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Changes in Biochemical, Sensory and Microbiological Quality Indices of Common Sole (*Solea solea*) from the Mediterranean Sea, During Ice Storage

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Abstract

The quality of wild common sole (*Solea solea*) stored in ice was investigated by chemical (*K* value, biogenic amines, protein degradation (SDS and SDS-PAGE), pH, peroxide value (PV), free fatty acids (FFA), total volatile basic nitrogen (TVB-N), tiobarbituric acid (TBA) analyses), sensory (raw and cooked fish) and microbiological methods. The quality of common sole decreased on day 16 (fair quality- B) and they were no longer acceptable on day 20 (unacceptable quality-C). When common sole were considered as fair quality (B) by assessors on ~16-18 days, the average *K* value was ≥ 80%. Among the investigated biogenic amines, histamine was not detected. No major change in the protein profiles of common sole was observed. Sensory, microbial and biochemical results revealed that the shelf life of common sole was approximately ~16-18 days. Among the chemical parameters, the most consistent indicator to measure the change in freshness was *K* value that showed linear response with storage time.

Keywords: Common sole, freshness indicators, shelf life, *K* value, biogenic amines, protein degradation.

Akdeniz'de Doğadan Yakalanan ve Buz İçerisinde Depolanan Dil Balığı (*Solea solea*)'nın Biyokimyasal, Duyusal ve Mikrobiyolojik Kalitesinde Değişiklikler

Özet

Doğadan yakalanan ve buz içerisinde depolanan dil balığının kalitesi kimyasal (K değeri, biyojenik amin, protein denatürasyonu (SDS ve SDS-PAGE), peroksit değeri (PV), serbest yağ asit (FFA), toplam uçucu bazik nitrojen (TVB-N), tiyobarbitürik asit (TBA) analizleri), duyusal (çiğ ve pişmiş olarak) ve mikrobiyolojik metodlarla araştırılmıştır. Dil balığının duyusal kalitesi 16. günde düşmeye başladığı (iyi kalite-B) ve 20. günde artık kabul edilemez duruma geldiği belirlenmiştir (kabuledilemez-C). Dil balığı panellistler tarafından iyi kalite (B) olarak değerlendirildiğinde (yaklaşık olarak 16-18 günde), ortalama K değeri > %80 olarak bulunmuştur. Araştırılan biyojenik aminler arasında histamin belirlenememiştir. Dil balığının protein profilinde önemli bir değişiklik gözlenmemiştir. Duyusal, kimyasal ve mikrobiyolojik sonuçlara göre dil balığının raf ömrü yaklaşık olarak 16-18 gün olarak bulunmuştur. Tazeliği belirlemede kullanılan kimyasal parametreler arasında, en iyi sonucu K değerinin verdiği ve depolama zamanına bağlı olarak linear olarak arttığı saptanmıştır.

Anahtar Kelimeler: Dil balığı, tazelik göstergeleri, raf ömrü, K değeri, biyojen aminler, protein bozulması.

Introduction

In seafood consumption, a great growth has been observed on account of changes in consumer attitudes aspects of health and nutrition (Cespedes *et al*., 1999). The common sole, *Solea solea*, belonging to Soleidae family (Amara *et al*., 1998), is a flatfish of major commercial interest in the Eastern Atlantic and in the Mediterranean countries (Campillo, 1992; Merigot *et al*., 2007). This species is important for commercial culture due to delicate texture and flavor. However, little information is available regarding the quality changes of common sole (*Solea solea*). A number of studies were conducted in relation to the quality index of different species such as Senegalese Sole (Gonçalves *et al*., 2007; Tejada *et al*., 2007). The effect of CO_2-enriched atmospheres on both the bacterial flora and chemical parameters during refrigerated storage of common sole were studied by López-Gálvez *et al.* (1998). Nucleotide catabolism products of rock and yellowfin soles (Greene *et al*., 1990), fat and protein contents of dover and wedge sole were determined by Soriguer *et al*. (1997). Quality changes and parameters of other flatfish such as turbot, (Aubourg *et al*., 2005; Rodriguez *et al*., 2006; Ozogul *et al*., 2006), flounder (Massa *et al*.,

2005) and halibut (Olsson *et al*., 2003; Guillerm-Regost *et al*., 2006) were also investigated. The aim of this work was, therefore, to investigate the shelf life and also sensory, chemical and microbiological quality changes of *Solea solea* (common sole) during iced storage period.

Materials and Methods

Sample Preparation and Storage of Common Sole

Common sole (*Solea sole)* were caught by bottom trawling. They were immedialtely iced in a box and transported to laboratory in ice. Fish were 1-day post capture on arrival at the laboratory in ice. The average weight and lenght of common sole were 186±23.44 g and 28.83±2.75 cm, respectively. The fish were gutted and washed and then stored in ice at a fish-to-ice ratio of 2:1 (w/w). The fish were kept in polystyrene boxes (three boxes containing 10 fish per box) with ice in cold storage at 4±1°C. During storage, ice was replenished when necessary. All boxes were closed and had perforated bottoms to allow drainage of melted ice. Sensory and chemical analyses were performed every four or five days. Data were obtained using at least three fish from the same box, which were minced for each sampling.

Proximate Analysis

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

Sensory Analysis

For sensory analysis, triplicate samples were taken at regular intervals. Sensory analysis was assessed using the official European method for freshness quality grading of chilled fish. Each assessment was carried out by a minimum of 6 trained panellist. 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 fish and 3 indicating a spoiled, putrid fish. To prepare the cooked fish sample, fish were cooked in a microwave for 2 minutes at medium temperature (600 W). The cooked samples were served hot to panellists.

Analytical Methods

The TVB-N content of common sole 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 *et al*. (1960) in fish fillets to evaluate the oxidation stability during storage period and the results expressed as TBA value, miligrams of malondialdehyde per kg flesh. Free fatty acid analysis (FFA), expressed as % of oleic acid was determined by AOCS (1994). Peroxide value (PV) expressed in miliequivalents of peroxide oxygen per kilogram of fat was determined according to AOCS (1994). The pH of fish 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 (Ozogul *et al*., 2000). The *K* value was calculated by the procedure described by Saito *et al*. (1959).

Biogenic amines were analysed using an HPLC method (Ozogul *et al*., 2002). Benzoyl chloride as a derivatization reagent was used and the derivatization procedure was based on that of Redmond and Tseng (1979).

Apparatus

High-performance liquid chromatography (HPLC) was used 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, 250x4.6 mm, particle diameter 5 µm (Mecherey-Nagel, Duren, Germany) For nucleotide determination, the column was a Sphereclone ODS 2 C18, 150x4.60 mm, particle diameter 5 µm micrometer (Phenomenex, Macclesfield, Cheshire, UK).

Sodium Dodecyl Sulphate (SDS)-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Fish muscle tissue (0.5 g) was homogenized in 10 ml of Tris buffer (50 mM Tris-HCl, 1 mM EDTA, 0.01% (w/w) BHT, pH 7.4) using a homogeniser (Ultra- turrax, Ika T8) for 1 min at 20,000 rpm. Protein concentration was determined by the method of Lowry *et al.* (1951). Bovine serum albumin was used as a standard. Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) in the absence and presence of β-mercaptoethanol was used to monitor polymerization or fragmentation of proteins using a BioRad Mini vertical gel electrophoresis. SDS-PAGE was performed in a 10% resolving gel and 4% stacking gel according to the method of Laemmli (1970). Gels were stained with a solution of 0.025% Coomassie Blue R-250, 40% methanol and 7% acetic acid, and de-stanined with a solution of 5% methanol and 7% acetic acid. Protein molecular weight standards (BioRad-161-0309) consisting of myosin (211.24) kDa), β-galactosidase

(117.76 kDa), bovine serum albumin (99.81 kDa) and ovalalbumin (49.78 kDa) were used.

Microbiological Analysis

Samples from each of three different fish (triplicate) were taken to estimate total viable counts (TVC). Ten grams of fish muscle were mixed with 90 ml of Ringer solution and then homogenised for 3 minutes. 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. This method was carried out under hygienic conditions, particularly during sampling procedure. They were then incubated for 2 days at 30°C.

Statistical Analysis

The data were subjected to analysis of variance. A SPSS statistical package (version 8.0) adapted to a personal computer (PC). The data were subjected to analysis of variance at the significant level of 5%.

Results and Discussion

Sensory Analyses

Table 1 shows the results of the sensory analysis of common sole stored in ice. Fish maintained high (E) and good quality (A) during the first 16 days of chilled storage. After that, the quality of fish started to decrease due to formation of mucus, loss of pigmentation, odour in the gills, belly cavity and flesh (B). Fish were no longer acceptable on 20 days (C). The earliest change was observed in the appearance of eyes, which were cloudy throughout the storage period. Skin aspects and consistency were still acceptable at 20 days of storage period. According to sensory analyses, the limit for acceptability of common sole stored in ice was 16-18 days. Gonçalves *et al.* (2007) reported a shelf life of 15 days for farmed Senegale sole (*Solea senegalensis*) stored in ice.

Figure 1 shows sensory evaluation score of cooked fish 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 16 days. Off-flavours and off-odours were detected on 20 day of storage trial due to bacterial metabolites. The rejection point for the cooked fillets was below 5 at 20 days. Tejada *et al*. (2007) carried out a quality test for cooked farmed Senegalese sole (10 min at 91°C using steam oven) to establish rejecting point and found around 22-25 days of ice storage for cooked fillets.

Chemical Assessment

Common sole was found to have the following proximate composition: 17.02±0.12% protein, 0.83±0.04% lipid, 79.60±0.31% moisture and 1.32±0.1% ash.

TVB-N and especially TMA-N are associated with seafood spoilage and regarded as unreliable for

Table 1. Changes in TVB-N, TBA, pH, PV, FFA values, in common sole over the period of iced storage

Days	TVB-N (mg/100g)	TBA (mg MA kg^{-1})	pH	PV (meq/kg)	FFA (% of oleic acid)
0	13.4±1.1	0.1±0.0	6.7±0.0	15.0±1.4	9.9±2.5
6	9.7±0.0	0.2±0.0	7.0±0.0	18.5±1.2	10.0±0.0
10	12.9±0.3	0.2±0.0	6.8±0.0	22.9±2.4	10.7±1.1
16	11.9±0.8	0.1±0.0	7.2±0.0	35.9±2.2	12.2±2.5
20	34±2.1	1.2±0.2	7.8 ±0.0	29.8±1.4	12.8±0.9
24	32.1±1.4	2.1±0.1	7.9±0.0	23.2±0.1	13.3±1.5

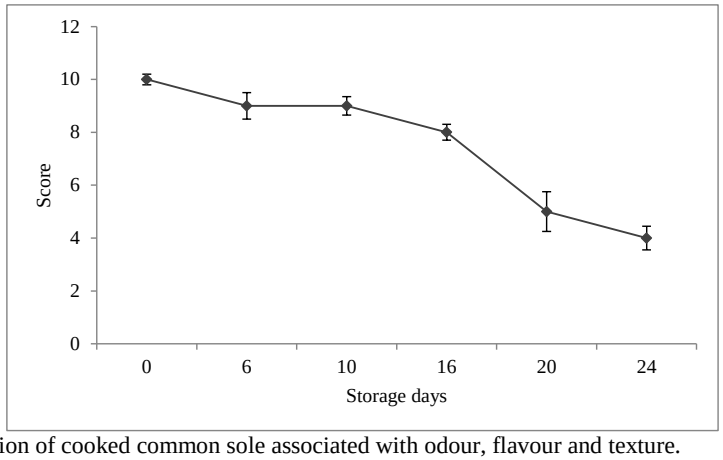

Figure 1. The sensory evaluation of cooked common sole associated with odour, flavour and texture.

the measurement of spoilage for the first 10 days of chilled storage for some fish species (Huss, 1995; Ólafsdóttir *et al*., 1997). TVB-N concentrations of fish stored in ice are shown in Table 2. The TVB-N values showed fluctuations during storage of common sole in ice. At the begining of storage, the TVB-N value was 13.44 mg 100 g^{-1} flesh. This value decreased to 9.73 mg TVB-N 100 g^{-1} flesh on day 6, thereafter significantly increasing to 34.03 mg TVB-N 100 g^{-1} ($P<0.05$) by day 20, when common sole were rejected by sensory assessment. TVB-N limits were in the range of 25-35 mg N per 100 g muscle considering the limits of acceptability for some fish species (Commission Decision 95/149/EC. 1995). In the present study, since the TVB-N level showed fluctuations during storage period, TVB-N could not provide a good index of common sole quality. Similar results were obtained for some fish species (Ólafsdóttir *et al*., 1997; Papadopoulos *et al*., 2003; Tejada and Huidobro, 2002). In addition, farmed Senegalese sole stored in ice did not produce TVB-N and TMA-N during the period of sensory-acceptable quality (Gonçalves *et al*., 2007; Tejada *et al*., 2007).

Mean pH measurements over the period of storage in ice are shown in Table 2. The initial pH value of 6.65 significantly increased to 7.02 ($P<0.05$) on day 6 and then, the pH value decreased on day 10 ($P<0.05$). After that, it increased steadily until the end of storage period. Similar results were obtained for farmed Senegalese sole (Tejada *et al*., 2007), wild turbot in ice (Ozogul *et al*., 2006). Post mortem pH has been reported to vary from 6.0 to 7.1, depending on season, species and other factors (Simeonidou *et al*., 1998).

Hydroperoxide quantified by peroxide value (PV) emerge as a result of the oxidation of fat and oil, while FFA are formed by the hydrolysis of fat and oil. Slightly oxidized fat and oil having PV at levels of only 100 meq kg^{-1} are reported to be neurotoxic (Gotoh *et al.*, 2006; Gotoh and Wada, 2006). In this study, the initial PV value was 15.02 meq kg^{-1} for the common sole stored in ice (Table 2) and significantly increased to maximum value of 35.87 on day 16 ($P<0.05$) and then decreasing to 23.22 meq kg^{-1} at the end of storage period ($P<0.05$). The initial PV values have been reported for a number of species e.g 0.8-1.2 meq kg^{-1} for herring (Smith *et al*., 1980), 5.60 meq kg^{-1} for wild turbot (Ozogul *et al*., 2006) and 27.6 meq kg^{-1} for fresh sardine (Cho *et al*., 1989).

The release of FFA significantly increased from the initial value of 9.86 (expressed as % of oleic acid) to the final value of 13.25 ($P<0.05$) during the storage period. Since the release of FFA content increased with time as found in this study (Table 2), it is reported that there is a relationship between FFA release and loss of freshness (Barassi *et al*., 1987; Ozogul *et al*., 2005). Lipid hydrolysis occured at a lower rate for common sole compared to the results for wild turbot (Ozogul *et al*., 2006).

The TBA index is widely used as an indicator of degree of lipid oxidation. It was 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, phospholipids and aldehydes which are end products of lipid oxidation. This interaction can vary with fish species (Aubourg, 1993). Nishimoto *et al.* (1985) reported for mackerel 4 and 27 mg malonaldehyde (MA) kg^{-1} muscle for good and low quality fish, respectively. The initial TBA value of 0.07 mg MA kg^{-1}, in this study, significantly increased to 2.06 mg MA kg^{-1} ($P<0.05$) at the end of storage period (Table 2). Tejada *et al.* (2007) reported that no rancidity was found after 28 days of chilled storage of farmed Senegalese sole (<1 mg MA kg^{-1}).

Nucleotide Degradation and *K* value

The patterns of ATP breakdown products in common sole stored in ice are shown in Figure 2. The main changes are the loss of IMP and the increase in Hx with storage. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) levels were very low (0.06, 0.18 and 0.07 μmol g^{-1}, respectively) at the beginning of the storage period and then decreased over the storage period. However, Gonçalves *et al*. (2007) reported that ATP, ADP, and AMP were not detected in iced farmed Senegalese sole. This results from the rapid post-mortem dephosphorylation and deamination of adenine nucleotides through inosine monophosphate (IMP) by autolytic process (Huss, 1995). IMP is a flavor enhancer and strongly associated with acceptability in fish (Fletcher and Statham, 1988; Bremner *et al*., 1988). The initial level

Table 2. The formation of biogenic amines and TMA (mg/100 g) in common sole kept in ice

Storage days	PUT	CAD	SPD	SPN	TMA
0	5.6±0.9	0.1±0.0	0.2±0.1	0.0±0.0	0.2±0.09
6	8.3±0.8	0.6±0.2	0.5±0.0	0.1±0.1	0.7±0.06
10	7.7±0.6	0.3±0.1	0.7±0.0	0.4±0.0	0.7±0.0
16	11.0±0.8	1.1±0.5	1.1±0.1	0.1±0.0	0.9±0.1
20	11.3±1.0	1.8±0.5	0.4±0.0	0.2±0.0	1.6±0.3
24	13.8±0.7	2.7±0.6	0.6±0.1	0.2±0.0	3.1±0.2

PUT, putrescine; CAD, cadaverine; SPD, spermidine; SPN, spermine; TMA, trimethylamine; ±, standard deviation. Histamine; tyramine, tryptamine, 2-phenylethylamine were not detected.

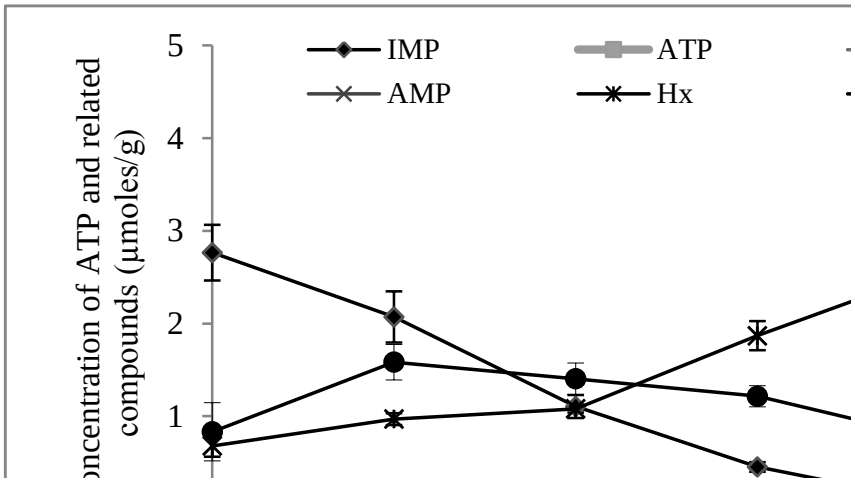

Figure 2. The pattern of nucleotide degradation products in common sole stored in ice.

of IMP in common sole stored in ice was 2.77 µmol g^{-1} and significantly decreased sharply to 1.11 µmol g^{-1} ($P<0.05$) on day 10 and 0.45 μmol g^{-1} on day 16. It was reported that farmed Senegalese sole contained 6.5 μmol g^{-1} and decreased to 3.7 µmol g^{-1} at the end f storage trial (16-17 days) (Gonçalves *et al*., 2007). The variation of initial nucleotide contents is associated with differences among species, season, catching gear, and stress during fish death, water temperature, and the time between catch-slaughter and storage (Huss, 1995).

During the metabolism of ATP post-mortem, hypoxanthine (Hx) is formed, which is bitter and regarded as a contributor to off-flavours. In this study, Hx concentration increased with the increase of storage period as reported for other fish species (Greene *et al*., 1990; Price *et al*., 1991; Kyrana *et al*., 1997; Ozogul *et al*., 2000; Gonçalves *et al*., 2007; Tejada *et al*., 2007). The initial levels of Hx in common sole stored in ice were 0.68 µmol g^{-1} and significantly increased to maximum levels of 1.67 µmol g^{-1} and 4.27 µmol g^{-1} ($P<0.05$) at the end of storage period. However, these values were much higher than those reported for farmed Senegalese sole (Gonçalves *et al*., 2007; Tejada *et al*., 2007).

The rate of nucleotide degradation is usually expressed by the *K* value (*K-value* (%)=[(Hx+Ino)/(ATP+ADP+AMP++IMP+Hx+Ino)] x 100) since it reflects the formation of Hx and Ino and the decrease of the nucleotide levels, which indicates a good freshness indicator (Saito *et al*., 1959). *K* value increased linearly with storage time in common sole stored in ice, reaching 97.88% at 24 days from the initial value of 32.92%. Although in ground fish ATP, ADP and AMP are almost completely converted to IMP within 24 h postmortem, the rate of accumulation of nucleosides may vary considerably among species, depending on the activity of the 5'-nucleotidase and nucleoside phosphorylase systems. For example, Hx levels increase at reasonably steady rates in chill-stored lemon sole, plaice and winter flounder (Kassemsarn *et al*., 1963; Jones *et al*., 1964; Burt, 1977; Dingle and Hines, 1971), but remain practically unchanged in petrale sole, English sole and witch flounder (Spinelli *et al*., 1964; Shaw *et al*., 1977). However, despite the observed species differences in the patterns of nucleotide metabolism, it would be rather an exaggeration to propose a *K* value of 33% for day 0 fish; a k-value of 20% is generally regarded as the freshness limit for fish destined for sashimi preparation, 60% being the rejection point for most species (Ehira and Uchiyama, 1987). In this study, when common sole stored in ice were considered at the limit of acceptability (B) by assessors on ~16-18 days, the average *K* value was ≥ 80%. Ozogul *et al.* (2006) reported that *K* value increased with storage time, reaching 90% from the initial value of 19% in wild turbots stored in ice during 19 days of storage. Aubourg *et al.* (2005) also reported that *K* value of farmed turbot increased from the initial value of ~6% to ~75% after 19 days. However, *K* value of ≥40% was reported to be unacceptable quality for farmed Senegalese sole (Gonçalves *et al*., 2007; Tejada *et al*., 2007). In this study, the *K* value provided a useful indicator for freshness in common sole stored in ice. Similar results were found with farmed Senegalese sole, wild turbot (Gonçalves *et al*., 2007; Tejada *et al*., 2007; Ozogul *et al*., 2006; Aubourg *et al*., 2005).

The concentrations of the biogenic amines and TMA present in the muscle of common sole stored in ice are given in Table 3. Eight biogenic amines were investigated namely, histamine, putrescine, cadaverine, spermidine, spermine, tryptamine, tyramine, and 2-phenylethylamine but histamine, tyramine, and tryptamine and 2-phenylethylamine were not detected. As storage time progressed, putresine and cadaverine became the dominant amines reaching 13.81 mg kg^{-1}, and 2.67 mg kg^{-1}, respectively at 24 days of storage in ice. When

common sole in ice (on day 20) were rejected by sensory panel, the level of putresine was 11.29 mg kg^{-1}, and cadaverine level was 1.77 mg kg^{-1}. Spermidine and spermine contents of common sole showed fluctuations, reaching a maximum level of 1.07 mg kg^{-1} on day 16 and 0.43 mg kg^{-1} on day 10, respectively. In our previous research with wild turbot (Ozogul *et al*., 2006), the levels of biogenic amines were higher than those found in common sole. It was also reported that biogenic amines were below the detection limit in the farmed Senegalese sole, probably due to the low amount of free amino acids (Gonçalves *et al*., 2007).

The spoilage caused by microorganisms, often detected as a fishy odour, is due to the decomposition of trimethylamine oxide (TMAO) by the enzyme TMAO demethylase. TMA can be used as a spoilage indicator since it appears after 3 or 4 days of storage. Fresh fish have very low amounts of TMA with values less than 1.5 mg TMA 100 g^{-1} in fresh cod, but values increase during spoilage. The fish is considered as stale when the amount of TMA is higher than 30 mg/100 g cod (Bonnell, 1994), but the levels between 10-15 mg of TMA-N 100 g^{-1} of fresh were considered as the limit for fresh fish (Connell, 1995). In this study, the level of TMA increased steadily from the initial value of 0.18 mg N kg^{-1} to the maximum value of 3.05 mg N kg^{-1} ($P<0.05$), which was lower than the limit**.** These results are in accordance with others obtained for farmed Senegalese sole, farmed turbot, in which significant TMA-N formation was not observed during acceptable sensory period (Gonçalves *et al*., 2007; Rodríguez *et al*., 2003; Rodríguez *et al*., 2006).

Protein Denaturation

Sodium dodecyl sulphate -polyacrylamide gel electrophoresis (SDS-PAGE) patterns of proteins of common sole during ice storage are shown in Figure 3. Electrophoretic results in the presence and absence of reducing agent showed that the intensity of bands slightly decreased on day 6 of storage in the absence of β-mercaptoethanol and then almost remained unchanged. In the presences of β-mercaptoethanol, the intensity of bands below 48.78 kDa (last three bands) weakened on day 10, 16 and 20 of the storage. The other bands did not change and new bands also did not appear during 24 days of iced storage. The decrease of the bands intensity both in the presence and absence of reducing agent during post mortem storage could reflect to degradation and/or digestion of myofibriller and sarcoplasmic proteins by proteolysis during storage (Tokur and Polat, 2010; Ladrat *et al*., 2003; Lund and Lielsen, 2001; Benjakul *et al*., 1997). Enzymatic proteolysis of fish meat during post mortem storage cause disruption of the structure of the myofibrillar proteins (Jasra *et al*., 2001; Verrez-Bagnis *et al*., 1999) and is thought to cause post-mortem softening, which is one of the most important quality attributes of fish muscle during cold storage. The degradation and/or digestion of proteins by proteolysis as a consequence of *post-mortem* changes have been monitored by SDS-PAGE

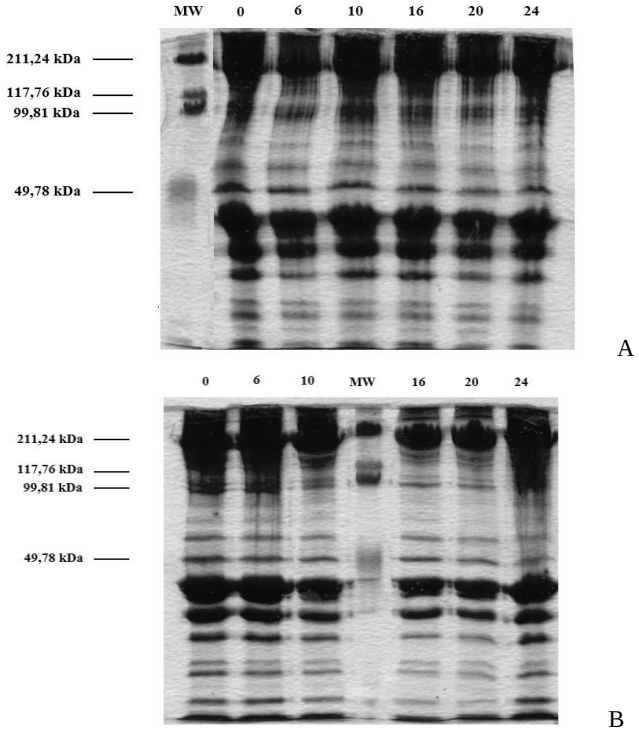

Figure 3. .Electrophoretic patterns of SDS-PAGE gels of common sole stored on ice. Electrophoresis of samples was performed in the absence (A) and presences of β-mercaptoethanol (B). (MW: molecular weight).

(Munasinghe *et al*., 2005; Bonnal *et al*., 2001; Verrez-Bagnis *et al*., 2001; Sompongse *et al*., 1996). These studies show that the rate of proteolysis varies among species (Papa *et al*., 1996; Tejada *et al*., 2002). In this study, although the intensity of some bands decreased during the storage periods, generally no major change in the protein profiles was observed, thereby indicating that proteolysis occurred in common sole during ice storage was of minor importance. Another possible explanation for this might be that polypeptides formed during proteolysis could not bind to the dye used in electrophoretic staining (Tejada *et al*., 2002) and they did not appear on the gel.

Microbiological Assessment

Microbial counts on the common sole kept in ice are shown in Figure 4. Initial total viable counts of whole gutted common sole was below 4 log cfu g^{-1} (day 0) and population of microorganisims significantly ($P<0.05$) increased to 8.3 log cfu g^{-1} (day 24) over the period of storage. On day 16 of storage TVC was 5.49 cfu g^{-1}. A somewhat higher (10^7 CFU g^{-1}) microbial safety criterion is normally applied for determining storage life of fresh seafood (IFST, 1999). The shelf life of common sole was approximately ~16-18 days, indicating that sensory analysis of common sole correlated well with microbiological analysis. Similar results were obtained for others species (Rodríguez *et al.*, 2006; Ozogul *et al*., 2006).

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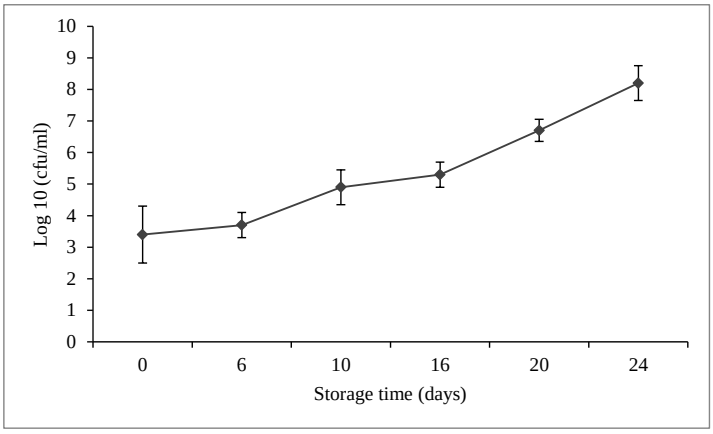

Figure 4. TVC content of the common sole stored in ice.

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