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Nucleotide degradation and biogenic amine formation of wild white grouper (*Epinephelus aeneus*) stored in ice and at chill temperature (4 °C)

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Abstract

Sensory (cooked and uncooked), chemical (proximate composition, TVB-N, nucleotide degradation products and biogenic amines) and microbiological quality (TVC and total coliform) changes were investigated during storage of ungutted white grouper kept in ice and at chill temperature (4 °C). According to the sensory assessment, the shelf life of white grouper was 16 days in ice and 4 days for fish stored at chill temperature. TVB-N values increased with storage time. Amines found in white grouper stored in ice were TMA, putrescine, cadaverine, 2-phenylethylamine, dopamine, agmatine, tryptamine and serotonin. Histamine, spermine, spermidine were never detected with either storage condition. The acceptability limit in terms of microbial count was exceeded at 8 days in ice and at 4 days for fish stored at chill temperature. Total coliform count was 2.8 $\log_{10}$ cfu/ml at 1 day and reached 10^5 cfu/ml for both storage conditions.

Keywords: White grouper; TVB-N; Biogenic amines; Nucleotide degradation products; *K* values

1. Introduction

Grouper species of the Serranidae family are considered to be a highly desirable fish in Asia and around the world (Lin & Shiau, 2003). It is estimated that grouper production world-wide was about 10,000 tons in 2000 (Lupatsch & Kissil, 2005). White grouper are a promising species for aquaculture because of their high market value and good taste, rapid growth rates, hardiness and disease resistance (Hassin et al., 1997; Fukuhara, 1989; Kuo, 1995; Lupatsch & Kissil, 2005. Although global demand for grouper has increased, it is reported that these fish are among the most endangered marine fish species in many parts of the world (Sadovy, 1989; Zabala, Garcia-Rubies, Louisy, & Sala, 1997). The *Epinephelus* genus of groupers, a threatened grouper genus (Maggio, Andaloro, Hemidac, & Arculeoa, 2005; Morris, Roberts, & Hawkins, 2000), are mostly tropical species with a few found in subtropical and temperate waters, e.g., only five species are found in Mediterranean waters (Dulcic, Tutman, & Caleta, 2006; Heemstra & Randall, 1993). Gorshkova, Protas, Ben-Atia, and Gorshkov (2002) reported that the white grouper, *Epinephelus aeneus,* is widely known and appreciated in the Mediterranean basin, where they commonly live on sandy, silty or muddy bottoms (Maggio et al., 2005; Tortonese, 1970).

Biogenic amines, especially histamine and tyramine, are toxic substances causing illness in man and animals following ingestion of foods containing them (Shalaby, 1996). Biogenic amines are produced by microbial decarboxylation of amino acids in food products. The most significant

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biogenic amines occurring in foods are histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, spermidine and agmatine.

Many studies have been done related to the culture, nutrition and hybridization of white grouper. However, there were no papers in the literature about the nutritive value and quality assessment of grouper with storage in ice. White grouper (*E. aeneus*) are important in Turkey because of their high market value. They are retailed whole in ice and obtain a relatively high price. The aim of this study was to investigate white grouper's nutritive value and quality parameters using sensory, chemical and microbiological methods.

2. Materials and methods

2.1. Fish storage

Wild white grouper (*E. aeneus*) were obtained from the Mediterranean Sea. They were caught in January 2007 by trawlnet. The weight and length of the samples were 500–600 g and 25–30 cm. They were stored in boxes with ice on-board after catching and delivered to the laboratory in ice. Wild white grouper were left ungutted as is the custom in Turkey and divided into two lots. One lot was surrounded by flake ice at a 2:1 fish to ice ratio and stored in a refrigerated box at 4 °C. Flake ice was added to the boxes as required. The second lot was placed in the refrigerated box without ice. Data were obtained using three white grouper for each of the assay at 1, 4, 8, 12, 16, 19 and 22 days of storage.

2.2. Proximate analysis

The white grouper samples were analyzed in triplicate for their proximate composition. Lipid content was done according to the Bligh and Dyer (1959) method. Moisture content was determined according to Mattissek, Schnepel, and Steiner (1992) method. Total crude protein and ash content were determined by using the Kjeldahl method (AOAC, 1998a, chap. 35) and the AOAC method (AOAC, 1998b, chap. 35), respectively.

2.3. Sensory analysis

Triplicate samples from each of the two storage conditions were taken at regular intervals for sensory analysis. The sensory assessment was conducted for ungutted raw white grouper using the Tasmanian Food Research Unit Scheme as modified by Alasalvar et al. (2001). The panel consisted of seven regular assessors; each was trained in fish quality assessment before the experiment. Each assessor was responsible for evaluating five simple parameters (appearance, eyes, gills, belly and vent condition), scoring demerit points from 0 to a maximum of 3, where 0 represented best quality and a higher score indicated poorer quality. For determination of the shelf life of the fish, the panel members were asked to state whether the fish were acceptable or not.

Cooked white grouper were analysed using the Torry Scheme (Howgate, 1982). A scale from 10 to $\leqslant 3$ was used, 10 showed absolutely fresh and $\leqslant 3$ completely putrid or spoiled. Fish fillets were cooked in a M1610N Samsung microwave (Kuala Lumpur, Malaysia) for 2.5 min (600 W) and then served to the panelists to assess.

2.4. Analytical method

2.4.1. General

The TVB-N content of white grouper was determined according to the method of Antonocopoulus (1973), and expressed as mg TVB-N per 100 g white grouper muscle. ATP and its breakdown products were analyzed according to the method of Özogul, Taylor, Quantick, and Özogul (2000). Biogenic amines analysis was done using the method of Özogul, Taylor, Quantick, and Özogul (2002).

2.4.2. Apparatus and columns

A Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector and two binary gradient pumps (Shimadzu LC-10AT), autosampler (SIL 20AC), column oven (CTO-20AC), and a communication bus module (CBM-20A) with valve unit FCV-11AL was used. For the biogenic amine analyses, the column was a reverse-phase, Spherisorb 5 Si C18 pH-St, 250 × 4.6 mm (Phenomenex, Macclesfield, Cheshire, UK). For nucleotide determinations, the column was a Prodigy 5μ ODS (2), 150 × 4.60 mm, (Phenomenex). All nucleotides and biogenic amines standards were purchased from Sigma–Aldrich (Munich, Germany). For both analyses, the mobile phase consisted of acetonitrile and HPLC grade water.

2.4.3. Sample preparation for nucleotides analysis

Extraction was carried out according to Ryder (1985). Five grams meat from white grouper without skin were hand chopped and extracted with 25 ml of 0.6 M perchloric acid using an Ultra-Turaks (T 25 basic IKA-WERKE, Staufen, Germany) for 1 min in an ice bath. The extraction mixture was filtered through filter paper (Scleicher & Schuell, 5951/2 110 mm) and after that, 10 ml of filtrate was quickly neutralized to pH 6.5–6.8 with 1 M KOH using 315*i* pH meter (Weilheim, Germany). The neutralized supernatant was allowed to stand for 30 min in an ice bath to precipitate most of the potassium perchlorate, which was then removed by filtration using filter paper (Scleicher & Schuell, 5951/2 110 mm). The filtrate solution was made up to 20 ml and then stored at −18 °C until analysed.

K, K_i, H and G values were calculated by the procedures described by Saito, Arai, and Matsuyoshi (1959), Karube, Matsuoka, Suzuki, Watanabe, and Toyama (1984), Luong, Male, Masson, and Nguyen (1992), and Burns, Kee, and

Irvine (1985), respectively, and expressed as percentages. The formulas used are as follows:

$$K \text{ value } (\%) = [(\text{Hx} + \text{INO})/(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Hx} + \text{INO})] \times 100$$

$$K_i \text{ value } (\%) = [(\text{Hx} + \text{INO})/(\text{IMP} + \text{Hx} + \text{INO})] \times 100$$

$$G \text{ value } (\%) = [(\text{Hx} + \text{INO})/(\text{AMP} + \text{IMP} + \text{INO})] \times 100$$

$$H \text{ value } (\%) = [(\text{Hx})/(\text{IMP} + \text{Hx} + \text{INO})] \times 100$$

2.4.4. Sample preparation for biogenic amine analysis

A rapid HPLC method was used for biogenic amine determinations. Fish muscle (5 g) was taken from the dorsal part of the fish fillet without skin and transferred to a 250 ml centrifuge tube. The sample was then homogenized using the Ultra-Turax with 20 ml 6% TCA for 3 min, centrifuged using a Hettich 32R centrifuge (Tuttlingen, Germany) at 10,000 rpm/11,180*g* for 10 min at 4 °C and filtered through Whatman No. 1 filter paper (Maidenstone, UK). The aliquot was brought to 50 ml with distilled water and was stored in a freezer (−18 °C) until derivitisation. Before injection into the HPLC, 2 ml of fish aliquot were mixed with benzoyl chloride.

2.4.5. Microbiological analysis

White grouper samples were taken to estimate total viable counts (TVC) from each of the three different fish stored under two storage conditions. Dorsal fish muscle (10 g) were mixed with 90 ml of Ringer solution [Merck, 1.15525.0001, Darmstadt, Germany] and then Stomachered [IUL instrument, Barcelona, Spain] for 3 min. Further decimal dilutions were made, and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar [Merck, 1.05463] plates, in triplicate, after which they were incubated for 2 days at 30 °C.

For total coliform bacteria, Violet Red Bile Agar (VRBA, Oxoid, CM0107, Hampshire, England) was used and prepared according to the manufacturer's instructions. Fish samples (10 g) were mixed with 90 ml of Ringer solution and then Stomachered for 3 min. Further decimal dilutions were made. Two pour plating methods were used (FDA, 1998). One milliliter aliquots of each dilution were transferred to petri dishes and VRBA poured on the aliquots and allowed to solidify. Additional VRBA was poured once again into the plates. They were incubated for 24 h at 30 °C. Red colour colonies were examined after incubation (Bridson, 1988).

2.5. Statistical analysis

For data analysis, the Student *t*-test, standard deviation and coefficient of variance were used. Significance of differences was defined at $P < 0.05$. Statistical comparisons were based on three samples for each treatment for each specific storage time.

3. Results and discussion

3.1. Proximate composition

The proximate composition of white grouper is given in Table 1. The results showed that the protein level of the white grouper is high, while lipid content is low.

Other fish species such as wild turbot caught in the Black sea (Özogul et al., 2006), wild and cultured sea bass (Beklevik, Polat, & Özogul, 2005; Erkan & Özden, 2007, wild sea bream (Kuley, Ozogul, & Ozogul, 2005) and European eel (Özogul, Özyurt, Özogul, Kuley, & Polat, 2005) caught in the Mediterranean sea, Mediterranean horse mackerel (Tzikas, Amvrosiadis, Soultos, & Georgakis, 2007) and fresh water fish species such as rainbow trout (Michalczyk & Surówka, 2007) have lower protein and higher lipid content than those of white grouper. Lin and Shiau (2003) reported that protein content of grouper (*Epinephelus malabaricus*) fed different lipids were between 16.0% and 16.5%. The chemical composition of fish varies widely, not only for the same species, but also within the individual fish depending on age, sex, migratory behaviour, environment and seasonal variation.

3.2. Sensory assessment

Fig. 1 shows the total demerit points of raw white grouper stored in ice for 22 days and in a refrigerator

Table 1
Proximate composition of white grouper

Proximate composition	Mean value (%)	SD	%CV
Protein	22.4	0.1	0.44
Lipid	0.61	0.03	4.91
Moisture content	75.2	0.2	0.26
Ash content	1.17	0.03	2.56

Data are expressed as mean value of three samples. SD, standard deviation; CV, coefficient of variance.

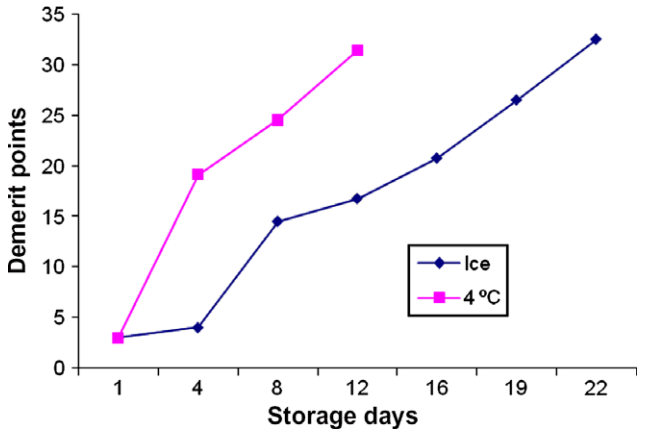

Fig. 1. Demerit points for raw white grouper during storage in ice and at 4 °C.

(4 °C) for 12 days. The initial quality characteristics of the white grouper were very bright appearance, fresh odours, firm texture and normal vent conditions. Demerit points increased with storage time, especially at 4 °C without ice. The acceptable shelf life of white grouper was found to be 16 days for iced and 4 days at 4 °C, corresponding with a demerit score of 20.8 and 19.1, respectively.

Significant differences ($P < 0.05$) were found in the demerit points between white grouper stored in ice and at 4 °C for all days except day 1. Lougovois, Kyranas, and Kyrana (2003) reported a shelf life of 16.5 days for iced ungutted seabream according to the Quality Index Method (QIM). Chytiri, Chouliara, Savvaidis, and Kontominas (2004) for iced ungutted trout and Özogul, Özogul, Kuley, et al. (2006) for iced gutted trout observed 15–16 and 12–15 days of acceptable shelf life, respectively. A shelf-life of 10 and 7 days for iced whole Mediterranean horse mackerel and blue jack mackerel were found, respectively (Tzikas et al., 2007). Sardines stored at 4 °C had a shelf life of 3 days (Özogul, Polat, & Özogul, 2004).

Changes of the sensory quality of cooked white grouper are shown in Fig. 2. Acceptability scores for odor, taste and texture of white grouper decreased with time of storage. Iced fish samples had higher scores than those at 4 °C. At the time of rejection, the demerit points were 6.25 at 19 days for iced and 5.30 at 8 days for uniced. Alasalvar et al. (2001) observed that a score of 4 was not acceptable for panelists evaluating cooked sea bream stored in ice.

3.3. Chemical analysis

3.3.1. TVB-N

The mean TVB-N values are shown in Tables 2 and 3. Although TVB-N concentration fluctuated during iced storage, it increased with storage time at 4 °C, which is in agreement with other studies (Grigorakis, Taylor, & Alexis, 2003; Özogul et al., 2004; Özogul, Özyurt, et al., 2005; Tejada & Huidobro, 2002).

Table 2
TVB-N values of iced white grouper

Storage Time (days)	Mean value (mg/100 g)	SD	% CV
1	15.4	0.69	4.48
4	20.5	1.72	3.40
8	21.7	0.49	2.26
12	29.9	1.36	4.54
16	28.8	1.40	4.86
19	50.5	0.92	1.82
22	47.9	0.89	1.85

Table 3
TVB-N values of white grouper stored at 4 °C

Storage time (days)	Mean value (mg/100 g)	SD	% CV
1	15.4	0.69	4.48
4	22.8	0.50	2.18
8	73.3	3.71	5.06
12	122.	1.39	1.14

When fish was rejected in terms of sensory assessment, TVB-N value was 28.8 mg/100 g for iced storage conditions and 22.8 mg/100 g for uniced. When the level of TVB-N reaches 35–40 mg/100 g of fish muscle, the fish is usually regarded as spoiled (Lakshmanan, 2000). In this study those values were not reached when the microbial count became 10^7 at 4 days for chill temperature and 8 days for iced. Thus, TVB-N does not seem to be the major cause of spoilage of whole white grouper, which is in agreement with other studies for fish species such as sea bass (Kyrana & Lougovois, 2002), turbot (Özogul, Özogul, Kuley, et al., 2006), and gilthead sea bream (Tejada & Huidobro, 2002).

3.3.2. Nucleotide degradation products

The nucleotide breakdown patterns in the white muscle of white grouper stored in ice and at 4 °C are shown in Figs. 3 and 4, respectively. Nucleotide degradation was

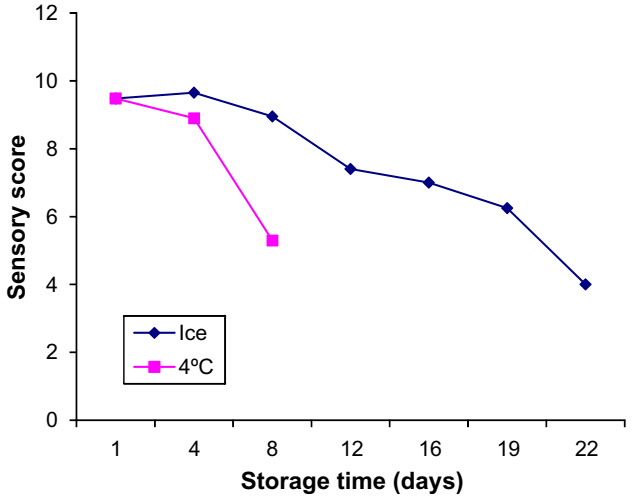

Fig. 2. Changes in sensory quality of cooked white grouper during storage.

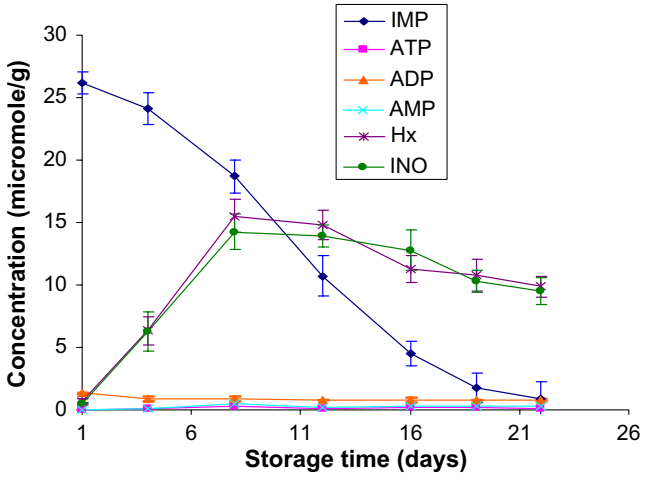

Fig. 3. Changes in the nucleotide degradation products of white grouper stored in ice. Each point presented is the mean value of three determinations for each sampling. Bars represent the standard deviation.

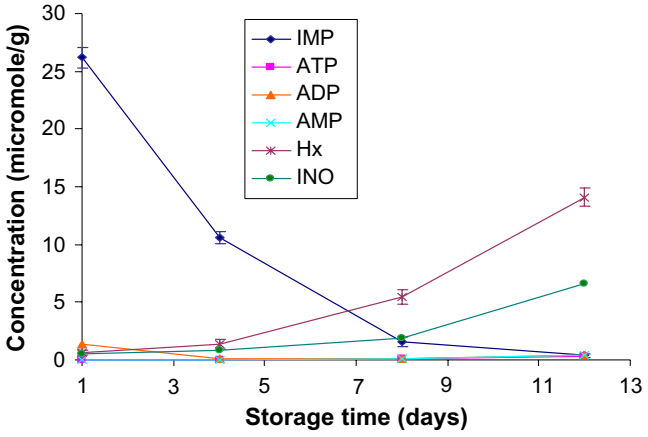

Fig. 4. Changes in the nucleotide degradation products of white grouper stored at 4 °C. Each point presented is the mean value of three determinations for each sampling. Bars represent the standard deviation.

found to be more rapid in white grouper stored uniced than iced. ATP and AMP levels were found to be very low (0.03 μmol/g), while ADP concentrations was 1.35 μmol/g on day 1. For cultured sea bream stored in ice (Alasalvar et al., 2001; Grigorakis et al., 2003; Kuley et al., 2005) and for wild sea bass stored in ice (Özogul, Gökbulut, Özyurt, Özoğul, & Dural, 2005) low values of ATP, ADP and AMP were also obtained. IMP, which provides the characteristic sweetness of fresh fish muscle (Church, 1998; Mazorra-Manzano, Pacheco-Aguilar, Díaz-Rojas, & Lugo-Sánchez, 2000), decreased sharply from an initial concentration of 26.2 μmol/g to 0.89 μmol/g during iced storage and 0.37 for uniced at the end of storage. In other studies, smaller IMP changes were observed, ranging from below 6 μmol/g to below 4 μmol/g for sea bass and sardine (Özogul, Gökbulut, et al., 2005; Özogul, Özoğul, & Kuley, 2007).

Hx accumulation in fish tissue reflects the initial phase of autolytic deterioration as well as bacterial spoilage (Mazorra-Manzano et al., 2000; Woyewoda, Shaw, Ke, & Burns, 1986). Hx was found to be useful as an index of the freshness quality of sea bass (Özogul, Gökbulut, et al., 2005). Huidobro, Pastor, and Tejada (2001) reported that the change from Ino to Hx was very fast, although the change slowed down by the end of storage for iced whole and gutted seabream. For grouper samples kept at 4 °C, the initial level of Hx (0.66 μmol/g) and INO (0.48 μmol/g) constantly increased and reached 14.1 μmol/g and 6.60 μmol/g at 12 days, respectively. However, Hx and INO values increased sharply until 8 days and gradually dropped to 9.9 and 9.5 μmol/g, respectively, at the end of iced storage. Similarly, Özogul et al. (2007) reported that the value of Hx showed an increase until 10 days and then a drop in sardines stored without ice at 4 °C. Contrary to those stored at 4 °C, Hx for iced white grouper was not a useful freshness indicator, in agreement with other studies (Alasalvar et al., 2001; Kyrana, Lougovois, & Valsamis, 1997).

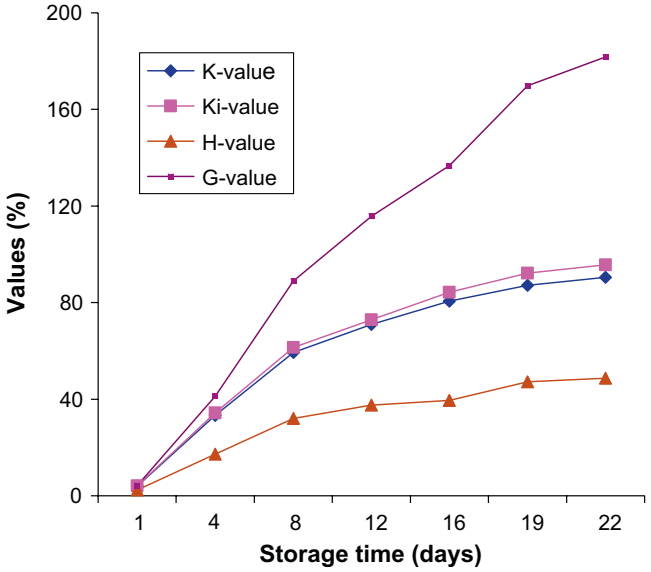

Fig. 5. K, K_i, G and H value changes of white grouper stored in ice.

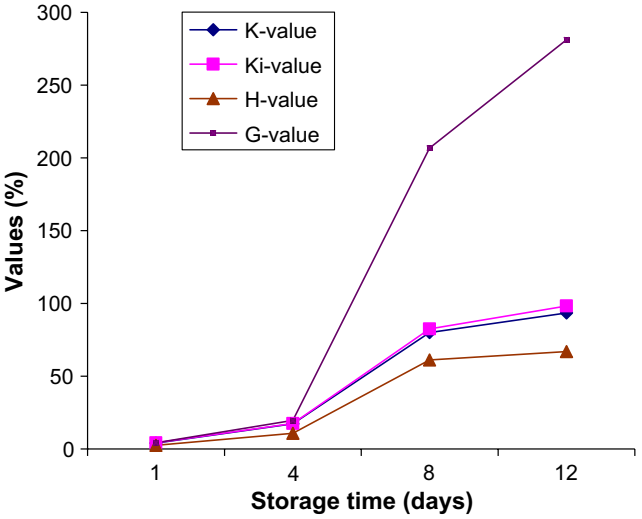

Fig. 6. K, K_i, G and H values changes of white grouper stored at 4 °C.

3.3.3. K and related values

The K, K_i, G, and H values of white grouper stored in ice and without ice are shown in Figs. 5 and 6, respectively. Linear increases were observed in the K and related values with storage time in white grouper stored under both conditions. However, these increases were rapid in samples stored at 4 °C after 4 day. Initial K, K_i, and G values for each storage condition were similar (~4%), but lower for the H value (~2%). After 4 days for fish stored at 4 °C, the highest increases were observed for G values. H values rose slower than other values. The observed maximum values of K, K_i, H and G were calculated as 90%, 96%, 49%, and 182% for iced (22 days) and 93%, 98%, 67%, and 281% for fish stored at 4 °C (12 days) at the end of their storage period.

At the limit of acceptability K, K_i, H and G value were approximately 81%, 84%, 39%, and 137%, respectively, for iced white grouper (16 days). The K, K_i, H and G values were low for samples stored at 4 °C and thus is absolutely not corresponded to sensory analysis. When bacterial counts exceed 10^7 cfu/g (after 4 days) these values rapidly increased and reached approximately 80%, 82%, 61% and 207%, respectively, at 8 days storage. The rapid increase of the K and related values is due to the sharp drop of IMP in the fish flesh. Lougovois et al. (2003) reported that Ki values lower than 10% represented very fresh farmed gilthead sea bream, while K_i values of 33–35% would indicate fish at the end of its shelf life (16 days). These values of 33–35% were exceeded when the iced white grouper still seemed to be acceptable. In other studies related to fish species such as sea bass, sea bream, eel, and sardine (Alasalvar et al., 2001; Grigorakis et al., 2003; Özogul, Gökbulut, Özoğul, & Özyurt, 2006; Özogul et al., 2004; Özogul, Özogul, & Gökbulut, 2006), K, K_i, H and G value were found as 22–85%, 70–85%, 4–15%, and 54–81% on the day of marginal acceptability, respectively.

3.3.4. Ammonia and biogenic amines formation

Tables 4 and 5 show the ammonia and biogenic amine contents of ungutted white grouper muscle. A slight increase in the amount of ammonia was observed during storage. Initial ammonia amounts of 0.02 mg/100 g reached 1.76 mg/100 g for fish stored at 4 °C and 3.54 mg/100 g for iced storage at the end of the storage period. Biogenic amines in muscle were only found in small amounts at the beginning of storage. Biogenic amines found in iced white grouper mainly consisted of TMA, putrescine, cadaverine, 2-phenylethylamine, dopamine, agmatine, tryptamine and serotonin. Tyramine was only found in white grouper stored at 4 °C. Histamine, spermine, spermidine and tryptamine were not found in any sample.

Marks (Rupp) and Anderson (2005) reported that histamine is not always found in spoiled fish, and that putrescine and cadaverine may be better markers of decomposition. Lakshmanan, Shakila, and Jeyasekaran (2002) observed that cadaverine and putrescine-forming bacteria could survive and multiply rapidly between 9 and 12 days, and contribute to the formation of amines during the ice storage of emperor fish and of shrimp. In this study, although putrescine and cadaverine were not found in the beginning of the storage, putrescine increased faster than cadaverine during storage. At the end of the experiment when concentrations of cadaverine and putrescine reached 8.04 mg/100 g for iced (at 22 days) and 7.79 mg/100 g for fish stored at 4 °C, the total viable counts (TVC) corresponded to 8.61 $\log_{10}$ cfu/ml and 9.06 $\log_{10}$ cfu/ml, respectively.

At the time the fish were still evaluated as acceptable, biogenic amines having the highest values in white grouper stored in ice and at 4 °C were TMA (7.85 vs. 2.63 mg/100 g), putrescine (3.48 vs. 2.05 mg/100 g) and serotonin (1.02 vs. 0.39 mg/100 g), respectively.

Table 4
The concentration of ammonia and biogenic amines (mg/100 g) of white grouper stored in ice

Storage days	AMN	PUT	CAD	2-Phenyl.	SPD	HIS	SPN	TMA	TYR	TRP	DA	AGM	SER
1	0.02[a] ± 0.01[b]	–	–	–	–	–	–	0.17 ± 0.17	–	–	–	–	0.01 ± 0.01
4	0.12 ± 0.05	1.75 ± 0.84	0.11 ± 0.01	–	–	–	–	2.05 ± 0.94	–	–	0.01 ± 0.01	0.01 ± 0.01	0.36 ± 0.28
8	0.26 ± 0.02	2.55 ± 0.40	0.30 ± 0.12	0.05 ± 0.01	–	–	–	3.00 ± 1.10	–	–	–	–	–
12	0.94 ± 0.43	3.04 ± 0.90	0.42 ± 0.22	0.08 ± 0.03	–	–	–	5.52 ± 0.81	–	–	0.01 ± 0.01	0.01 ± 0.01	0.18 ± 0.13
16	2.01 ± 1.20	3.48 ± 1.09	0.74 ± 0.17	0.05 ± 0.01	–	–	–	7.85 ± 2.63	–	–	0.13 ± 0.09	0.05 ± 0.02	1.02 ± 0.21
19	2.20 ± 1.12	5.33 ± 2.02	0.67 ± 0.15	0.04 ± 0.03	–	–	–	12.01 ± 2.01	–	–	–	0.03 ± 0.02	0.30 ± 0.16
22	3.54 ± 1.08	8.04 ± 1.75	0.85 ± 0.29	0.03 ± 0.02	–	–	–	17.38 ± 3.12	–	0.01 ± 0.01	0.12 ± 0.05	0.04 ± 0.02	0.79 ± 0.53

AMN, ammonia; PUT, putrescine; CAD, cadaverine; 2-Phenyl, 2-Phenyl-ethylamine; SPD, spermidine; HIS, histamine; SPN, spermine; TMA, trimethylamine; TYR, tyramine;TRP, tryptamine; DA, dopamine, AGM, agmatine; SER, serotonin.

–, not detected.

[a] Mean value.

[b] Standard deviation ($n = 3$).

Table 5
The concentration of ammonia and biogenic amines (mg/100 g) of white grouper stored at 4 °C

Storage days	AMN	PUT	CAD	2-Phenyl.	SPD	HIS	SPN	TMA	TYR	TRP	DA	AGM	SER
1	0.02[a] ± 0.01	0.00 ± 0.00	–	–	–	–	–	0.17 ± 0.15	–	–	–	–	0.01 ± 0.01
4	0.25 ± 0.05	2.04 ± 1.03	0.16 ± 0.06	0.01 ± 0.01	–	–	–	2.63 ± 1.05	–	–	0.01 ± 0.01	0.08 ± 0.06	0.39 ± 0.28
8	0.37 ± 0.07	4.47 ± 1.29	0.26 ± 0.20	0.03 ± 0.01	–	–	–	9.38 ± 2.43	0.02 ± 0.02	–	0.04 ± 0.02	0.36 ± 0.16	0.69 ± 0.26
12	1.75 ± 0.21	7.79 ± 1.66	0.34 ± 0.14	0.05 ± 0.00	–	–	–	15.98 ± 3.67	–	–	0.02 ± 0.00	0.25 ± 0.04	0.54 ± 0.12

AMN, ammonia; PUT, putrescine; CAD, cadaverine; 2-Phenyl, 2-Phenyl-ethylamine; SPD, spermidine; HIS, histamine; SPN, spermine; TMA, trimethylamine; TYR, tyramine; TRP, tryptamine; DA, dopamine, AGM, agmatine; SER, serotonin.
[a] Mean ± standard deviation ($n = 3$); –, not detected.

TMA concentration increased with storage time and reached 7.85 vs. 2.63 mg/100 g before rejection in terms of sensory analysis. Phenylethylamine was not found at 1 day. Both of these amines increased slowly and at the end of acceptable shelf life reached 2.63 and 7.85 mg/100 g for iced and uniced fish, respectively. Tyramine was only found in low concentration (0.02 mg/100 g) for spoiled white grouper stored at 4 °C at 8 days.

3.4. Microbiological quality

The initial quality of the fish used in this study was good, as indicated by a low initial number of bacteria (10^3 cfu/g) before the fish were stored. Bacteria grew most quickly in white grouper at 4 °C (Fig. 7). The spoilage levels of 10^7 cfu/g reported by IFST (1999) were reached when the white grouper stored at chilled temperature was considered spoiled according to the sensory panel. Thus, the microbiological changes of the fish stored at 4 °C were in good agreement with the results of the sensory evaluation. Similar results were found by Özogul et al. (2004) for gutted sardines stored in boxes at 4 °C. However, the level of 10^7 had already been reached in iced white grouper before the rejection time. The result obtained from sensory evaluation, after iced treatment, showed a longer shelf life when compared with microbiological assessment (16 vs. 8 days). Initial aerobic plate count of 3.91 log10 cfu/ml reached 7.15 log10 cfu/ml at 8 days and towards the end of storage only minor changes occurred (9.61 log10 cfu/ml at 22 days) for iced white grouper samples. For iced gutted turbot, a longer shelf life (13–14 days) was reported according to the microbiological acceptability limit (Özogul, Gökbulut, et al., 2006). Total viable counts of the fresh Mediterranean horse mackerel muscle were between 3.09 and 4.95 log10 cfu/g (Tzikas et al., 2007). Lakshmanan et al. (2002) found that total mesophilic bacteria in fresh emperor fish was 10^5 cfu/g at 0 day and iced storage conditions decreased the bacterial counts.

For iced whole gilthead sea bream Lougovois et al. (2003) reported lower microbial counts (10^8–10^9 cfu/g) at the end of the trial (18 days).

Changes of total coliform bacteria present in white grouper muscle are given in Fig. 8. Initial total coliform counts were 2.8 $\log_{10}$ cfu/ml, and these remained below the level of 10^6 in all analyzed samples during the storage periods. At the end of the storage time, maximum coliform bacterial counts were calculated as 5.26 $\log_{10}$ cfu/ml for iced at 22 days and 5.41 $\log_{10}$ cfu/ml for 4 °C at day 12.

Sensory assessment showed that white groper stored in ice and at chill temperature (4 °C) had a 16 day and 4

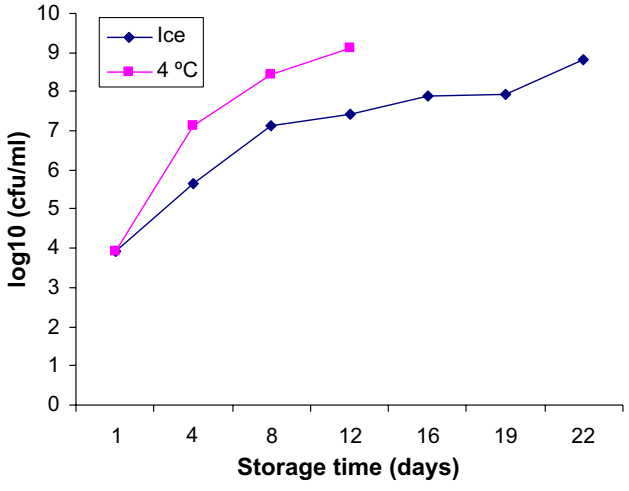

Fig. 7. Total viable count (TVC) of white grouper stored in ice and at 4 °C.

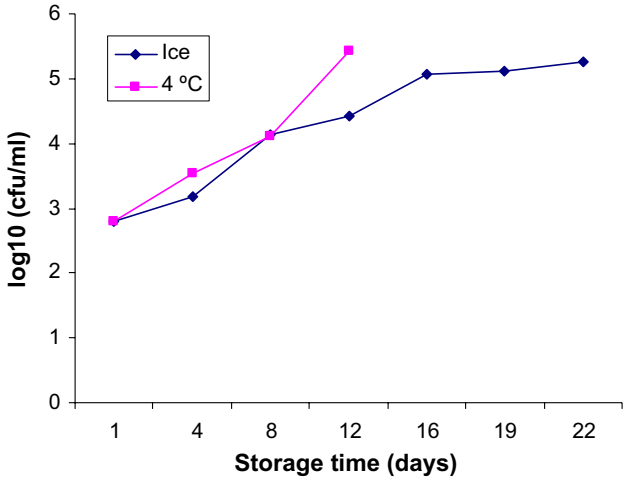

Fig. 8. Total coliform bacteria in white grouper stored in ice and at 4 °C.

day shelf life, respectively. Sensory evaluation was in good agreement with the results of the microbiological assessment for white grouper stored at 4 °C but not for iced white grouper. TVB-N were not be correlated with sensory and microbiologic assessment and does not seem to be the major cause of spoilage. Hx could be usefull freshness indicator for 4 °C but not for iced white grouper. At the limit of acceptability K, K_i, H and G value were found approximately 81%, 84%, 39%, and 137%, respectively, for iced white grouper (16 days) Histamine, being biologically active amine, were not found.

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