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Full Length Research Paper

Quality evaluation of tray-packed tilapia fillets stored at 0℃ based on sensory, microbiological, biochemical and physical attributes

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The quality deterioration of tray-packed tilapia (genetically improved farmed tilapia strain of *Oreochromis niloticus*) fillets stored at 0℃ were studied by integrated evaluations of sensory, microbiological, biochemical and physical analysis, in order to expound the mechanism of fish spoilage and develop the most reliable indicators for quality assessment. The results showed that four quality index as *Pseudomonas* counts, total volatile basic nitrogen (TVBN), cadaverine (CAD) and K value were highly correlated (r > 0.90) with storage time and sensory acceptability. Protein degradation was visible on SDS-PAGE when microbiological load exceeded 6 log cfu/g. Thiobarbituric acid reactive substances (TBARS) value remained at a very low level throughout the storage, suggesting low lipid oxidation in muscle. Hardness decrease tested by texture machine was consistent with texture softening of fillets in the sensory evaluation. Considering fish freshness and microbiological safety, the shelf life of tilapia fillets stored at 0℃ was approximately 10 - 12 days.

Key words: Tilapia fillet, chilled storage, quality, spoilage, shelf life.

INTRODUCTION

Fish is highly perishable, due to its high water activity (a_w) and protein content, neutral pH and presence of autolytic enzymes which cause fish spoilage. The rate of fish spoilage is affected by species, fat content, fishing and slaughter method, hygiene manipulation, postmortem handling and many other factors (Huss, 1995). Post-mortem fish undergoes four stages as rigor mortis, dissolution of rigor mortis, autolysis and bacterial spoilage. The initial loss of freshness is caused by endogenously autolytic enzymes in muscle and the subsequent spoilage is usually due to microbial activities, especially for the rapid proliferation of specific spoilage organisms (SSO) (Huss, 1995). Interaction between microbial metabolism and physiochemical reactions accelerate fish quality deterioration as amines formation, lipid oxidation, nucleotide and protein degradation, contributes to off-odors, off-flavors and texture softening (Ozogul et al., 2006; Alasalvar et al., 2001; Hernandez et al., 2009). Varieties of quality attributes have been used to assess fish freshness in many cold water fish species as sea bream, sea bass, sardine and European eel (Alasalvar et al., 2001; Alasalvar et al., 2002; Ozogul et al., 2004; Ozogul et al., 2006; Hernandez et al., 2009). However, few researches were reported on quality assessment for tropical freshwater fish species (Chytiri et al., 2004).

Tilapia are ranked as the second most widely farmed fish in the world. They are farmed in at least 85 countries, with most production coming from Asia and Latin America (Eknath et al., 2007). In 2007, tilapia production of China reached 1,210,000 tons, approximately up to 49% of the global yield (Li and Cai, 2008). The majority (approximately 66.7%) of tilapia production in China is sold alive in domestic market and the remaining are frozen for

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Table 1. Sensory scheme of tilapia fillets.

Scores	Sensory properties
5	Fresh seaweedy odor, bright red color without mucus, stiff texture without finger-pressed concave, distinct sweetness and meaty flavor
4	Slight seaweedy and earthy odor, bright red color with slight transparent or water mucus, firm and elastic texture with slight finger-pressed concave, weak sweetness
3	Slight fishy, slight dark red color with moderate transparent mucus, less elastic and slight clammy, no sweetness, no off-flavor
2	Fishy with intense off-odor, dark red color with slight opaque mucus, softer and clammy, sight bitterness, off-flavor
1	Spoiled with stronger off-odor, brownish red with more opaque and milky mucus, very soft and strong clammy, amines and woody
0	Putrid and rotten, brownish red with more opaque and milky mucus, sunken and stronger slimy, putrid

exportation or used for further processing (Li and Cai, 2008). Low production of chilled tilapia is mainly limited by its high perishability and poor system of quality evaluation. Many researches on tilapia emphasize particularly on their breeding performance and variety improvements (Wang et al., 2000; Yi and Lin, 2001; Gall and Bakar, 2002, Eknath et al., 2007). Some reports on the storage quality of frozen/chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (Eves et al., 1995; Al-Kahtani et al., 1996; Korel et al., 2001; Arannilewa et al., 2005; Yanar et al., 2006; Sil et al., 2008). Genetically improved farmed tilapia (GIFT) strain of *oreochromis niloticus* introduced from the world fish center (ICLARM -International Center for Living Aquatic Resources Management) exhibited higher growth performance and salinity tolerance than any other Nile tilapia strains (Eknath et al., 2007). It has been promoted and widely cultured in China and produces a significant market value. However, no studies were reported on its quality assessment and microbiological safety during chilled storage.

The objective of this study was to expound the spoilage mechanism of postmortem tilapia (GIFT) during chilled storage by integrated evaluations of sensory, microbiological, biochemical and physical analysis and to develop the most reliable indicators for further rapid prediction of fish quality and shelf life.

MATERIALS AND METHODS

Preparation of tilapia fillets and storage condition

50 tails of tilapia (GIFT strain of *O. niloticus*) were obtained from a local freshwater pond located at the suburb of Beijing in North China. The newly- caught fish (length of 34 ± 3 cm, weight of 517 ± 82 g) were anesthetized by immersing in ice-cold water mixture, then beheaded, gutted, filleted, peeled, washed and drained. The skinless fillets (weight of 112 ± 25 g) were packed in sterile trays and covered with a thin polyethylene film of high oxygen permeability (equal to aerobic package). The tray-packed fillets were kept in insulated polystyrene boxes with ice/fillets ratio of 2:1 to keep temperature at 0 - 4°C. The fish samples were transported to the laboratory within 12 h after capture and immediately were stored in an isothermal cold-room with air blow at 0°C. Samples were taken for analysis at storage day 0, 3, 6, 9, 13, 17, 21, 25 and 29. On each sampling occasion, eleven random-chosen fillets were divided into four parts, 3, 2, 3 and 3 fillets. These were subjected to sensory evaluation, microbial enumeration, biochemical analysis and physical measurement. All analysis was performed thrice except physical measurement.

Sensory evaluation

The panel consisted of six students from the laboratory of fishery science, age 24 - 35 years old. They had high sensory sensitivity and were trained in fish quality assessment according to Stone and Sidel (2004) before experiment. The panel performed the sensory evaluation without information about the experimental design and all the sample were coded with three-digital random numbers. They assessed the raw and cooked fillets according to a modified scheme from Ruiz-Capillas and Moral (2001). A 5-point scoring scale was used for quantitative evaluation of overall acceptability, the scale points were: excellent, 5; good, 4; moderately good (acceptable), 3; poor, 2; very poor, 1; putrid, 0 (Table 1). On each sampling day, three random fillets were individually presented to the panel. They were firstly asked to evaluate the odor, appearance and texture of raw fillets in the trays and subsequently tasted the cooked samples after cooking in boiling water for two minutes and finally judged the overall acceptability. Newly-caught tilapia was used as reference for sensory analysis each time. The shelf life was defined as onset of sensory rejection by at least half of panels.

Microbiological enumeration

Twenty five gram muscle were taken aseptically from the anterior dorsal region of fillet and then homogenized with 225 ml sterile physiological saline in a laboratory blender. The homogenates were serially diluted with saline and plated in duplicate by the pour-plate method and then were incubated aerobically. The microbiological analysis was according to previous reports with some modifications (Chytiri et al., 2004). For mesophilic and proteolytic bacteria, plate count agar and casein-agar medium were used, respectively, and the plates were incubated at 30°C for 3 days. Psychrotrophic bacteria and *Pseudomonas* sp. colonies were counted on crystal violet tetrazolium agar and cetrimide fusidin cephaloridine agar (CFC, CM 559, supplemented with SR 103, Oxoid, UK) after 3-day incubation

at 22°C, respectively. Only plates with 30 - 300 colonies were counted and the microbial counts were transformed to be log10 values for statistical analysis.

Measurement of pH, thiobarbituric acid reactive substances (TBARS), water holding capacity (WHC) and total volatile basic nitrogen (TVBN)

The pH was determined in muscle homogenate with 10 times of distilled water as previously reported (Hernandez et al., 2009). TBARS was measured spectrophotometrically according to the procedure described by Siu and Draper (1978) and expressed in mg Malonaldehyde/kg of muscle. WHC was carried out by the high-speed centrifugation (10000 × g for 15 min at 4°C) method (Cheret et al., 2005). TVBN was measured by the modified methods from Malle and Tao (1987). Briefly, PCA-fish extract (muscle homogenate/0.6M perchloric acid =1/10) was performed using Kjeldahl steam distillation with 30% (w/v) aqueous NaOH solution and 4% (v/v) aqueous boric acid as received solution. Finally, 0.01 M HCl solution was used for neutralization titration. The amount of TVBN was calculated from the volume of 0.01 M HCl used for titration and the results were expressed in mg nitrogen/100 g of sample.

Analysis of biogenic amines (BAs) and ATP breakdown compounds by HPLC

BAs measurement was performed by the method of Sil et al. (2008) while the ATP breakdown compounds were determined according to the method of Ryder (1985). Both perchloric acid-extract for BAs and ATP determinations were kept at - 80°C until analysis and all mobile phase and sample extracts were filtered through a 0.45 µm aqueous or organic filter before use. Analysis were performed on a Schimadzu LC-10 AT (Shimadzu, Kyoto, Japan) HPLC instrument equipped with a UV detector and LC column of C18-Diamondsil ^{TM} (25 cm × 4.6 mm, 5 µm). Chromatographic conditions for BAs and ATP were: flow rate of 1 ml/min, wavelength of 254 nm and injecttion of 20 µl aliquot of the sample extracts. All standards were purchased from Sigma-Aldrich. For the freshness indicator, K value was calculated as K (%) = [Inosine (Ino) + Hypoxanthine (Hx)] × 100 / (ATP + ADP + AMP + IMP + Ino + Hx) (Ozogul et al., 2006).

Protein solubility and SDS-PAGE for protein degradation

Protein solubility was measured as salt soluble protein (SSP) by method of Duun and Rustad (2007). Myofibrillar protein was prepared from tilapia muscle as reported by Ogata et al. (1998). The myofibrillar protein degradation was analyzed using SDS-PAGE according to the method of Laemmli (1970). Approximately, 15 µg protein extractions were separated by 10% SDS-PAGE gel using a Mini-Protean III^{TM} Gel electrophoresis system (Bio-Rad Laboratories Ltd). Following electrophoresis, the gel was fixed and stained with Coomassie blue R-250.

Physical analysis

Texture measurements were conducted according to the method of Cheret et al. (2005) with some modifications using a texture testing instrument (STEVENS QTS 25, UK). The dorsal muscles of fillets were cut into cubes (approximately, length × width ×height = 1.5 × 1.5 × 1.1 cm). The hardness and springiness were measured by two consecutive 50% compressions of the cube, parallel to muscle fibre orientation at a constant speed of 1 mm/s. The delay between two compressions was 30 s under a trigger point of 50 g. The data were calculated according to Cheret et al. (2005). Eight independent determinations were carried out for the test.

Statistical analysis

Data were analyzed by the statistical software of SPSS 11.5 for windows. The differences between quality indicator and storage time were analyzed by analysis of variance (ANOVA) using Duncan's differential test. Means □ SD were reported and considered different when $P < 0.05$. Correlations were calculated using the parametric Pearson's correlation in statistical software of SAS 9.1.

RESULTS AND DISCUSSION

Sensory evaluation

The decrease of sensory scores was highly correlated ($r = - 0.95$) with storage time (Figure 1), indicating quality deterioration. Fresh seaweedy odor, bright red, firm texture and no off-odors were observed in fresh raw fillets and intense sweetness and meaty flavor were tasted in cooked samples. Raw fillets maintained good quality within 9-day storage and were acceptable up to day 13. After that, the quality deterioration was observed as off-odors, opaque mucus, discoloration and texture softening, which limited overall acceptability of raw fillets. Similarly, in the taste of cooked samples, characteristic flavor and sweetness were strong before 6 day, decreased gradually in intensity and became flavorless on day 9. On day 13, slight off-flavor was rejected by the panel and then no further taste test was performed. The inspection of raw fillet was still performed until the end of storage to expound the characters of fillet spoilage. The shelf life of tilapia fillets stored at 0°C ranged 9 - 13 days considering fish freshness.

Microbiological analysis

Various microflora counts were increased ($P < 0.05$) with storage time (Figures 2A and B). Both mesophilic and psychrophilic bacteria grew exponentially from initial load of 3 - 4 log cfu/g, reaching 7.4 log cfu/g on day 13 and up to about 9.0 log cfu/g at the end of storage (Figure 2A). About 4 log cfu/g of initial mesophilic bacteria indicated good quality of fillets initially as it ranged from 2 - 6 log cfu/g in many freshwater fish (Huss, 1995). As proposed by International Commission on Microbiological Specifications for Foods (ICMSF, 1986), the microbial upper limit for fresh fish was 7 log cfu/g of aerobic mesophilic counts. In this study, mesophilic and psychrophilic bacteria reached about 5.9 log cfu/g on day 9 and 7.4 log cfu/g on day 13 (sensory rejection), respectively. The shelf life of tilapia fillets were about 10-12 days considered for microbial safety and sensory acceptability. Consistent with this study, similar shelf life and microbial counts at sensory rejection point were obtained in both trout and meager fillets stored in flake ice aerobically (Chytiri et al., 2004; Hernandez et al., 2009).

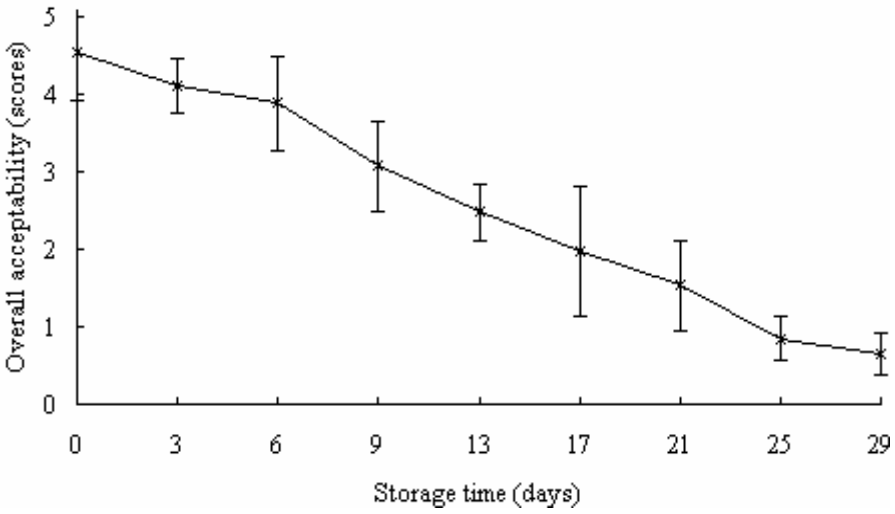

Figure 1. Sensory acceptability of tilapia fillets during storage at 0℃.

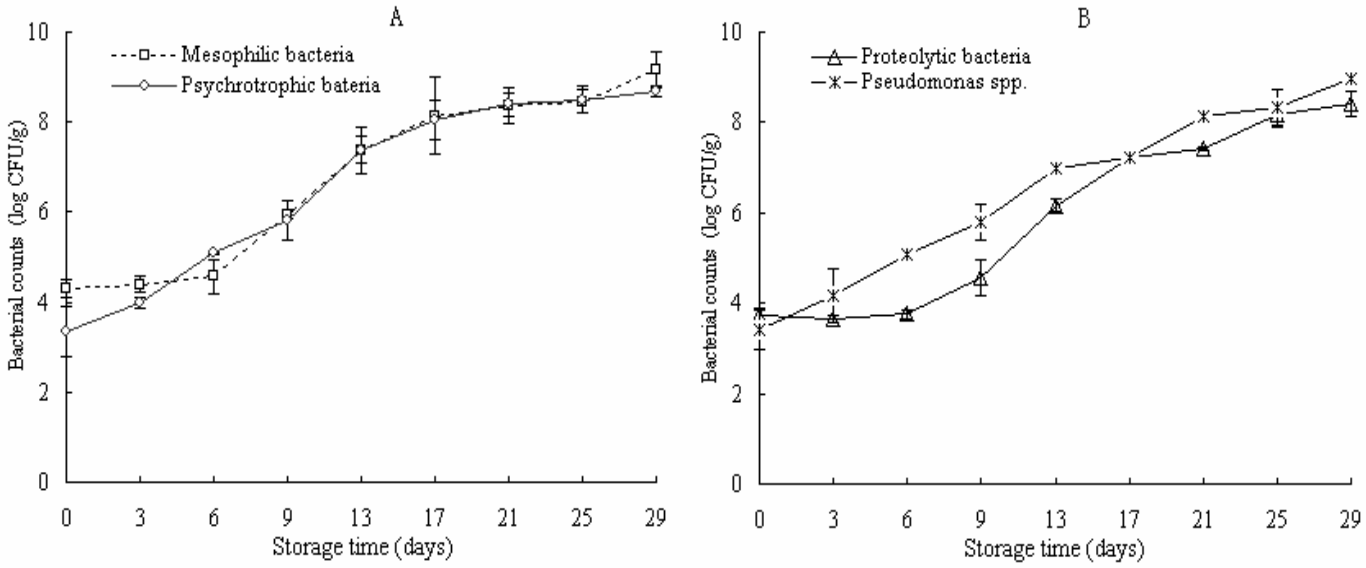

Figure 2. Growth of various microflora in tilapia fillets during storage at 0℃.

Proteolytic bacteria were increased from initial level of 3.7 to 6.2 log cfu/g on day 13 and up to 8.4 log cfu/g at the end of storage (Figure 2B); suggesting bacterial proteinases might contribute to protein degradation during chilled storage. Similarly, proteolytic bacteria contributed to muscle spoilage of whole hake during flake-ice storage (Rodriguez et al., 2004). *Pseudomonas* sp. displayed the typical growth pattern of the psychrotrophic bacteria without a lag phase (Figure 2B), increasing from initial counts of 3.4 to 7.0 log cfu/g on day 13 and up to 9.0 log cfu/g at the end of storage. They were strong spoilage bacteria which produced intense off-odors, large amounts of TVBN and secreted huge amounts of proteolytic enzymes and considered as the specific spoilage organisms (SSO) in various fish species from temperate and tropical waters, under aerobic refrigeration or ice storage and their abundance were used to predict the shelf life of fish (Gram and Huss, 1996). In this study, *Pseudomonas* sp. could be considered as SSO of the chilled tilapia fillet due to the fact that their growth significantly correlated with storage time ($r = 0.97$) and sensory acceptability ($r = -0.92$), as well as good correlation ($r > 0.92$) with proteolytic bacteria, TVBN and CAD.

Changes of pH and TVBN

pH value of tilapia fillet was increased ($P < 0.05$) with

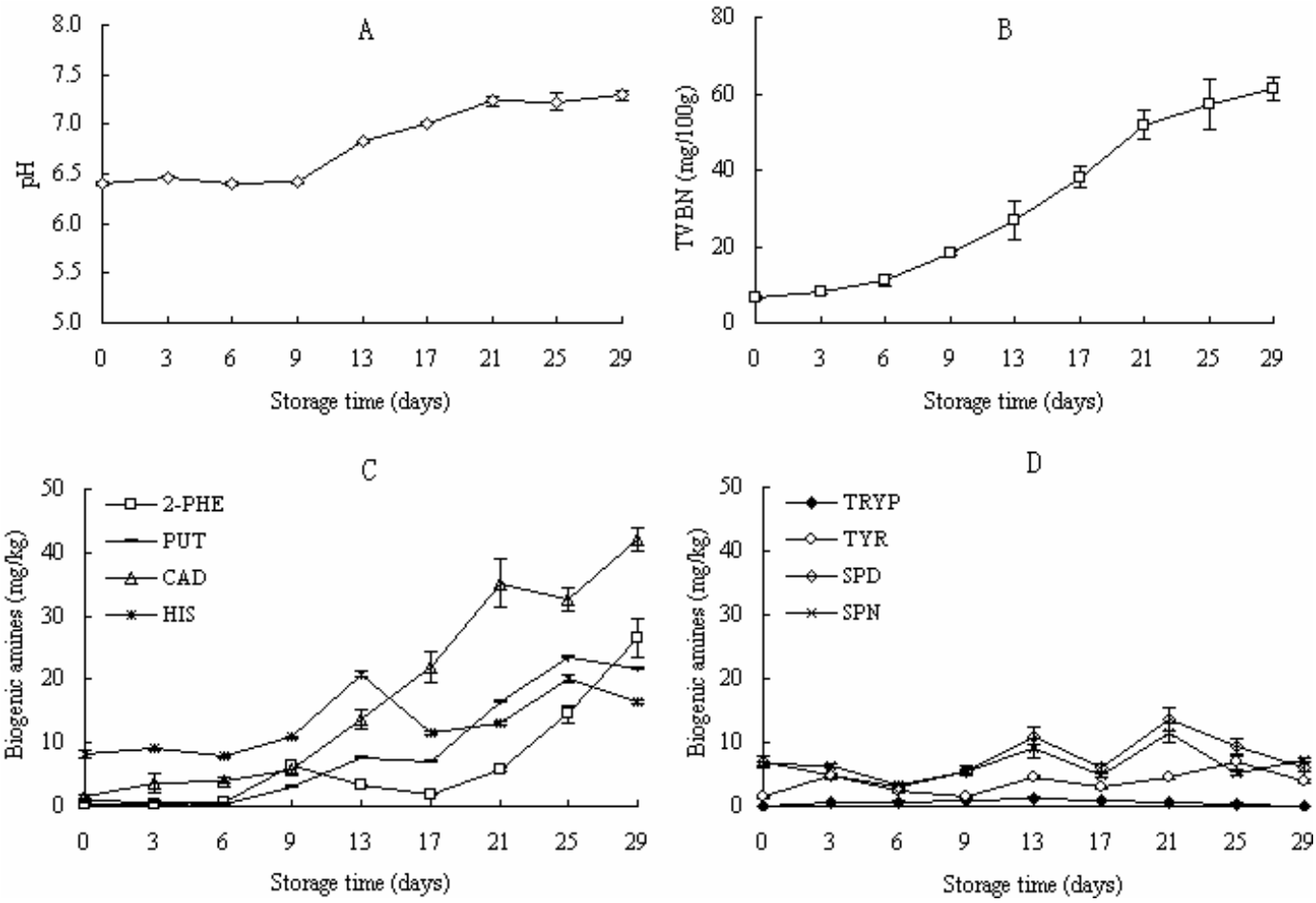

Figure 3. Changes in pH and amines formation of tilapia fillets during storage at 0°C. TRYP = tryptamine; 2 -PHE = 2 -phenylethylamine; PUT = putrescine; CAD = cadaverine; HIS = histamine; TYR = tyramine; SPD = spermidine; SPN = spermine.

storage time (Figure 3A), indicating that alkaline compounds were accumulated through autolytic activities or microbial metabolism (Pons-Sanchez-Cascado et al., 2006).

TVBN had been widely proposed as fish spoilage indicator since it showed a close relationship with the sensory score (Pons-Sanchez-Cascado et al., 2006). The content of TVBN in freshly-caught fish was typically 5 - 20 mg/100 g fish and 30 - 35 mg/100 g of muscle which were generally regarded as the limit of acceptability for ice-stored cold water fish (Huss, 1995). TVBN, including trimethylamine, dimethylamine, ammonia and other volatile basic nitrogen compounds, was produced mainly by bacterial decomposition of fish flesh. In many studies, TVBN level was very close to the rejection level when bacterial counts reached 7 log cfu/g (Koutsoumanis and Nychas, 2000). Previous studies indicated that TVBN value fluctuated in both whole ungutted and filleted rainbow trout during chilled storage and it was proposed as a poor indicator of fish freshness (Chytiri et al., 2004). In this study, TVBN was increased from initial 6.5 to 61.3 mg/100 g of flesh at the end of storage (Figure 3B) and correlated well with storage time (r=0.98), sensory acceptability (r = - 0.93) and bacterial counts (r > 0.90), indicating TVBN to be a good indicator for quality deterioration in chilled tilapia fillets.

Change of BAs

BAs could be used for estimating the freshness or the degree of spoilage in fish since they were found at low levels in fresh fish and associated with bacterial spoilage (Veciana-Nogues et al., 1997). Various levels of BAs from tilapia fillets were presented in Figures 3C and D. Among them, tryptamine (TRYP), 2 -phenylethylamine (2-PHE), putrescine (PUT), cadaverine (CAD) and tyramine (TYR) showed very low content initially, slight high initial level of histamine (HIS). Spermidine (SPD) and spermine (SPN) was normally considered as the natural amines in fish muscle due to the physiological or environmental factors (Veciana-Nogues et al., 1997). TYR, SPD and SPN showed obvious fluctuation with a maximum level of 15 mg/kg (Figure 3D) and low correlation (r < 0.56) with

storage time, TRYP was also at a very low concentration throughout the storage, they were not suitable as quality indicators. HIS was regarded as a hazardous index and very important in fish species as tuna and sardine (Veciana-Nogues et al., 1997). In this study, HIS was a dominant BA within the shelf life of fillets, but its level fluctuated during the storage, suggesting that it was unsuitable for quality assessment. Both PUT and 2-PHE level was also low before 17 days and then increased sharply beyond the shelf life (Figure 3C), they were not good quality indicator as reported previously in iced tilapia and chilled tuna (Sil et al., 2008; Veciana-Nogues et al., 1997). CAD increased steadily from low to high level throughout the storage (Figure 3C) and became the dominant BAs, reaching a final concentration of 42.1 mg/kg at the end of storage. Furthermore, CAD was increased significantly with storage time ($r = 0.97$) and correlated well with acceptability ($r = - 0.91$) and bacteria load ($r > 0.90$). It could be considered as a good quality indicator. Similar results were obtained by previous studies on tuna stored at 0 and 8°C, as well as anchovies stored in ice (Veciana-Nogues et al., 1997; Pons-Sanchez-Cascado et al., 2006).

BAs level obtained in this study was some higher than those reported in ice-stored tilapia (*oreochromis mossambicus* cultured in brackish water) (Sil et al., 2008), which might be affected by differences in fish species, farm environment (water salinity), especially the post-harvest handling. The formation of BAs was also dependent on the free amino acids content and the decarboxylation by endogenous enzymes and BA-producing bacteria activities (Ozogul et al., 2002). Multiplicate micro-organisms could produce BAs, the study of relationship between BAs and dominant spoilage bacteria on horse mackerel showed that high levels of PUT, CAD and HIS were detected when *Pseudomonas* sp., *Vibrio* sp. and *Photobacterium* sp. were predominant microflora (Flick et al., 2001). This was similar to our results that CAD level was highly correlated ($r = 0.92$) with dominant spoilage microflora, *Pseudomonas* sp.

Changes of nucleotide degradation products

The nucleotide breakdown patterns in chilled tilapia fillets were shown in Figure 4A. The main changes were the loss of IMP and the increment of Ino and Hx with storage time. Nucleotide degradation (ATP → ADP → AMP → IMP → Ino → Hx) products, especially for the concentrations of IMP, Ino and Hx, had been utilized extensively as indices of fish freshness before bacterial spoilage (Alasalvar et al., 2002; Ozogul et al., 2006). The pattern and rate of nucleotide degradation differed between and within fish species. The conversion of ATP to IMP was usually complete within 1 or 2 day, which was presumed to be a totally autolytic process, the subsequent breakdown of IMP to Hx was slow and was caused by both autolytic and microbial enzymes (Alasalvar et al., 2002). As shown in Figure 4A, IMP was the predominant nucleotides initially and then steadily degraded into Ino and Hx over storage. Ino concentration was increased by rapid IMP decrement and then decreased when IMP was depleted. Accordingly, Hx was increased continually with Ino metabolism. IMP was reported to strongly associate with pleasant flavor intensity and acceptability in fish freshness and the decrease or disappearance of IMP was correlated with the loss of fresh fish flavor in most species (Alasalvar et al., 2002; Ozogul et al., 2006). Inversely, Hx contributed to the progressive loss of desirable flavor and the development of bitter off-flavors. Its accumulation in fish tissue reflected the initial phase of autolytic deterioration as well as subsequent bacterial spoilage. Hx had been considered as an index for fish freshness, but the Hx content varied both between species and within given species between light and dark muscle (Ozogul et al., 2006).

K value used commonly for fish freshness was increased linearly ($r = 0.97$) with storage time (Figure 4B) and corrected well with sensory deterioration ($r = - 0.94$). During first 3 days, fillets were in excellent quality and the initial K value was less than 20% (Figure 4B). Generally, the initial K value did not exceed 10% in immediately post-capture fish, which was mainly due to endogenous enzymatic degradation and the K value showed another rapid increment caused by bacterial activities. The stressed fish also showed fast increase of K value (Pacheco-Aguilar et al., 2003). K value reached 68% on day 13 when the sensory panel rejected the fillet sample (Figure 4B). Wide variations of K value on the sensory rejection point among different fish species were reported ranging from 39 ~ 80% in chilled storage (Alasalvar et al., 2001; Ozogul et al., 2004). In the present study, K value provided a useful indicator for freshness evaluation, which was consistent with other fish species such as Sea bass and European eel stored in ice (Alasalvar et al., 2002; Ozogul et al., 2006).

Protein degradation

Protein degradation was detected by SDS-PAGE pattern of myofibril proteins (Figure 5) and protein solubility (Table 2). Myosin heavy chain (MHC, 200 kDa) and actin (45 kDa) were two major components visible in the protein pattern throughout the storage (Figure 5). There were no visible changes of myofibrillar protein pattern before 13-day storage and then continuous hydrolysis of MHC caused gradual appearance of low molecular weight bands (200 →45 kDa and < 29 kDa) after 13 days. However, only slight changes of actin were observed on SDS-PAGE throughout the storage. It was reported that the MHC of Pacific whiting muscle protein was highly hydrolyzed over 8-day iced storage and the endogenous proteinases made some contributions though the hydrolysis

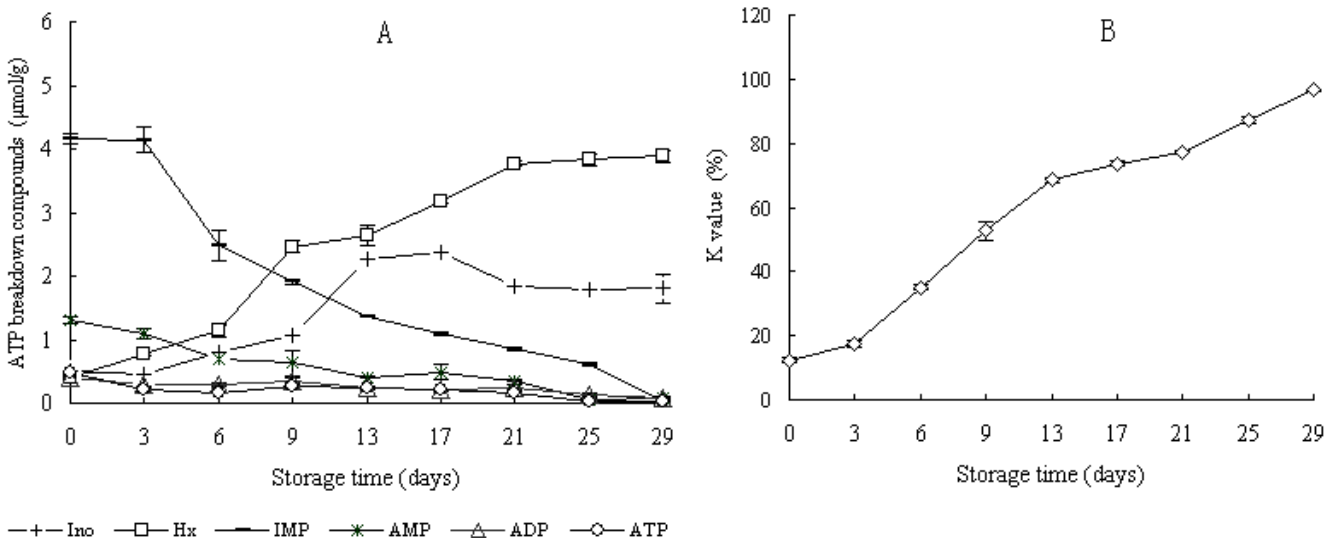

Figure 4. Nucleotide degradation products and K value of tilapia fillets during storage at 0℃. Ino = Inosine; Hx = Hypoxanthine.

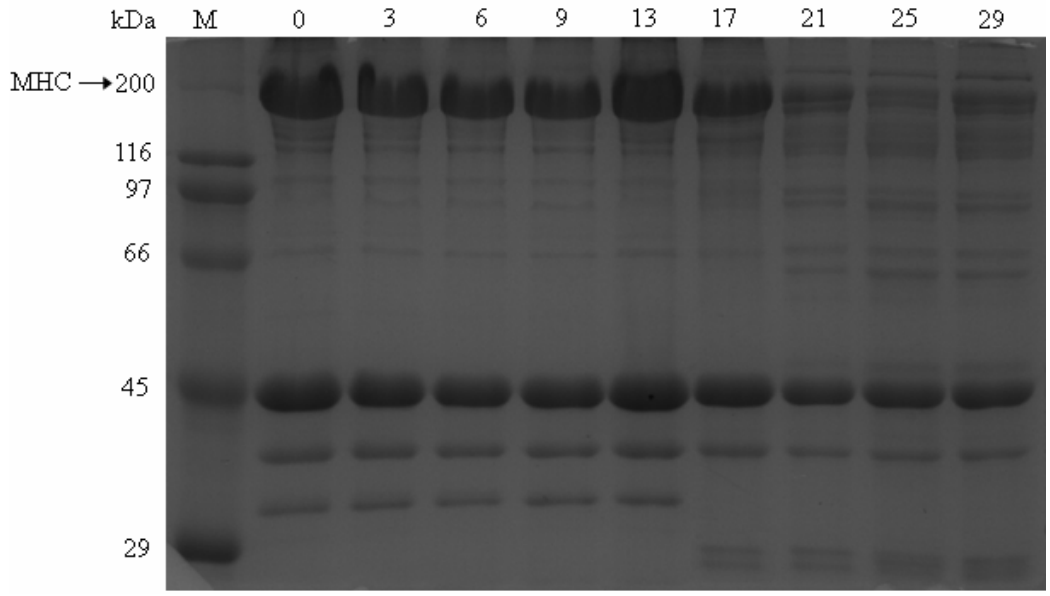

Figure 5. SDS-PAGE pattern for myofibrillar proteolysis of tilapia fillets during storage at 0℃. Lane M = molecular weight standards (29 - 200 kDa); Lanes 0 – 29 = protein profile at different storage time (from day 0 to day 29); MHC = myosin heavy chain (200 kDa).

rate was low. Microbial proteases might also be a potential source of protein degradation, especially for some psychrotrophic proteolytic bacteria (Benjakul et al., 1997). In this study, myofibrillar proteolysis was more significant than those in salmon stored at 0℃ for 23 days (Lund and Nielsen, 2001). It could be deduced that progressive protein degradation were dominated by microbial activities other than endogenous proteinases, since the obvious proteolysis on SDS-PAGE were observed only when microbial load exceeded 6 log cfu/g in this study (Figure 5). Therefore, protein degradation pattern was not an excellent indicator for quality assessment due to its hysteresis.

SSP was reduced from 9.5 to 5.7% as shown in Table 2. The decrease of SSP solubility might result from myofibril degradation, since the myofibril protein was the major component of SSP. Similar to the present results, the SSP content was decreased in minced channel catfish stored at 4℃ (Suvanich et al., 2000) and frozen minced silver carp (Siddaiah et al., 2001). However, increment of SSP was observed in iced chilled Atlantic cod and fluctuation in superchilled and frozen samples

Table 2. Changes in various physicochemical indices of tilapia fillets during storage at 0°C.

Days	SSP (%)[1]	TBARS(mg MA/kg)[2]	WHC(%)[3]	Hardness (kg)	Springness (mm)
0	9.54 ± 0.55^{de}	0.11 ± 0.01^{a}	87.89 ± 0.81^{c}	3.18 ± 0.13^{c}	2.95 ± 0.58^{c}
3	11.17 ± 0.49^{e}	0.14 ± 0.04^{a}	84.36 ± 0.69^{a}	3.02 ± 0.46^{c}	2.08 ± 0.53^{b}
6	8.16 ± 0.49^{c}	0.14 ± 0.03^{a}	86.51 ± 1.55^{b}	2.67 ± 0.28^{bc}	2.12 ± 0.22^{b}
9	7.22 ± 0.10^{b}	0.35 ± 0.06^{ab}	86.84 ± 0.52^{bc}	2.96 ± 0.58^{c}	2.02 ± 0.43^{b}
13	8.04 ± 0.77^{bc}	0.28 ± 0.03^{ab}	94.78 ± 0.17^{d}	2.65 ± 0.49^{bc}	1.93 ± 0.34^{b}
17	7.70 ± 0.74^{bc}	0.27 ± 0.01^{ab}	98.54 ± 0.68^{fg}	2.92 ± 0.55^{c}	1.79 ± 0.44^{b}
21	7.66 ± 0.33^{bc}	0.41 ± 0.06^{b}	99.42 ± 0.14^{g}	2.29 ± 0.37^{ab}	1.73 ± 0.18^{ab}
25	6.37 ± 0.26^{a}	0.88 ± 0.24^{c}	96.5 ± 0.78^{e}	2.15 ± 0.45^{ab}	1.62 ± 0.28^{ab}
29	5.73 ± 0.20^{a}	1.35 ± 0.27^{d}	97.7 ± 0.17^{ef}	2.05 ± 0.23^{a}	1.27 ± 0.25^{a}

$^{a-g}$ = Values sharing no common superscript within a column were significantly different ($P < 0.05$) and all data were shown as mean values ± standard deviation; [1]SSP = salt soluble protein; % = w/w, wet weight of fish muscle; [2] TBARS = thiobarbituric acid reactive substances; mg MA/kg = mg Malonaldehyde/kg of muscle; [3] WHC = water holding capacity; % = w/w, wet weight of fish muscle.

(Duun and Rustad, 2007). SSP solubility could be affected by many factors including hydrophobic effect of lipid on proteins, interaction of lipid oxidation with amino acids in fish muscle (Siddaiah et al., 2001). Since the myofibrillar network retained most of the water in the muscle, the properties of the SSP were important for the WHC. The myofibrillar proteolysis and loss of SSP resulted in inferior processing properties of fish muscle (Duun and Rustad, 2007).

Change of TBARS

Fish lipid typically contained high percentage of poly-unsaturated fatty acids and was consequently prone to oxidation (Huss, 1995). The TBARS value was widely used for measuring lipid oxidation in fish and fish products (Siu and Draper, 1978; Al-Kahtani et al., 1996; Yanar et al., 2006). Present TBARS value was at a very low level throughout the chilled storage without exceeding 1 mg MA/kg until day 29 (Table 2). The low TBARS value during storage might result from the direct microbial utilization of malonaldehyde and other TBARS, or result from reactions between these TBARS and the amine compounds produced by bacterial metabolism (Rhee et al., 1997). However, the TBARS content in present study was similar to those in chilled tilapia at 2 ± 2°C after irradiation (Al-Kahtani et al., 1996) but lower than the refrigerated hot-smoked tilapia at 4°C (Yanar et al., 2006). The difference might be affected by different storage temperature and processing methods.

Texture analysis

Texture softening was mainly influenced by the autolysis and denaturation of muscle protein during the chilled and frozen storage (Tsuchiya et al., 1992; Benjakul et al., 1997). Textural differences occurred between and within species due to differences in protein structure as muscle fiber diameter and chemical contents and measurement methods (Jonsson et al., 2001). Haard (1992) suggested that texture of fish flesh was influenced by many factors including postmortem pH decline, proteolysis, fat content, composition and its distribution in the fish muscle. As shown in Table 2, hardness and springness tested by texture instrument were in the decrease, while water holding capability (WHC) was at first decreased and then increased throughout the storage. This was not consistent with previous reports that showed hardness reduction, springness increase and fluctuant WHC in ice-stored meager fillets for 18 days (Hernandez et al., 2009). However, hardness reduction tested by instrument agreed well with texture softening of raw fillets judged by sensory evaluation. The WHC of muscle was regarded as an essential quality parameter and great influence to muscle texture concerned by both industry and the consumers (Ofstad et al., 1993; Olsson et al., 2003). WHC reflected the free or mobilizable water as a textural parameter in muscle and affected sensory perception of juiciness of the fillet (Jonsson et al., 2001). The WHC improvement could be affected by bacterial growth and protein degradation, the disintegration of collagen fibrils and cleavage of cross-links was mainly due to bacterial proteinases, contributing to texture softening (Olsson et al., 2003).

Conclusion

Postmortem changes of tilapia muscle indicated that microbial deterioration processes were major contribution to fish spoilage during chilled storage. The bacterial metabolites of TVBN and BAs were increased throughout the storage. The content of nucleotide breakdown components and K value were good indicators in monitoring the decrement of freshness. Protein fraction and texture analysis indicated myofibrillar protein degradation and contributed to texture softening. TBARS value remained

very low level throughout the storage. All the microbiological and physicochemical changes resulted in sensory quality deterioration and the shelf life of tray-packed tilapia fillet stored at 0°C was about 10 - 12 days when freshness and microbiological safety is being considered. In this study, the most reliable quality indicators were *Pseudomonas* counts, TVBN, CAD and K value, which might be used for further research on rapid assessment of fish quality.

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REFERENCES

Alasalvar C, Taylor KDA, Oksüz A, Garthwaite T, Alexis MN, Grigorakis K (2001). Freshness assessment of cultured sea bream (*Sparus aurata*) by chemical, physical and sensory methods. Food Chem. 72: 33-40.

Alasalvar C, Taylor KDA, Oksüz A, Shahidi F, Alexis M (2002). Comparison of freshness quality of cultured and wild sea bass (*Dicentrarchus labrax*). J. Food Sci. 67: 3220-3226.

Al-Kahtani HA, Abu-Tarboush HM, Bajaber AS, Atia M, Abou-Arab AA, El-Mojaddidi, MA (1996). Chemical changes after irradiation and post-irradiation storage in Tilapia and Spanish mackerel. J. Food Sci. 61: 729-733.

Arannilewa ST, Salawu SO, Sorungbe AA, Ola-Salawu BB (2005). Effect of frozen period on the chemical, microbiological and sensory quality of frozen tilapia fish (*Sarotherodun galiaenus*). Afr. J. Biotechnol. 8: 852-855.

Benjakul S, Seymour TA, Morrissey MT, An H (1997). Physicochemical changes in Pacific whiting muscle proteins during iced storage. J. Food Sci. 62: 729-733.

Cheret R, Chapleau N, Delbarre-Ladrat C, Verrez-Bagnis V, Lamballerie M (2005). Effects of high pressure on texture and microstructure of Sea bass (*Dicentrarchus labrax* L.) fillets. J. Food Sci. 70: E477-483.

Chytiri S, Chouliara I, Savvaidis IN, Kontominas MG (2004). Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. Food Microbiol. 21: 157-165.

Duun AS, Rustad T (2007). Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. Food Chem. 105: 1067-1075.

Eknath AE, Bentsen HB, Ponzoni RW, Rye M, Nguyen NH, Thodesen J, Gjerde B (2007). Genetic improvement of farmed tilapias: Composition and genetic parameters of a synthetic base population of *Oreochromis niloticus* for selective breeding. Aquaculture, 273: 1-14.

Eves A, Turner C, Yakupitiyage A, Tongdee N, Ponza S (1995) The microbiological and sensory quality of septage-raised Nile tilapia (*Oreochromis niloticus*). Aquaculture 132: 261-272.

Flick GJ, Oria MP, Douglas L (2001). Potential hazards in cold-smoked fish: biogenic amines. J. Food Sci. 66: S1088-1099.

Gall GAE, Bakar Y (2002). Application of mixed-model techniques to fish breed improvement: analysis of breeding-value selection to increase 98-day body weight in tilapia. Aquaculture, 212: 93-113.

Gram L, Huss HH (1996). Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33: 121-137.

Haard NF (1992). Biochemical reactions in fish muscle during frozen storage. In: Bligh EG (Eds), Seafood Science and Technology, Canada Institute of Fisheries Technology, pp.176-209.

Hernandez MD, López MB, Alvarez A, Ferrandini E, Garcia Garcia B, Garrido MD (2009). Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. Food Chem.114: 237-245.

Huss HH (1995). Quality and quality changes in fresh fish. FAO Fisheries Technical Paper 348. FAO. Rome. Italy.

International Commission on Microbiological Specifications for Foods (ICMSF), (1986). Sampling plans for fish and shellfish, In: Microorganisms in Foods. Sampling for Microbiological Analysis: Principles and Scientific Applications, Vol. 2, 2nd Edition. University of Toronto Press, Toronto, Canada, pp. 181-196.

Jonsson A, Sigurgisladottir S, Hafsteinsson H, Kristbergsson K (2001). Textural properties of raw Atlantic salmon (*Salmo salar*) fillets measured by different methods in comparison to expressible moisture. Aquacult. Nutr. 7: 81-89.

Korel F, Luzuriaga DA, Balaban MO (2001). Objective quality assessment of raw tilapia (*Oreochromis niloticus*) fillets using electronic nose and machine vision. J. Food Sci. 66: 1018-1024.

Koutsoumanis K, Nychas GJE (2000). Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. Int. J. Food Microbiol. 60: 171-184.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature, 227: 680-685.

Li SF, Cai WQ (2008). Varieties characteristics and cultured techniques of GIFT strain of Nile tilapia (*Oreochromis niloticus*). Scientific fish aquiculture, 5: 21-22.

Lund KE, Nielsen HH (2001). Proteolysis in salmon (*Salmo salar*) during cold storage: effects of storage time and smoking process. J. Food Biochem. 25: 379-395.

Malle P, Tao S (1987). Rapid quantitative determination of trimethylamine using steam distillation. J. Food Prot. 50: 756-760.

Ofstad R, Kidman S, Myklebust R, Hermannson AM (1993). Liquid holding capacity and structural changes during heating of fish muscle: cod (*Gadus morhua* L.) and salmon (*Salmo salar*) muscle. Food Structure, 12: 163-174.

Ogata H, Aranishi F, Hara K, Osatomi K (1998). Proteolytic degradation of myofibrillar components by carp cathepsin L. J. Sci. Food Agric. 76: 499-504.

Olsson GB, Ofstad R, Lødemel JB, Olsen RL (2003). Changes in water-holding capacity of halibut muscle during cold storage. LWT Food Sci. Technol. 36: 771-778.

Ozogul F, Polat A, Ozogul Y (2004). The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). Food Chem. 85: 49-57.

Ozogul F, Taylor KDA, Quantick P, Ozogul Y (2002). Changes in biogenic amines in herring stored under modified atmosphere and vacuum pack. J. Food Sci. 67: 2497-2501

Ozogul Y, Ozogul F, Gokbulut C (2006). Quality assessment of wild European eel (*Anguilla anguilla*) stored in ice. Food Chem. 95: 458-465.

Pacheco-Aguilar R, Ocaño-Higuera VM, Castillo-Yañez FJ, Moran-Palacioe EF, Marquez-Rios E, Lugo-Sanchez ME (2003). Changes in postmortem quality indices in finescale triggerfish muscle stored in ice. J. Food Biochem. 27: 333-352.

Pons-Sanchez-Cascado S, Veciana-Nogues MT, Bover-Cid S, Marine-Font A, Vidal-Carou MC (2006). Use of volatile and non-volatile amines to evaluate the freshness of anchovies stored in ice. J. Sci. Food Agric. 86: 699-705.

Rhee KS, Krahl LM, Lucia LM, Acuff GR (1997). Antioxidative/antimicrobial effects and TBARS in aerobically refrigerated beef as related to microbial growth. J. Food Sci. 62: 1205-1210.

Rodriguez Ó, Losada V, Aubourg SP, Barros-Velazquez J (2004). Enhanced shelf-life of chilled European hake (*Merluccius merluccius*) stored in slurry ice as determined by sensory analysis and assessment of microbiological activity. Food Res. Int. 37: 749-757.

Ruiz-Capillas C, Moral A (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. Food Res. Int. 34: 441-447.

Ryder JM (1985). Determination of adenosine triphosphate and its breakdown products in fish muscle by high-performance liquid chromatography. J. Agric. Food Chem. 33: 678-680.

Siddaiah D, Sagar Reddy GV, Raju CV, Chandrasekhar TC (2001). Changes in lipids, proteins and kamaboko forming ability of silver

carp (*Hypophthalmichthys molitrix*) mince during frozen storage. Food Res. Int. 34: 47-53.
Siu GM, Draper HH (1978). A survey of the malonaldehyde content of retail meats and fish. J. Food Sci. 43: 1147-1149.
Sil S, Joseph J, Kumar KA (2008). Changes in biogenic amines during iced and ambient temperature storage of tilapia. J. Sci. Food Agric. 88: 2208-2212.
Stone H, Sidel JL (2004). Descriptive analysis. Sensory evaluation practices, third edition. Elsevier, Academic Press, pp. 201-244.
Suvanich V, Jahncke ML, Marshall DL (2000). Changes in selected chemical quality characteristics of channel catfish frame mince during chill and frozen storage. J. Food Sci. 65: 24-29.
Tsuchiya H, Kita S, Seki N (1992). Postmortem changes in α-actinin and connectin in carp and rainbow trout muscles. Nippon Suisan Gakkaishi 58 : 793-798.
Veciana-Nogues MT, Marine-Font A, Vidal-Carou MC (1997). Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts, ATP related compounds, volatile and organoleptic changes. J. Agric. Food Chem. 45: 2036-2041.
Wang Y, Cui YB, Yang YX, Cai FS (2000). Compensatory growth in hybrid tilapia, *Oreochromis mossambicus×O. niloticus*, reared in seawater. Aquaculture, 189: 101-108.
Yanar Y, Celik M, Akamca E (2006). Effects of brine concentration on shelf-life of hot-smoked tilapia (*Oreochromis niloticus*) stored at 4 □C. Food Chem. 97: 244-247.
Yi Y, Lin CK (2001). Effects of biomass of caged Nile tilapia (*Oreochromis niloticus*) and aeration on the growth and yields in an integrated cage-cum-pond system. Aquaculture, 195: 253-267.