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Assessment of the freshness changes in lessepsian lizardfish (*Saurida lessepsianus,* n. sp.) during storage in ice

Bewertung der Frischeveränderungen bei Lessepsian Lizardfish (Saurida lessepsianus, n. Sp.) bei Eislagerung

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Summary

Freshness changes in lessepsian lizardfish (*Saurida lessepsianus,* n. sp.) was investigated during ice storage of 18 days through chemical, microbiological, and sensory analyses. In contrast to K and related values, proposed Fr_i value decreased with the loss of freshness. Average K value was 6 % prime freshness soon after the catch, and reached to 30 % in limit of acceptability on 15th day. TVB-N value increased from 16.9 mg TVB-N 100 g^{-1} to 32 mg TVB-N 100 g^{-1} in spoiled fish on the 18th day. Initial pH value of the flesh was by beginning 6.46 and increased to 7.5 on the 18th day. Total microbial load was by beginning day 3.45 log cfu g^{-1} and had a very slow increase until the 5th day (4.49 log cfu g^{-1}), and it reached 7.02 log cfu g^{-1} at the end of the storage period. The results of sensory scores agreed with those of chemical and microbiological analyses.

Keywords: Lizardfish, shelf-life, TVB-N, sensory analyses

Zusammenfassung

Während einer 18tägigen Eislagerung wurden die Veränderungen des Frischegrades von Lessepsian Lizardfisch (*Saurida Lessepsianus,* n. Sp.) anhand chemischer, mikrobiologischer und sensorischer Analysen untersucht. Im Gegensatz zum K-Wert und anderen Frischewerten nahm der vorgeschlagene Fr_i-Wert mit dem Verlust der Frische ab. Der durchschnittliche K-Wert betrug kurz nach dem Fang 6 % und erreichte am 15. Tag eine Akzeptanzgrenze von 30 %. Der TVB-N-Wert stieg von 16,9 mg TVB-N/100 auf 32 mg TVB-N/100 bei verdorbenem Fisch am 18. Tag. Der anfängliche pH-Wert des Fleisches betrug zu Beginn 6,46 und stieg am 18. Tag auf 7,5 an. Die gesamte mikrobielle Belastung betrug anfänglich 3,45 log KbE und hatte einen sehr langsamen Anstieg bis zum fünften Tag (4,49 log KbE) und erreichte am Ende der Lagerzeit 7,02 log KbE. Die Ergebnisse der sensorischen Bewertungen stimmten mit denen der chemischen und mikrobiologischen Analysen überein.

Schlüsselwörter: Eidechsenfisch, Haltbarkeit, TVB-N, sensorische Analysen

Introduction

Lessepsian lizardfish (*Saurida lessepsianus,* n. sp.), formerly known as *Saurida undosquamis,* has spread along the Mediterranean Sea after opening Suez Canal in the 19th century. It became one of the economically important fish species caught off not only in the shores of Iskenderun Bay (north east Mediterranean, Turkey) but also South cost of Turkey. Since lizarfish has white flesh, mild flavour, desirable aroma and low fat content, it is commonly accepted by local consumers. Beside these desirable properties, freshness of lizardfish is one of the main concerns since limited numbers of studies are available on degree of frehness for lessepsian lizardfish. Past studies are mostly on growth, stock assessment and mortality of lizardfish in the Mediterranean Coast (Erguden et al., 2009; Gabr and Mal, 2017; Gökçe et al., 2007). Previous studies describe lizardfish in the Mediterranean sea as *Saurida undosquamis,* but recent molecular study revealed that the Red Sea and Mediterranean populations are genetically distinct from specimens of *S. undosquamis* and *S. macrolepis* from their respective type localities north-western Australia and Japan (Tikochinski et al., 2016). Therefore, new description of lizardfish in the Red Sea and Mediterranean Sea specimens as a new species (Russell et al., 2015) will be mentioned in this study as a lessepsian lizardfish (*Saurida lessepsianus,* n. sp.).

Chemical, physical, microbiological, and sensory methods have been applied to evaluate the freshness or spoilage of many marketable fish species that are currently used by both industry and research purposes as freshness indices. The most accepted and satisfactory method of evaluating the freshness quality of fish is sensory analyses. Sensory analyses are based on measuring changes in appearance, odour, flavour, and texture over a certain period of time. While sensory methods provide fast, simple, and sensitive measures, they rely on human judgment that requires proper training of panels (Sims et al., 1992; Strachan and Nicholson, 1992). Therefore, sensory methods are sometimes considered as subjective. Another method of measuring the freshness quality of fish is chemical testing that mainly measures the amounts of ATP breakdown products derived from enzymatic, bacterial or oxidation activity. In this method, freshness quality of fish is related to chemical changes taking place during the post-mortem period, and the concentration of ATP and its breakdown/degradation products such as ADP, AMP, IMP, Ino, and Hx are used as indicators of freshness quality for different kind of fish species. Beside this method, new freshness assessment methods are also developed such as Colour Calibration Method (Costa et al., 2013) and multicolour biosensor based on hypoxhanthine concentration (Chen et al., 2017).

Fishing ground and environmental factors affect the microbiological conditions of fish (Fraizer and Westhoff, 1988; Hayes, 1985). The bacterial flora on newly caught fish depends on the environment in which the fish are caught rather than on the fish species (Shewan, 1977). Fish and crustaceans caught in very cold, clean waters have lower number of bacterial flora than fish caught in warm waters (ICMSF, 2005). Low initial bacterial count in chilled fish may help to extend its shelf-life. Although the chemical and microbiological assessment methods are used to measure the freshness quality of fish, sensory methods are also needed to make sure that results from sensory method evaluation for freshness are in parallel with the results of the chemical and microbiological methods. Nevertheless, investigations of the freshness quality of lizardfish during the handling, distribution, and storage in ice are of considerable interest to both retailers and consumers, limited numbers of studies are available investigating chemical, microbiological, and sensory characteristics of lizardfish during ice storage. The objective of this study is to determine the degree of freshness and its changes of lizardfish by all three methods mentioned above.

Material and methods

Catching, storage conditions and sampling of the fish samples

Lessepsian lizardfish were caught in Iskenderun Bay by trawl net (north eastern Mediterranean Sea, Turkey). Towing was carried out in between at 36°.32.488'N, 35.56.048'E and ended at 36.29.849' N, 35° 57.082'E in March 2014. Fish samples were taken from the last towing (duration 2 hrs). As soon as the fish were landed on board, they were stored in polystyrene box covered with ice. Time and temperature were monitored in order to know post-harvest history of the fish. The time elapsed between landing to the deck and first extraction of ATP breakdown compounds was less than 3 hours. The average body length weight was measured as 24±1.7 cm and weight was 120±22 g. Total 8 kg of lizardfish were stored in ice and used for experiment. Whole lizardfish were stored in ice over a period (3:1 fish ice ratio) of 18 days; and chemical, microbiological, and sensory analysis were performed on 0, 2, 5, 9, 12, 15 and 18th days.

Chemical analysis

Proximate composition

Proximate composition of the lessepsian lizardfish samples were analyzed in triplicate. Total crude protein (Nx6.25) content was determined by Kjeldahl procedure (Method 981.10, AOAC, 1995), moisture and ash contents were analyzed according to the method of AOAC procedures (Method 925.04, AOAC, 1995; 938.08, AOAC, 1995) respectively. Total lipid was determined by using the chloroform-methanol extraction procedure of Modified Bligh & Dyer method (Hanson and Olley, 1963).

pH measurement

Samples of lizardfish dorsal muscle were homogenized with distilled water in the ratio of 1:10 (w/v) by using IKA Ultra Turax at 2000 rpm for 30 second. Then pH of homogenate was measured by pH meter (ORION). Instrument was calibrated with buffer solutions (pH 4 and pH 7) before the measurements.

Determination of total volatile base- nitrogen

Total volatile base-nitrogen (TVB-N) of lizardfish was determined according to Botta et al. (1984). Basically, 10 g of fish sample was homogenized with 40 mL of distilled water and 2 g of MgO was added. A few drops of antifoam emulsion (Sigma A8582) were added into the homogenate, then the distillation was performed using Kjeldahl steam distillation unit (Buchi KjelDigester K-446). After the distillation, the released volatile amines and ammonia were trapped in boric acid and titrated against 0.01N HCl until the end point. TVB-N content was calculated and expressed as mg N 100 g^{-1} sample.

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ATP breakdown compounds

Extraction and determination of ATP and its breakdown compounds were carried out with 5 g of homogenized lizardfish samples with 0.6M cold $HClO_4$ with a ratio of 1:5 (w/v) (Ryder, 1985). ATP breakdown compounds were separated by Shimadzu Prominence HPLC (LC-10AD SP model, Shimadzu, Japan) using a reverse phase column (Inertsil, ODS-2*150 mm) and detector (SPD–M20A DAD) set at 254 nm with a SCL10A VP system controller. Mobile phase consisted of 0.06 mM K_2HPO_4+0.04 M KH_2PO_4 dissolved in HPLC grade ultra-pure distilled water. Column temperature was maintained at 25°C with a flow rate of 1.5 mL min^{-1}. The results were calculated using external calibrations (0.05 to 7 µmolar) of targeted compounds and expressed as µmol g^{-1} of fish muscle.

K, K_i, H, G and P values as indices of ATP degradation products were calculated by following formulas. A new proposed Fr_i value was also included to this study.

Analysis

Raw fish: Modified quality assessment scheme was used to identify the quality index demerit score (Larsen et al., 1992). The fish were examined for changes in general appearance (e. g., skin, bloodspot on gill cover, stiffness), eyes (clarity and shape), and gills (colour and smell). Total of eight trained panellists participated to assess the freshness changes in raw and cooked fish. Freshness scores represented absolute freshness from zero (0) to 3 (distinct features of spoiled fish) and maximum demerit point 20. All sensory quality parameters, characteristics and scores are given in Table 1.

TABLE 1: *Sensory assessment scheme for raw lizard fish.*

Quality Parameters	Description	Score
General appearance	Very bright	0
	Bright	1
	Slightly dull	2
	Dull	3
Skin	Bright, shinning	0
	Bright	1
	Dull	2
Bloodspot in gill cover	Absent	0
	Small	1
	Moderate	2
	Large	3
Stiffness	Stiff, in rigor	0
	Elastic	1
	Firm	2
	Soft	3
Belly	Firm	0
	Soft	1
	Burst	2
Eyes clarity	Clear	0
	Cloudy	1
Shape	Normal	0
	Plain	1
	Sunken	2
Gills Colour	Characteristic, red	0
	Faded, Fresh, seaweed	1
Smell	metallic	0
	Neutral	1
	Sweaty/slightly rancid	2
	Sour, stink, stale, rancid	3
Total demerit points		(0–20)

Cooked fish: The sensory assessment of cooked lizardfish was conducted by using the Torry sensory scheme for cooked white fish (Archer, 2010). The whole fish were gutted, cleaned, and prepared as fillet. Each fillet was cooked by using microwave (Arcelik, Turkey) at 850W for 3 min. Panels were initially required to describe odour and flavour quality for cooked sample from a list of terms provided in Table 2. In this sensory analysis, a score with 10–9 considered as excellent, 8–7 for good, 6–5 for fair and acceptable. A score less than 5 indicate that the fish is not acceptable for consumption.

Microbiological analysis

For microbiological analysis, 25 g of sample was aseptically removed from dorsal fish flesh, cut into small pieces and then mixed with 225 mL of 0.1 % peptone water, and homogenized in 400 mL of 0.1 % peptone water in sterile stomacher bag (BagLight®) for 90 sec, and serial dilutions from microbial extracts were performed in 0.1 % peptone water. Total viable psychrophilic bacteria count (TPB), total coliform count (TC), and total mould and yeast count (TMYC) were determined by the pour plate method using Plate Count Agar (PCA) at 10±2°C for 48 h, Violet Red Bile Dextrose Agar (VRBA) at 35±2°C for 48–72 h, and 10% tartaric acid added Potato Dextrose Agar (PDA) at 22°C for 3–5 days, respectively. All analysis was carried out in triplicate. PCA, PDA, VRBA, peptone and tartaric acid were bought from Sigma-Aldrich Chemie GmbH (Munich, Germany).

Data analysis

Data were analyzed on the basis of mean, standard deviation, variation of coefficient, one-way analysis of variance and Duncan multiple comparison test, and linear regression analysis. A significance level of 5% was used. For the analytical work three specimens (n = 3) were sampled at days 0, 2, 5, 9, 12, 15 and 18.

Results and Discussion

Proximate composition of fish samples

Biometric measurements of the fish samples revealed that average weight and length were 120±24 g and 24±1.7 cm, respectively. Moisture, protein, lipid, and ash contents of lizardfish were calculated as 78.3±0.5%, 19.5±0.1%, 1.2±0.2%, and 1.4±0.1%, consequently. Lessepsian lizardfish is a white fleshed fish, and its lipid content does not change greatly, as do in many pelagic fatty fish species. Lessepsian lizardfish can be classified as a lean fish having <2% of lipid in their muscle (Ackman, 1994). Protein content of some fish species such as sea bass and sea bream were 20.35% and 19.81%, respectively (Erkan and Ozden, 2007). Proximate composition of fish varies greatly from one species to another depending on age, sex, spawning, season, and environment. Therefore, a substantial normal variation can be observed for the composition of fish muscle. Data obtained in this study were in the range of data reported in previous studies conducted with lizardfish as well as some other white fish species (Ackman, 1994; Erkan and Ozden, 2007). Proximate composition of lessepsian lizardfish was similar to other lizardfish species *(Saurida tumbil)* with the same size caught in Indian Ocean (Meena et al., 2005).

TABLE 2: *Quality assessment scheme used to identify the quality index demerit score (Archer, 2010) adopted for lizard fish.*

Score	Odour	Flavour	Texture (mouth feel)	Score
10	Initially weak odour of sweet, Starch followed By strengthening of these odours	Watery, metallic, starchy. Initially No sweetness but meaty Flavours with slight sweetness may develop	Dry, crumbly with short, tough fibres	10
9	Shellfish, seaweed, boiled meat	Sweet, meaty, creamy, green plant, characteristic	Dry, crumbly with short, fibrous, succulent	9
8	Loss of odour, boiled milk, boiled potato	Sweet and characteristic flavours but reduced in intensity	Dry, less succulent, fibrous, stick	8
7	Wood shaving, wood sap, boiled potato	Neutral	Slightly dry, less succulent, sticky, fibrous	7
6	Condensed milk, caramel, toffee-like	Insipid	Slightly dry, less succulent, sticky, fibrous	6
5	Milk jug odours, boiled potato, boiled clothes-like	Slight sourness, trace of off flavours	Less succulent, less fibrous	5
4	Lactic acid, sour milk, "byre-like', stale grass	Slight bitterness, sour, off flavours	Initial firm going softer with storage	4
3	Lower fatty acids (e.g acetic acid or butric acids), composted grass, boiled clothes-like	Strong bitter, rubber, slight sulphide, putrid	Initial firm going softer with storage	3

Changes in pH

Lizardfish fish flesh initially showed a pH value of 6.46±0.03 and had a slow increase in pH up to 7.07±0.01 until day 12 (Fig. 1). Increase in pH indicates the accumulation of alkaline compounds, such as ammonia, derived from microbial action (Hebart et al., 1982). Usually the pH of a live fish flesh is very close to 7.0 and post mortem pH value of fish muscle ranges from 6.0 to 7.1, depending on season, species, and other factors (Simeonidou et al., 1997). The pH value in this study did not change significantly during the storage, except a rapid increase observed in late stage of storage, from 7.07±0.01 to 7.45±0.01 between 12th and 18th day (Fig. 1). This phenomenon may be related to breakdown of nitrogenous compounds either by microbial or enzymatic reactions in fish muscle and causing a rise in pH. The increase in pH after initial period may be associated with the state of rapid spoilage of fish (Kyrana, Vasiliki R. and Lougovois, Vladimiros P., 2002). However, pH analysis by itself may not be a good indicator to determine the freshness of lizardfish and other chemical indicators such as TVB-N, TMA, or K value, should be used in addition to microbiological count to determine the freshness changes for lizardfish (Simeonidou et al., 1997).

Nucleotide degradation products

ATP breakdown compounds have been widely used as an objective method to determine the degree of freshness in many marine and freshwater fish species. This method is able to monitor degree of fish freshness from their prime freshness until spoilage of fish. In fish muscle, ATP degrades by a series of enzymatic and microbial reactions to hypoxhantine (Hx) and the ratio of their concentrations give a valuable freshness index. Figure 2 shows the changes of ATP and its breakdown compounds in lizardfish during ice storage. At the beginning of storage, ATP concentration was 0.116±0.04 µmol g^{-1} muscle and this value decreased to 0.075±0.03 µmol g^{-1} muscle after day 18 (R^2=56.99 %; R^2_{adj}=38.56 %; $p > 0.05$). While the initial ADP concentration of 0.243±0.10 µmol g^{-1} increased to 0.275±0.10 µmol g^{-1} (R^2=62.17 %; R^2 adj=45.96 %; $p > 0.05$); the initial AMP concentration of 0.127±0.019 µmol g^{-1} decreased to 0.067±0.029 µmol g^{-1} muscle (R^2=54.41 %; R^2_{adj}=34.87 %; $p > 0.05$). ATP rapidly breakdown to ADP; AMP and their concentrations were nearly constant to zero and did not change much during the storage (Fig. 2). In spite of minor fluctuations during storage, average ATP, ADP, and AMP content of the fish samples remained nearly constant, and their levels were 0.091±0.035, 0.240±0.091, and 0.115±0.042 µmol g^{-1}, respectively.

Degradation of ATP and ADP occurs rapidly and disappears at 0 °C at approximately 24 h after death (Watanabe et al., 2005). Our previous experience showed that, moderately high level of ATP (2.8 µmol g^{-1}) was observed only in pre-rigor state rainbow trout and degraded to almost zero level within 24 hours (Öksüz and Garthwaite, 1998). A high level of ATP may be observed soon after the death and may disappear within 24 h in ice storage and its concentration immediately after death was reported to be 6 µmol g^{-1} and rapidly decreased in struggling fish within 6 hours (Mishima et al., 2005). The methods of catch and storage temperature have a substantial effect on post-harvest breakdown of nucleotides in fish muscle. Since, lizardfish were caught by beam trawl, and struggling in the net, duration of towing may accelerate the degradation of ATP, thus causing early onset of rigor mortis in lizardfish. There was no single alive lizardfish in landing, whereas

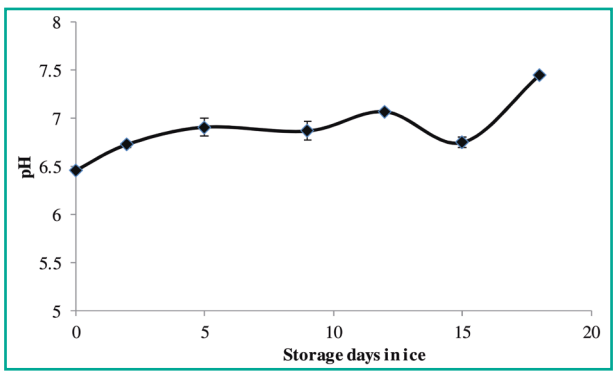

FIGURE 1: *Changes in pH value of lizardfish stored in ice.*

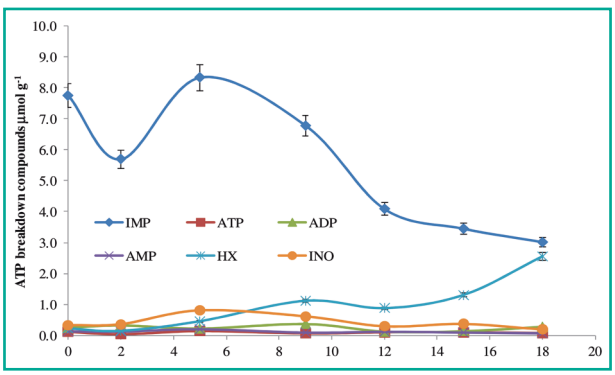

FIGURE 2: *Changes in ATP breakdown compounds during ice storage of lizardfish.*

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some other species such as threadfin bream, red snapper and horse mackerel were seen alive. Low level of ATP was also reported in cazon fish (Ocano-Higuera et al., 2009); and in ray (Ocano-Higuera et al., 2011).

At the beginning of the storage, IMP was the major nucleotide breakdown compound, and its average level was 7.76±3.06 µmol g^{-1}, then degraded gradually down to 3.42±0.69 µmol g^{-1} over 18 days storage period in ice (Fig 2).

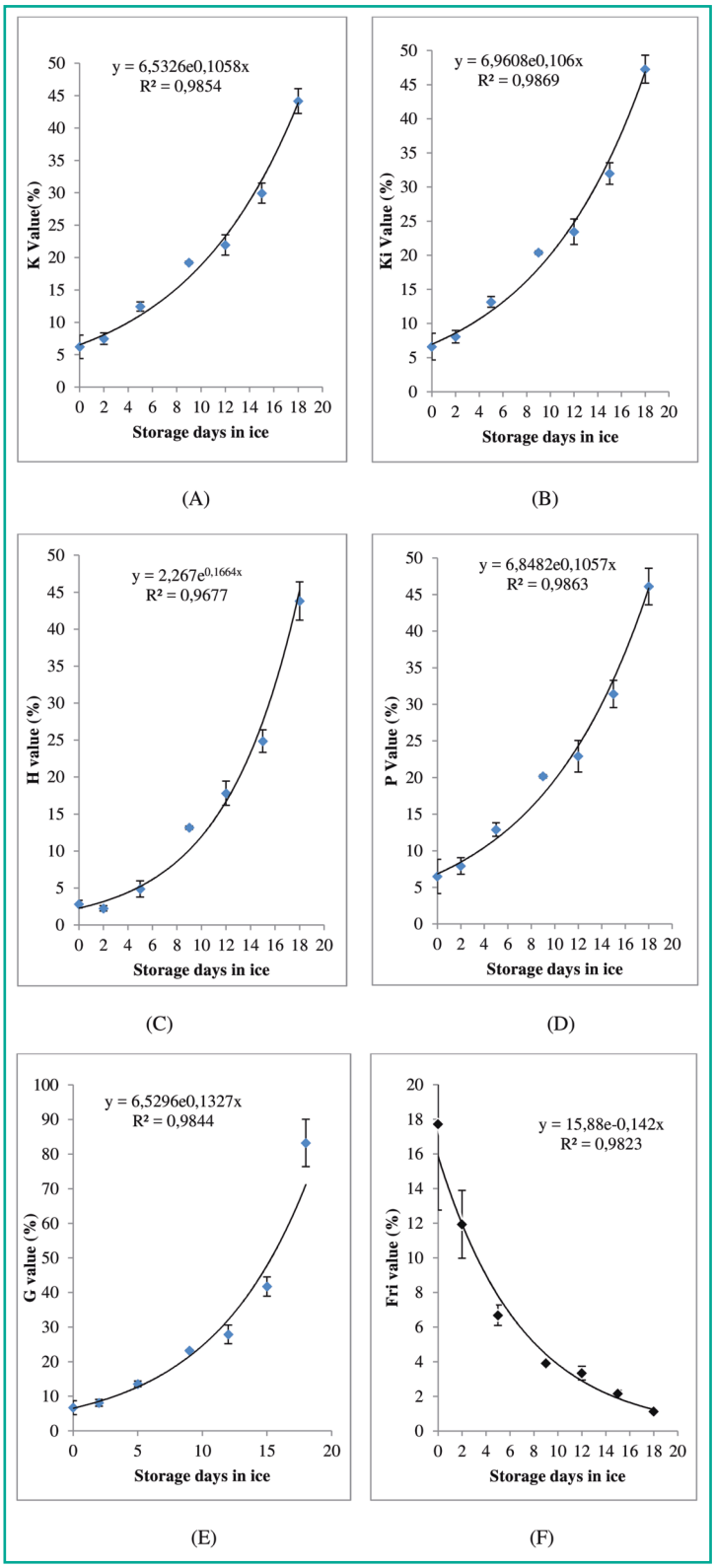

FIGURE 3: *Changes in K (A) and related values of Ki (B), H (C), P (D), G (E) and Fri (F) during ice storage of lizardfish.*

The concentration of IMP soon after death was reported to be less than 1 µmol g^{-1} and reached its maximum level within 18 h in horse mackerel (Mishima et al., 2005). This large range of IMP concentration in fish muscle may be explained by onset of rigor mortis begun much earlier than in other studies, and as a consequence of rapid ATP breakdown, accumulation of IMP occurred. High level of IMP maintained up to day 5 (8.33±0.62 µmol g^{-1}), and then decreased gradually over the period of ice storage. Breakdown of IMP in lizardfish muscle was rather slow in ice storage compare to ATP, ADP and AMP in this study. Similarly, degradation of IMP in the lizardfish muscle showed a comparable pattern in ice such as mullet and pearl spot (Lakshmanan et al., 1996); sea bream and sea bass (Alasalvar et al., 2001; Alasalvar et al., 2002), and amberjack and red sea bream (Ahimbisibwe et al., 2010). Initial level of inosine (0.332±0.286 µmol g^{-1}) also continued to decrease during storage period and resulted in 0.215±0.059 µmol g^{-1} at the end of the storage (R^2=58.46 %; R^2_{adj}=40.66 %; $p \leq 0.05$). Almost 58 % of initial INO concentration was depleted at the end of 18th day (Fig 2). Hx level of lizardfish at the beginning of ice storage (0.224±0.101 µmol g^{-1}) significantly increased to 2.87±1.08 µmol g^{-1} at the end of the 18th day (R^2=79.92 %; R^2_{adj}=71.32 %; $p \leq 0.05$) (Fig 2). The level of Hx during ice storage of European catfish (Massa et al., 2005; Ozogul et al., 2009) was much greater than in the present study.

ATP degradation products are used to determine freshness and/or spoilage of fish. Hx is a better indication of fish spoilage because Hx accumulation starts shortly after rigormortis; thus it is better indication than both TVB-N and TMA-N as they are more related to the microbial activity and do not increase at the beginning of storage (Spinellj, 1967). High level of Hx in fish muscle is not desirable, since it contributes bitter off taste in fish muscle (Fletcher and Statham, 1988). In contrast to Hx, IMP is known for its flavour enhancer properties, and thus, loss of IMP may accompany with loss of fish flavour. As a consequence of ATP breakdown in fish muscle, some fish species accumulate Hx, some species accumulates Ino or both. Therefore, depending on the accumulation of Ino or Hx, other freshness indexes such as H (Luong et al., 1992) and G values are also described beside the K value. According to current findings, lizardfish seems to be an Hx accumulating fish species. Although formation of Hx may range among fish species, fishing methods and post-mortem conditions, accumulation of Hx lizardfish during ice storage was much higher than ice-stored ray fish (Ocaño-Higuera et al., 2011; Rzepka et al., 2013) but lower in current study than Ozogul et al. (2009).

Changes in K and related values of Ki, H, P, G and Fri in lizardfish are demonstrated in Figure 3. The percentage of K, Ki, H, P, and G values in lizardfish increased during the storage with R^2 values of 0.95, 0.98, 0.96, 0.98 and 0.98 %, respectively (Fig 3a–e). These values indicate that there is good relationship between storage time and calculated values. These increments were exponential from beginning of ice storage until the end of the storage. H value increased linearly from day 2, until day 12.

Considering overall storage, Figure 3 (a–e) shows a significant and exponential increase in K and related values, and K value starting from 4–10 % to the advance

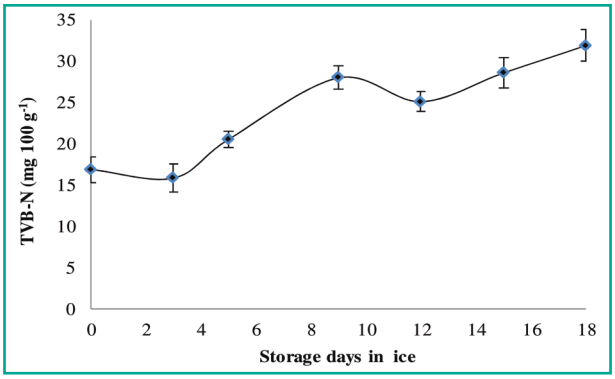

FIGURE 4: *Changes in TVB-N of lizardfish stored in ice.*

spoilage value of 38–45 % values over the period of storage. Based on changes in IMP, Hx and Ino levels, new Fr_i value was proposed since the concentration of IMP decreases with loss of freshness. Fr_i value can be described as the ratio of IMP to the sum of Hx and Ino concentrations. The initial Fr_i value (18 %) decreased exponentially down to 1 % at the end of storage. The limit of acceptability of the lizardfish was 15 days in ice according sensory panel and corresponding K value (30 %). In order to observe advance spoilage of the fish samples, storage period of fish samples was expanded up to 18 days in ice. At this stage, rapid increase was observed in K value, and it rose up from 30 % to 40 %. Therefore, the acceptability limit of lizard fish in relation to K value was much lower than for some other fish species. Gutting may be suggested to extend freshness of lizardfish a few more days further, but this application may not be practical for small size fish species.

Total volatile base-nitrogen content

TVB-N is one of the chief indicators for assessment of quality in seafood products and seems as the most common chemical indicators of marine fish spoilage. Post mortem reduction of TMA-O via bacterial enzymes may leads to a remarkable increase in a significant production of ammonia and other basic nitrogenous compounds such as TMA and DMA which are collectively known as TVB-N (Huss, 1995; Sallam, 2007). In general, it is expected that TVB-N value would increase during storage of fish samples as freshness decreases. The initial TVB-N value of lizardfish was 16.9±0.77 mg N $100g^{-1}$ which is a good start for a white muscle marine fish and did not exceed 20±0.97 mg TVB-N $100g^{-1}$ until day 5. It reached to nearly 28±1.43 mg N $100g^{-1}$ on day 9, and had a smooth increase until day 15 (28.5±0.94 mg N $100g^{-1}$) which was the limit of organoleptic acceptability. TVB-N value reached up to 32±1.9 mg N $100g^{-1}$ in spoiled lizardfish (Fig. 4). TVB-N limit of acceptability for different fish species ranges between 25 to 35 mg N $100g^{-1}$ muscle where organoleptic assessment has raised doubts as to their freshness. (EC/EU, 2005). It is difficult to set certain acceptability limit based on TVB-N value for lizardfish but 30 mg TVB-N $100g^{-1}$ may be considered as a borderline.

Measurement of TVB-N to determine fish freshness is generally considered as an accurate method, except some studies for European sea bass (Castro et al., 2006); and for sea bream (Kyrana and Lougovois, 2002). Other than that, TVB-N content in this study seemed to be a valid method for lizardfish since the findings for TVB-N showed a gradual and uniform increase during the storage period. The amount of TVB-N content in fresh fish is typically in the range between 5 to 20 mg TVB-N $100g^{-1}$, while the amount of 30–35 mg TVB-N $100g^{-1}$ fish muscle is generally regarded as the limit of acceptability for ice stored fish (Huss, 1988). Similar TVB-N values have been reported for a number of white muscle Mediterranean fish including sea bass (Kyrana and Lougovois, 2002). TVB-N value of lessepsian lizardfish may be comparable with ice stored lizardfish studied by some researchers. The initial level of TVB-N content of lizardfish was reported to be almost 14 mg N $100g^{-1}$ at the beginning of storage and reached to almost 30 mg N $100g^{-1}$ at the 14^{th} day of storage (Vittayanont et al., 2005). In another study, initial TVB-N value for lizardfish *(Saurida tumbil)* was 22.8 mg/100g and reached to 32.5 mg TVB-N/100g at the end of 18 days ice storage (Meena et al., 2005). This value correlated well with the current findings at rejection point for ice stored lizardfish.

Microbial changes

The initial total aerobic psychrophilic bacteria (TAPB), total mould and yeast count (TMY), and total coliform (TC) counts, at the beginning of the ice storage, were detected as 3.44±0.3, 3.64±0.69, and 4.65±0.41 log cfu g^{-1}, respectively (Fig. 5). According to Huss (1988) microbial counts from the investigation of the caught fish give an idea about environment, whether it is clean (low numbers of microbial count) or polluted (high numbers of microbial counts). Given this previous knowledge, it can be concluded that lizardfish used in this study was coming from clean environment. TMY count of the fish samples decreased a little; while TAPB and TC counts had a slow increase by day 2. TMY count had a smooth increase between second and ninth day (5.68±0.17 log cfu g^{-1}), and a sudden decrease by day 12 (3.71±0.68 log cfu g^{-1}). From day 12 to day 15, there was a slight decrease in TMY count to 3.60±1 log cfu g^{-1}. In addition, a quick increase in TMY count value in the fish samples was detected on day 18 and rapidly increased from 3.60±1.0 log cfu g^{-1} to 6.15± 0.16 log cfu g^{-1} (Fig. 5).

Initial TC of the samples increased slightly by day 2 and then decreased by day 5, where it had the value of 4.15±0.51 log cfu g^{-1}. After day 5, TC increased incrementally by the end of the storage to 6.33±0.33 log cfu g^{-1}. Initial TPMC had a slow increase until day 5 (4.48±0.18 log cfu g^{-1}) and then decreased by day 9 to 3.73±0.28 log cfu g^{-1}. After day 9, it increased sharply until day 15 to 7.02±0.55 log cfu g^{-1}, indicating spoilage (Fig. 5). TAPB count value at the end of the storage period was 7.02±0.55 log cfu g^{-1}, and this value is

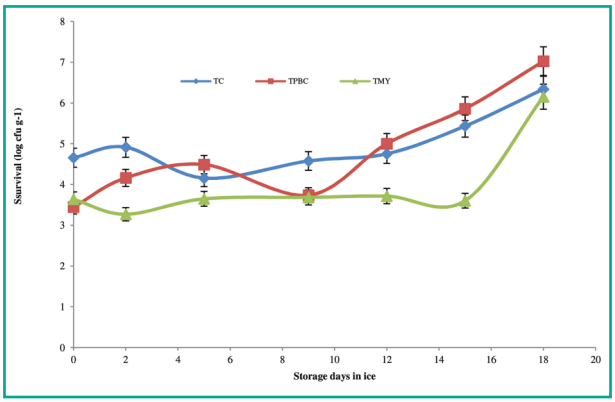

FIGURE 5: *Microbial analyses (TPMC: total aerobic psychrophilic bacteria count; TMY: total mold and yeast count; TC: total coliform count) lizardfish during ice storage.*

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considered above the maximum level of acceptability for freshwater and marine fish by (ICMSF, 1978).

Sensory analysis

Raw fish: Changes in sensory characteristics of the whole lizardfish (ungutted) during the storage period in ice were recorded using the descriptions given by the individual panel members (Fig. 6). Bright skin, firm belly, seaweed/metallic smell, clear eyes, and characteristic gill colour were considered as attributes of freshness while dull skin, blood-spot on gill cover, soft belly, belly burst, rancid smell, discoloured gills, cloudy and sunken eyes indicated stale fish. As it can be seen from Figure 6, very good scores for whole lizardfish were recorded during the first nine days of the storage period. After that, spoilage level started to increase by the end of the storage period. As it was mentioned above, belly burst is one of the remarkable indicators to measure the loss of freshness either caused by physical damage, microbial, or enzymatic action. Belly burst occurs in different times for different species. Belly burst was observed in some fish samples on day 5, although they showed no visual spoilage sign on the other part of the fish. When stomach content of such fish was dissected, some undigested crustacean and small fish were present in it. It is believed that digestive enzyme, which is still active in the digestion duct, may play a crucial role to digest bell wall and causing bell burst (Ghaly et al., 2010). In this case, the spoilage of fish may be accelerated by contaminating with gut content to other fish during storage if they are stored together.

In addition, fish skin became dull and slime was present on the skin after day 12. Furthermore, cloudy eyes and off-odour were also observed and smelled by the end of the storage (Fig. 6). All these undesirable changes are the results of microbial activity taking place during the storage period (Gram and Dalgaard, 2002). At the end of the storage period, microbial growth (PCA) already reached 7.02±0.55 log cfu g^{-1} causing microbial spoilage of the samples.

Cooked fish: According to the panellists' description, cooked fish was found very tasty and had a nice odour at the beginning of the storage and were scored as 10 out of 10 (meaning very pleasant). Sensory quality of cooked fish got considerably better score than that of raw fish (Fig. 6). It is quite possible that some undesirable volatile compounds may disappear by cooking. Scores of odour and flavour decreased to score of 4 at 15th day of storage, which was considered borderline of acceptable score by panellists. Similar scores were also previously reported by Alasalvar et al. (2001) for white fleshed fish for sea bream stored in ice.

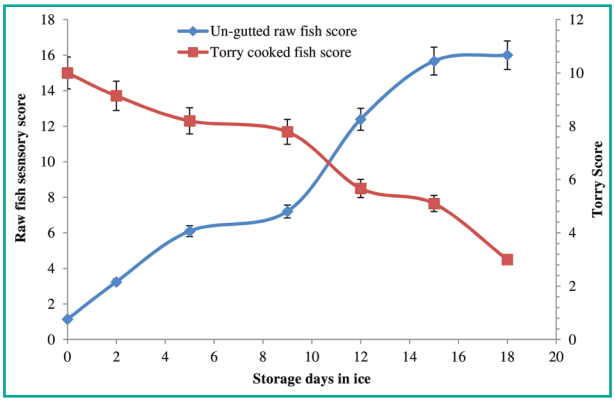

FIGURE 6: *Sensory evaluation of ungutted and cooked lizardfish.*

Conclusions

Un-gutted lizardfish remained fresh up to 12 days in ice storage. In comparison to other chemical indices such as TVB-N and pH, analysis of ATP breakdown compounds seem to be a better freshness indicator showing the changes from initial freshness up to advance spoilage. K value at rejection point in lizardfish was much lower than other fish species indicated in the literatures. Therefore, the use of ATP breakdown compounds as a freshness indicator is species dependant in particular at rejection point. Progress of ATP breakdown compounds in lean fish or white fleshed fish should be reconsidered in order to set the limit of acceptability based on K value. K value in ice stored fish species differs greatly at rejection point. Therefore, setting the degree of freshness based on K value must not be generalised in all fish species. Sensory score well correlated with loss of freshness (y=0.8674x+1.2632, R^2 = 0.9668) and with K value (R^2 = 0.9854).

Total microbial count rapidly increased towards the spoilage, and icing delayed the microbial growth. The lessepsian lizardfish maintained its freshness up to 12 days and reached acceptability borderline on day 15. However, this study was in controlled storage condition with a known catch and temperature history. In practical, shelf-life of lessepsian lizardfish may be less than indicated in this study. This study revealed how physical, chemical, microbial and sensory properties of lessepsian lizardfish changed during ice storage, but further studies are needed to determine how catching conditions effect the shelf life as these conditions play a vital role in the quality and shelf life in fishing industry.

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Conflict of interest

The authors declare no conflict of interest.

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