	9.98±0.16$^{a}_{D}$
	IAT	17.51±0.10$^{b}_{C}$	2.98±0.06$^{b}_{D}$	4.86±0.12$^{b}_{D}$
	RT	21.18±0.20$^{c}_{D}$	2.42±0.03$^{c}_{D}$	7.13±0.07$^{c}_{D}$
	IRT	14.01±0.12$^{d}_{B}$	0.86±0.14$^{d}_{C}$	1.18±0.10$^{d}_{D}$
5	C	119.07±0.16$^{a}_{E}$	5.89±0.21$^{a}_{E}$	14.31±0.08$^{a}_{E}$
	IAT	18.21±0.23$^{b}_{D}$	3.86±0.07$^{b}_{E}$	6.14±0.18$^{b}_{E}$
	RT	24.86±0.15$^{c}_{E}$	3.25±0.18$^{c}_{E}$	11.15±0.07$^{c}_{E}$
	IRT	16.11±0.10$^{d}_{C}$	1.01±0.09$^{d}_{D}$	1.38±0.16$^{d}_{E}$
6	IAT	23.81±0.14$^{a}_{E}$	5.14±0.22$^{a}_{F}$	7.67±0.03$^{a}_{F}$
	RT	29.40±0.20$^{b}_{F}$	4.67±0.12$^{b}_{F}$	13.24±0.05$^{b}_{F}$
	IRT	17.51±0.26$^{c}_{D}$	1.25±0.14$^{c}_{E}$	2.02±0.16$^{c}_{F}$
7	IAT	26.62±0.28$^{a}_{F}$	7.68±0.18$^{a}_{G}$	10.09±0.18$^{a}_{G}$
	RT	33.26±0.20$^{b}_{G}$	5.49±0.09$^{b}_{G}$	15.35±0.19$^{b}_{G}$
	IRT	20.31±0.17$^{c}_{E}$	1.40±0.03$^{c}_{F}$	2.46±0.16$^{c}_{G}$
8	IAT	32.92±0.15$^{a}_{G}$	8.12±0.07$^{a}_{H}$	11.72±0.17$^{a}_{H}$
	RT	42.82±0.20$^{b}_{H}$	6.99±0.08$^{b}_{H}$	18.36±0.25$^{b}_{H}$
	IRT	21.71±0.22$^{c}_{E}$	1.57±0.07$^{c}_{G}$	3.06±0.14$^{c}_{H}$
9	RT	37.82±0.22$^{a}_{H}$	9.28±0.16$^{a}_{I}$	13.19±0.28$^{a}_{I}$
	IRT	22.41±0.12$^{b}_{F}$	1.71±0.10$^{b}_{H}$	3.86±0.18$^{b}_{I}$
10	IRT	24.51±0.30$_{G}$	1.86±0.08$_{H}$	4.98±0.20$_{I}$
11	IRT	25.21±0.28$_{G}$	1.81±0.14$_{H}$	5.55±0.55$_{I}$
12	IRT	25.91±0.16$_{G}$	2.03±0.12$_{I}$	6.14±0.14$_{J}$
13	IRT	26.62±0.34$_{G}$	2.42±0.10$_{I}$	6.82±0.18$_{K}$

Different superscript small letters (a,b,c) represents statistical differences among groups ($p<0.05$). Different superscript capital letters (A,B,C,D,…) represents statistical differences amongst different days within the same group during storage ($p<0.05$).C: Control, IAT: Fish stored in ice at ambient temperature; RT: Refrigerated fish without ice; IRT: Fish stored in ice at refrigerator temperature. TVB-N: Total volatile basic nitrogen, TBA: Thiobarbituric acid value, TMA-N: Trimethylamine nitrogen.

The recommended limit for Total Viable bacteria Counts (TVC) for fresh fish consumption is reported to be between 6-7 log cfu/g (ICMSF 1992; CHOTIMARKORN, 2014). The results of this study suggest that although, mesophiles reached the unacceptable level on the 3rd day of storage for the control group, psychrophilic bacteria counts were still within an acceptable level for further storage, possibly due to unfavourable conditions. Better quality was obtained from the samples kept in ice at ambient conditions in comparison with samples stored without ice at refrigerated conditions, in terms of bacteria growth, indicating the washing effect of ice during melting at ambient conditions. TVC exceeded the limit for psychrophilic bacteria of the samples stored under refrigerated conditions without ice on the 7th day, while samples with ice at ambient conditions were still acceptable up to the 9th day. However, the best quality was found for shad which was kept in ice and under refrigerated conditions, the TVC was still within the acceptable level throughout the storage period. Sensory results supported TVC for control group and the bacteria load are still under the suggested limit for other groups within their acceptable sensory quality. With the exception of TVB-N, the results of mesophilic and psychrophilic viable counts did not support chemical quality parameters. UDDIN *et al*. (2001) observed a higher bacteria load from the muscle of Indian shad (*Tenualosa ilisha*) stored in ice, and placed in insulated polystyrene and wooden boxes. They also reported higher counts for fish stored in wooden boxes than fish stored in the initial. The total bacterial count in the muscle of the fish stored in insulated boxes was 3.5×10^4 cfu/g and it increased to 1.8×10^9cfu/g after 18 days, when the fish became organoleptically unfit for consumption. They also reported that the bacterial flora of the fishes stored in wooden boxes showed comparatively higher initial bacteria counts and increased rapidly during storage. No study exists on the microbial changes of fresh, spotless shad, either at ambient or chilled conditions. Therefore, this study presents new data on the possible microbial activities during storage of this species under different conditions.
Initial levels of HFB in fish is important since previously formed histamine decarboxylases, are likely to continue to decarboxylase histidine to histamine, even when histamine decarboxylase positive bacteria are no longer viable (KÖSE, 2010). In this study, initial HFB counts were 2.15 log cfu/g for mesophiles and 2.45 log cfu/g for psychrophilic bacteria. The counts also increased significantly throughout the storage period with the exception of mesophiles, which significantly dropped on the 1st day for samples stored in ice at ambient temperatures and without ice at refrigerated temperatures ($p<0.05$). Significant variations were observed between control group and other groups as well as within the experimental groups ($p<0.05$). The highest HFB counts was recorded for control group, while the lowest were observed in samples stored in ice at refrigerated temperatures.
The results of BAs contents are shown in Table 6. Biogenic amines contents varied significantly depending on storage life and the groups, with some exceptions ($p<0.05$). Histamine is the only BAs that is regulated for fish and fisheries products. The European Regulation (EU) permits up to 100 mg/kg for fresh and processed fish, and 200 mg/kg for fishery products which have undergone enzyme maturation treatment in brine (EU DIRECTIVE, 2005), while FDA allows for less than 50 mg/kg (FDA, 2011). Shad is listed among the regulated fish species due to its high histidine content (FDA, 2011). Histamine contents in samples representing control and in all experimental groups, were under the detection limit at the beginning of the storage trial and the 1st day of storage, and significantly increased throughout storage ($p<0.05$). Detectable levels started on the 2nd day for control group as 7.41 mg/kg and the values reached an unacceptable level set by the FDA on the 5th day. Detectable values of histamine were obtained on the 3rd day for the samples stored in ice at ambient temperature and without ice at refrigerated temperatures as 4.93 mg/kg and 1.50 mg/kg, respectively.

Table 5: Microbial changes of the shad groups stored under various conditions.

Day of storage	Sample type	Total Viable Bacteria (log cfu/g)		Total Histamine Forming Bacteria (log cfu/g)	
		Mesophiles	Psychrophilic	Mesophiles	Psychrophilic
0	Fresh	1.95 ± 0.07	2.18 ± 0.10	2.15 ± 0.23	2.45 ± 0.13
1	C	$3.12\pm0.14^{a}_{A}$	$2.20\pm0.09^{a}_{A}$	$3.36\pm0.12^{a}_{A}$	$2.74\pm0.04^{a}_{A}$
	IAT	$2.95\pm0.10^{a}_{A}$	$2.76\pm0.11^{b}_{A}$	$1.95\pm0.15^{b}_{A}$	$2.57\pm0.07^{b}_{A}$
	RT	$1.60\pm0.08^{b}_{A}$	$2.43\pm0.12^{a}_{A}$	<1.47	$2.72\pm0.16^{a}_{A}$
	IRT	<1.47	$2.72\pm0.07^{b}_{A}$	$2.36\pm0.17^{d}_{A}$	$2.30\pm0.15^{b}_{A}$
2	C	$5.22\pm0.13^{a}_{B}$	$2.24\pm0.10^{a}_{A}$	$4.41\pm0.23^{a}_{B}$	$2.98\pm0.10^{a}_{B}$
	IAT	$3.27\pm0.15^{b}_{B}$	$3.04\pm0.12^{b}_{B}$	$2.81\pm0.09^{b}_{A}$	$2.85\pm0.13^{a}_{B}$
	RT	$1.90\pm0.12^{c}_{B}$	$3.44\pm0.07^{c}_{B}$	$1.60\pm0.12^{c}_{B}$	$4.31\pm0.15^{b}_{B}$
	IRT	$1.60\pm0.05^{d}_{B}$	$2.61\pm0.20^{d}_{B}$	$1.78\pm0.08^{c}_{B}$	$2.18\pm0.02^{c}_{A}$
3	C	$6.34\pm0.25^{a}_{C}$	$2.26\pm0.04^{a}_{A}$	$5.18\pm0.13^{a}_{C}$	$3.10\pm0.09^{a}_{B}$
	IAT	$3.45\pm0.07^{b}_{C}$	$3.00\pm0.28^{b}_{B}$	$3.73\pm0.11^{b}_{B}$	$3.30\pm0.21^{a}_{C}$
	RT	$2.30\pm0.10^{c}_{C}$	$3.85\pm0.17^{c}_{C}$	$2.23\pm0.14^{c}_{C}$	$4.28\pm0.01^{b}_{B}$
	IRT	$1.85\pm0.03^{d}_{C}$	$2.68\pm0.12^{d}_{B}$	$1.95\pm0.10^{d}_{B}$	$2.89\pm0.12^{c}_{B}$
4	C	$7.26\pm0.29^{a}_{D}$	$2.43\pm0.12^{a}_{B}$	$5.58\pm0.27^{a}_{D}$	$3.32\pm0.14^{a}_{C}$
	IAT	$3.95\pm0.10^{b}_{D}$	$3.30\pm0.21^{b}_{C}$	$3.40\pm0.07^{b}_{C}$	$3.45\pm0.13^{a}_{C}$
	RT	$4.19\pm0.13^{c}_{D}$	$5.21\pm0.12^{c}_{D}$	$3.57\pm0.09^{b}_{D}$	$5.36\pm0.09^{b}_{C}$
	IRT	$2.20\pm0.09^{d}_{D}$	$2.80\pm0.06^{d}_{A}$	$2.48\pm0.23^{c}_{A}$	$2.93\pm0.12^{c}_{B}$
5	C	$9.43\pm0.21^{a}_{E}$	$2.38\pm0.04^{a}_{B}$	$6.23\pm0.17^{a}_{E}$	$3.40\pm0.18^{a}_{C}$
	IAT	$4.12\pm0.17^{b}_{D}$	$3.54\pm0.13^{b}_{D}$	$3.60\pm0.05^{b}_{B}$	$3.69\pm0.12^{a}_{D}$
	RT	$4.24\pm0.22^{b}_{D}$	$5.48\pm0.19^{c}_{E}$	$3.83\pm0.10^{b}_{E}$	$5.60\pm0.13^{b}_{D}$
	IRT	$3.02\pm0.18^{c}_{E}$	$2.93\pm0.04^{d}_{C}$	$2.57\pm0.20^{c}_{A}$	$3.19\pm0.06^{c}_{B}$
6	IAT	$4.42\pm0.05^{a}_{E}$	$3.70\pm0.09^{a}_{E}$	$3.85\pm0.14^{a}_{B}$	$4.26\pm0.30^{a}_{E}$
	RT	$5.04\pm0.17^{b}_{E}$	$6.30\pm0.32^{b}_{F}$	$3.60\pm0.13^{a}_{D}$	$5.90\pm0.15^{b}_{E}$
	IRT	$2.91\pm0.02^{c}_{E}$	$3.04\pm0.09^{c}_{C}$	$2.86\pm0.17^{b}_{C}$	$3.30\pm0.05^{b}_{C}$
7	IAT	$4.82\pm0.13^{a}_{F}$	$4.19\pm0.15^{a}_{F}$	$3.93\pm0.15^{a}_{B}$	$4.39\pm0.17^{a}_{E}$
	RT	$5.28\pm0.07^{b}_{F}$	$7.39\pm0.06^{b}_{G}$	$3.78\pm0.12^{a}_{E}$	$6.69\pm0.01^{b}_{F}$
	IRT	$2.48\pm0.11^{c}_{D}$	$2.77\pm0.07^{c}_{A}$	$2.48\pm0.16^{b}_{A}$	$3.37\pm0.15^{c}_{C}$
8	IAT	$5.60\pm0.06^{a}_{G}$	$4.24\pm0.11^{a}_{F}$	$4.20\pm0.29^{a}_{D}$	$5.48\pm0.04^{a}_{F}$
	RT	$5.85\pm0.09^{b}_{G}$	$7.85\pm0.13^{b}_{H}$	$4.40\pm0.08^{a}_{F}$	$6.48\pm0.09^{b}_{G}$
	IRT	$2.70\pm0.12^{c}_{D}$	$2.82\pm0.08^{c}_{A}$	$2.00\pm0.17^{b}_{B}$	$3.54\pm0.08^{c}_{D}$
9	IAT	$6.94\pm0.30^{a}_{H}$	$4.46\pm0.10^{a}_{G}$	$4.18\pm0.05^{a}_{D}$	$6.18\pm0.12^{a}_{G}$
	IRT	$2.95\pm0.11^{b}_{E}$	$3.11\pm0.10^{b}_{C}$	$2.30\pm0.12^{b}_{A}$	$3.27\pm0.06^{b}_{C}$
10	IRT	$2.90\pm0.08_{E}$	$2.98\pm0.11_{C}$	$3.11\pm0.09_{D}$	$3.59\pm0.08_{D}$
11	IRT	$3.18\pm0.07_{F}$	$2.89\pm0.06_{A}$	$3.30\pm0.07_{E}$	$3.79\pm0.10_{E}$
12	IRT	$3.10\pm0.10_{F}$	$3.04\pm0.03_{C}$	$3.18\pm0.05_{D}$	$3.28\pm0.09_{C}$
13	IRT	$3.00\pm0.11_{E}$	$3.29\pm0.14_{C}$	$3.23\pm0.16_{E}$	$3.48\pm0.07_{D}$

Different superscript small letters (a,b,c) represents statistical differences among groups ($p<0.05$). Different superscript capital letters (A,B,C,D,…) represents statistical differences amongst different days within the same group during storage ($p<0.05$).C: Control, IAT: Fish stored in ice at ambient temperature; RT: Refrigerated fish without ice; IRT: Fish stored in ice at refrigerator temperature.

According to FDA, the values were unacceptable on the 8th and 9th day, for samples stored at refrigerated temperature without ice and ambient temperature in ice, respectively. The lowest histamine values were recorded for the samples stored in ice at refrigerated temperature. The detectable levels were obtained for this group on the 7th day, and the values were under 10 mg/kg throughout the storage period. Therefore, this condition is found to be the most suitable method used to store fresh shad in order to avoid histamine health risk. Histamine values were well correlated with sensory and chemical quality parameters in terms of food safety. AL-BULUSHI *at al*. (2009) reported that mesophilic bacterial count of log 6–7 cfu/g has been associated with 50 mg/kg histamine. The suggested level reached by the control group on the 3rd day. On the other hand, the results of mesophilic HFB count on the 5th day were correlated with the amount of histamine obtained on the relating day for control group. The results of mesophilic bacteria counts obtained for samples stored in ice at ambient temperature supported the statement of AL-BULISHI *at al.* (2009), while the counts of psychrophilic total viable bacteria and HFB were in agreement with this statement, for the group which stored in refrigerator without ice, indicating a favourable temperature for such bacteria. Since both bacteria counts and histamine values were well below the permitted levels for the group stored in ice and at refrigerated temperature, the results gotten from this group also supports the above statement.

Although, significant variations were obtained for tryptamine levels of some groups on certain days, the values were found insignificant amongst the groups. Tryptamine values were observed to be below 7 mg/kg throughout the storage. The values of phenylethylamine decreased at the beginning of storage, thereafter, fluctuations in the levels occurred. Putrescine value of fresh fish was 0.73 mg/kg at the beginning of trial, then, the levels increased sharply up to 72.5 mg/kg for control group. Although, the putrescine levels of other groups also increased significantly, the rate was significantly slower for these groups, with the lowest values being at 2.49 mg/kg, representing the samples kept in ice at refrigerated temperature at the end of the storage period. Higher values were observed for cadaverine with the highest levels corresponding to control group. The values reached up to 166.69 mg/kg for control group on the 5th day, while the levels were 56 mg/kg and 120.02 mg/kg on the 8th and 9th day, for groups stored in ice at ambient temperature and without ice at refrigerated temperature, respectively. Fig 1. shows the samples stored in ice at refrigerated temperature which also contained the lowest cadaverine value and the result was 25.18 mg/kg at the end of storage.

Tyramine is a naturally occurring monoamine compound derived from the amino acid, tyrosine. Fresh fish contains little or no tyramine, but a large amount can be found in spoiled or fermented fish (FAO, 2013). Tyramine values also increased significantly during storage ($p<0.05$) with the highest values representing the control group at 54.63 mg/kg on the 4th day. The values of other groups were significantly lower in comparison to control group ($p<0.05$). The lowest values were attributed to the samples stored at refrigerated temperatures. Spermidine values of fresh fish was 12.32 mg/kg, and the levels decreased significantly in all groups throughout storage ($p<0.05$). Significant variations were also observed amongst the groups ($p<0.05$). The changes in the spermine values are usually insignificant up to the 6th day of storage with some exceptions, thereafter, fluctuations in the values were observed. The highest spermine level was determined to be 4.70mg/kg on the 7th day for the sample stored in ice at ambient temperature.

Biogenic amines in foods are reported to be indicators of freshness or spoilage in fish. Moreover, some migraines are known to BAs, particularly, tyramine and phenylethylamine. Although, tyramine might also act as a histamine potentiator (TAYLOR and LIEBER, 1979), the contribution of this biogenic amine to histamine poisoning is not clear. However, around 100-800 mg/kg of tyramine and 30 mg/kg of phenylethylamine have been reported to be toxic doses in foods (KORAL and KÖSE 2012). Our study showed that the levels of tyramine and phenylethylamine did not reach toxic levels within the storage period of this research

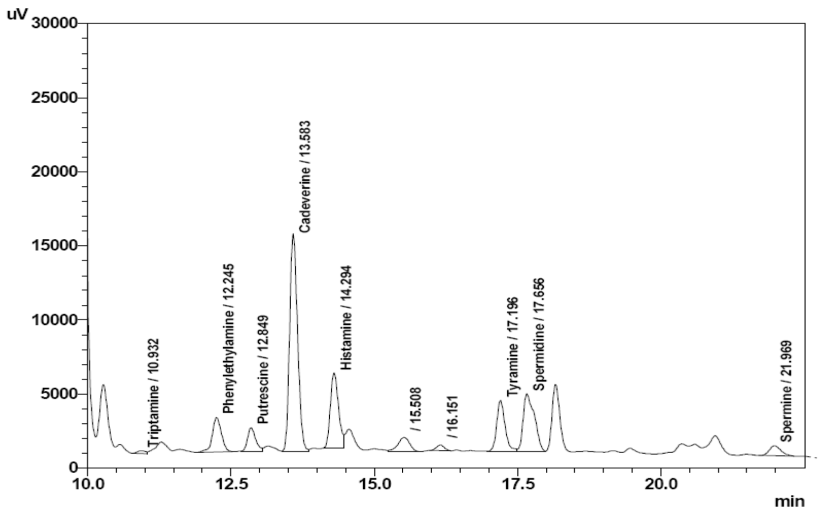

Fig. 1: Chromatogram belongs to shad samples (IRT group, 13th days).

Table 6: The values of biogenic amines for the shad samples stored under various conditions.

Day of Storage		Results of Biogenic amines (mg/kg)							
	Sample Groups	Tryptamine	Phenylethylamine	Putrescine	Cadeverine	Histamine	Tyramine	Spermidine	Spermine
0	**Fresh**	5.60±0.21	18.21±0.26	0.73±0.13	2.80±0.08	<0.86*	2.12±0.19	12.32±0.11	2.49±0.23
1	**C**	5.04±0.62$^{a}_{A}$	17.11±0.44$^{a}_{A}$	1.32±0.16$^{a}_{A}$	4.28±0.37$^{a}_{A}$	<0.86*	1.83±0.21$^{a}_{A}$	9.34±0.20$^{a}_{A}$	2.37±0.18$^{a}_{A}$
	IAT	6.45±0.50$^{b}_{A}$	15.82±0.54$^{b}_{A}$	1.37±0.14$^{a}_{A}$	4.82±0.23$^{b}_{A}$	<0.86*	2.10±0.15$^{a}_{A}$	11.70±0.42$^{b}_{A}$	2.67±0.10$^{a}_{A}$
	RT	5.98±0.31$^{b}_{A}$	16.07±0.35$^{b}_{A}$	0.95±0.12$^{b}_{A}$	5.35±0.29$^{c}_{A}$	<0.86*	1.95±0.21$^{a}_{A}$	10.52±0.34$^{a}_{A}$	3.34±0.19$^{b}_{A}$
	IRT	6.02±0.03$^{b}_{A}$	17.62±0.05$^{a}_{A}$	0.78±0.04$^{c}_{A}$	3.40±0.14$^{d}_{A}$	<0.86*	2.01±0.01$^{a}_{A}$	12.28±0.10$^{c}_{A}$	3.65±0.02$^{b}_{A}$
2	**C**	5.13±0.23$^{a}_{A}$	8,74±0,21$^{a}_{B}$	6,83±0,22$^{a}_{B}$	31,44±0,80$^{a}_{B}$	7.41±0.15$_{A}$	6,97±0,13$^{a}_{B}$	5,29±0,10$^{a}_{B}$	2,73±0,18$^{a}_{A}$
	IAT	5.43±0.18$^{a}_{B}$	16,83±0,39$^{b}_{B}$	2,94±0,24$^{b}_{B}$	15,60±0,71$^{b}_{B}$	<0.86*	4,74±0,22$^{b}_{B}$	9,75±0,36$^{b}_{B}$	2,67±0,31$^{a}_{A}$
	RT	5.85±0.37$^{a}_{A}$	17,75±0,49$^{b}_{B}$	1,66±0,11$^{c}_{B}$	9,25±0,37$^{c}_{B}$	<0.86*	2,82±0,20$^{c}_{B}$	9,74±0,35$^{b}_{A}$	3,08±0,11$^{b}_{A}$
	IRT	5.35±0.49$^{a}_{B}$	18,86±0,42$^{c}_{B}$	2,38±0,11$^{d}_{B}$	6,57±0,30$^{d}_{B}$	<0.86*	1,96±0,11$^{d}_{A}$	11,06±0,52$^{c}_{A}$	3,32±0,40$^{b}_{A}$
3	**C**	5.15±0.21$^{a}_{A}$	8,87±0,27$^{a}_{B}$	12,76±0,65$^{a}_{C}$	42,25±0,93$^{a}_{C}$	15.88±0.60$^{a}_{B}$	14,31±0,83$^{a}_{C}$	4,11±0,28$^{a}_{C}$	2,36±0,11$^{a}_{A}$
	IAT	4.93±0.32$^{a}_{C}$	12,59±1,00$^{b}_{C}$	8,89±0,44$^{b}_{C}$	35,70±0,71$^{b}_{C}$	4.93±0.13$^{b}_{A}$	6,67±0,47$^{b}_{C}$	6,18±0,28$^{b}_{C}$	1,81±0,11$^{b}_{B}$
	RT	5.73±0.38$^{b}_{A}$	18,06±0,77$^{c}_{B}$	1,70±0,11$^{c}_{B}$	7,59±0,52$^{c}_{C}$	1.55±0.11$^{c}_{A}$	3,27±0,16$^{c}_{C}$	9,30±0,44$^{c}_{A}$	2,85±0,21$^{c}_{A}$
	IRT	5.71±0.42$^{b}_{B}$	17,25±0,64$^{c}_{A}$	0,92±0,10$^{d}_{A}$	4,82±0,28$^{d}_{C}$	<0.86*	2,02±0,15$^{d}_{A}$	8,13±0,25$^{d}_{B}$	3,34±0,30$^{d}_{A}$
4	**C**	5.48±0.25$^{a}_{A}$	8,10±0,28$^{a}_{B}$	55.91±1,32$^{a}_{D}$	126.32±1,38$^{a}_{D}$	35.86±0.64$^{a}_{C}$	54,63±0,89$^{a}_{D}$	4,12±0,30$^{a}_{C}$	2,83±0,25$^{a}_{A}$
	IAT	4.91±0.13$^{b}_{C}$	12,20±0,40$^{b}_{C}$	3,94±0,11$^{b}_{D}$	25,83±0,86$^{b}_{D}$	8.26±0.60$^{b}_{B}$	6,57±0,33$^{b}_{C}$	6,35±0,37$^{b}_{C}$	3,10±0,14$^{a}_{C}$
	RT	5.17±0.23$^{a}_{A}$	16,90±0,28$^{c}_{A}$	2,70±0,12$^{c}_{C}$	10,41±0,61$^{c}_{B}$	1.88±0.14$^{c}_{A}$	3,16±0,28$^{c}_{C}$	7,53±0,32$^{c}_{B}$	2,92±0,16$^{a}_{A}$
	IRT	5.18±0.29$^{a}_{B}$	17,54±0,61$^{c}_{A}$	1,69±0,18$^{d}_{C}$	8,13±0,24$^{d}_{D}$	<0.86*	2,76±0,14$^{c}_{B}$	8,25±0,42$^{d}_{B}$	2,37±0,12$^{b}_{B}$
5	**C**	5.38±0.42$^{a}_{A}$	9,85±0,35$^{a}_{C}$	72,57±0,98$^{a}_{E}$	166,69±1,81$^{a}_{E}$	48.82±1.68$^{a}_{D}$	46,31±0,98$^{a}_{E}$	3,39±0,25$^{a}_{D}$	2,27±0,15$^{a}_{A}$
	IAT	4.92±0.47$^{a}_{C}$	16,04±0,80$^{b}_{B}$	4,36±0,29$^{b}_{E}$	27,80±0,71$^{b}_{E}$	16.88±0.25$^{b}_{C}$	6,37±0,36$^{b}_{C}$	7,65±0,40$^{b}_{D}$	2,44±0,30$^{a}_{A}$
	RT	5.25±0.34$^{a}_{A}$	16,76±0,39$^{b}_{A}$	3,36±0,21$^{c}_{D}$	12,70±0,57$^{c}_{D}$	2.59±0.18$^{c}_{B}$	3,16±0,25$^{c}_{C}$	8,50±0,72$^{b}_{B}$	2,64±0,31$^{a}_{A}$
	IRT	5.10±0.20$^{a}_{B}$	15,31±0,63$^{b}_{C}$	3,52±0,47$^{c}_{D}$	19,35±0,94$^{d}_{E}$	<0.86*	3,67±0,43$^{c}_{C}$	6,87±0,25$^{b}_{C}$	2,62±0,33$^{a}_{B}$

Different superscript small letters (a,b,c) represents statistical differences among groups ($p<0.05$). Different superscript capital letters (A,B,C,D,..) represents statistical differences amongst different days within the same group during storage ($p<0.05$).C: Control, IAT: Fish stored in ice at ambient temperature; RT: Refrigerated fish without ice; IRT: Fish stored in ice at refrigerator temperature*: LOD(Limit of detection for histamine). (LOD values for histamine:0.86; for putrescine:0.56; for tryptamine: 1.80; Phenylethylamine: 1.50; Cadeverine: 0.66; Tyramine: 0.87; Spermidine: 0.65; Spermine: 0.71 mg/kg.).

Limited studies exist for the biogenic amine contents of spotless shad. In our previous studies on salted shad products, obtained from Turkey and Greece, we determined histamine values to be below 20 mg/kg, although, higher amounts of tyramine and cadaverine which is about 85mg/kg and 100 mg/kg, respectively, were observed for products stored at ambient temperatures, while low values correspond to the samples kept at chilled conditions (KÖSE *et al*., 2011; KORAL *et al*., 2013).

5. CONCLUSIONS

Good sensory quality was observed in fresh shad with the addition of ice during refrigerated storage. Ice application and refrigerated storage increased the shelf-life for 10 days. The worst sensory results, represented control group which was without ice and ambient temperature storage shelf life less than three days. Storing fresh shad in ice also helped to improve its physico-chemical quality and decrease biogenic amine development. Therefore, keeping fresh shad in ice under refrigerated storage is advised, in order to delay histamine formation and retard quality loss prior to processing or fresh market. The results suggest that using ice can improve the shelf-life of shad, stored at ambient and refrigerated temperatures as relating to food quality and safety.

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