International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Determination of the Degree of Marine Fish Spoilage by Measuring Trimethylamine Contents and Physical Assessments

Ahmed Rashed Al-Najada

King Abdulaziz City for Science and Technology, P. O. Box 6086, Riyadh, 11442, Kingdom of Saudi Arabia

Corresponding Author: E-mail: ahmedrn[at]hotmail. com

Abstract: ***Changes in the shelf life indices of iced species from the Arabian Gulf Shaeri (Lethrinusminiatus) and Hamour (Epinephelus chlorostigma) were evaluated. Perishable food products can be determined through an assessment of the bacterial tests or by chemical tests such as the determination of Trimethylamine (TMA) which is produced by the reduction of trimethylamine oxide (TMAO), Trimethylamine contents and Physical assessments evaluations were performed at 0, 3, 5, 7, 10 and 13 days postmortem. Initially, trimethylamine (TMA) values were higher (P <.05) in marine species and TMA values increased with storage period, yet none of the species under investigation reached the rejection (30 mg TMA-N/100g) level. Sensoryly the keeping time of the different fish species were found to be around 6.33 and 10.9 days for Hamour and Shaeri respectively. Hamour had way less (P <.05) sensory scores than Shaeri. In general, torry meter scores for marine fish species decreased to varying degrees with storage period. Shaeri had higher values than Hamour. In the first three days of storage changes in torry meter values of Shaeri and Hamour were very minimal during this period. Initially, marine fish species under investigation had pH value higher than 6.8. Throughout the storage period marine fish species had Changes in the Hunter color values L*, a* and b* were followed for up to 7 days of storage. Generally, throughout the storage period tested marine fishes had hunter color values higher than (P <.05) L*. Marine species. Upon storage became darker i. e. their a* values increased with storage time to reach their peaks 8.69 and 10.67 for Hamour and Shaeri respectively. None of the two marine fish species had a_w below 0.9. However, throughout the storage period studied, no significant differences (P >.05) in moisture content were noticed in marine fishes, the initial protein content of Shaeri was slightly (P >.05) higher than the protein content of Hamour. Storage period had no effect (P >.05) on the protein content of either Shaeri or Hamour. The iron content of marine fish species (Hamour and Shaeri) had initially similar (P >.05) iron content. Upon storage (day 5) and showed substantial increases (P <.05) of 44% and 48% for Hamour and Shaeri respectively. The effect of storage on the copper content was similar to that of iron i. e. a decrease in content with storage except for Shaeri where a 45% increase.***

Keywords: Marine fish, Shelflife, postmortem changes, TMA,, Sensory evaluation and Hunter color values

1. Introduction

Fish is a highly perishable food ; it spoils quickly after the fish dies and therefore, series of changes can be responsible for the fish spoilage after their body defense mechanisms stop. These changes can be caused by many factors (microorganisms, enzymes, physical and chemical activities) which contribute to changes in edible and sensory qualities, including deterioration in color, texture, appearance, aroma and flavor. Trimethylamine (TMA) is produced by the reduction of trimethylamine oxide (TMAO), possibly by endogenous enzymes in fish, but mainly by the enzyme activity of certain bacteria, TMA is associated with the odor of fish spoilage and is clearly a part of the spoilage pattern of many fish species. The consumption of meat and meat products mostly depends on color, appearance, flavor and taste (Risvik, 1994; Van Oeckel et al., 1999; Davoli and Braglia, 2007). After death the quality of fish deteriorates, and as it does gaseous species are given off including trimethylamine, the concentration of which increases significantly as the freshness decreases. Thus there is a possibility of determining the freshness of fish by monitoring changes in the concentration of emitted trimethylamine. Fish freshness can be expressed by the K value, this being defined as the percentage of inosine and hypoxanthine present among the adenosine triphosphate related compounds in fish muscle. However, the storage times in ice are relatively longer, e. g., from 2 to more than 3 weeks. This difference in storage time is due to the nature of the microflora initially present in fish at the moment of capture (El Marrackchi, Bennour, Bouchriti, Hamama, & Tagafait, 1990). It is known that loss of freshness and spoilage pattern in fish markedly varies from species to species. Once the fish dies, several postmortem changes take place, which are due to the breakdown of the cellular structure and biochemistry as well as to the growth of microorganisms that are either naturally associated with the fish, or associated to contamination during handling (Ehira & Uchiyama, 1987). Many contaminating bacteria reduce trimethylamine oxide (TMAO) and form TMA, thus the presence of TMA in muscle can give a very good indication of the level of bacterial contamination (Beatty and Gibbons, 1937; Babbitt et al., 1972). However, it should be mentioned that the significance of the TMA content varies according to the species under consideration, and the amount of TMA produced is an indicator of the degree of spoilage and so is a measure of the activity of spoilage bacteria which is naturally present in the skin and in the guts of many marine fish species.

2. Materials

Marine fish species

1- Longface emperor (Lethrinusminiatus) (Shaeri)

2- Hexagonal spotted grouper (Epinepheluschlorostigma) (Hamour)

Marine water species. Longface emperor (Lethrinusminiatus) (Shaeri) and Hexagonal spotted grouper (Epinepheluschlorostigma) (Hamour) with a weight of about (350 – 400 g) were purchased from a local fish market. The fish were caught from the Arabian Gulf and immediately dispatched in plastic container filled with ice. Upon arrival at the laboratory, fishes were divided into five treatment groups and stored in crushed ice in five fish ice boxes (70 × 40 × 35 cmeach). Longface emperor (Shaeri) and Hexagonal spotted grouper (Hamour) were stored in box A, B, C, S, and H respectively. The ratio of fish to cruched ice was 1:1 (w/w) and storage temperature was about (3-4^{O}C) over the storage period. Whenever, necessary boxes were dranined of melted ice water and more crushed ice was added to the boxes to maintain the temperature at around 3-4 ^{o}C.

Methods of Analysis:

Fish meat was assessed initially and after 3, 5, 7, 10 and 13 days of cold storage. Assessments include chemical analysis of TMA for marine water fishes, color evaluation including saturation index and hue angle, pH, water activity and sensory evaluation.

Physical assessments:

1- Torry meter

Initially and at the end of each storage period the changes in the freshness of fish species with storage times were measured objectively using Distell freshness Meter " Torrymeter " device (Distell Industries LTD, Scotland, UK).

2- pH measurement

The pH was determined by adding 5 gm ground fish meat to 20 ml distill water. The mixture was well homogenized and the pH was measured using a digital pH meter (Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA).

3- Color measurement

Color of fish meat was measured objectively using

Hunter lab color measurement device (Hunter color Quest 45/0,
Hunter Associates Laboratory, Reston, VA. USA) to measure
Hunter values L*, a* and b*. These values were then used to calculate the saturation index and Hue angle according to Little (1975) as follows:

Saturation index: $SI = (a^2 + b^{2)\,½}$
Hue angle: $H = \frac{(\tan^{-1} b/a) \times 180}{\Pi}$

a = Hunter a* value
b = Hunter b* value
tan = tangent

4- Water activity measurement

Water activity reflects the active part of the moisture content as opposed to the entire moisture contents of a product. The water activity of fish meat from each storage period was determined with the A2101 water activity meter (Rotronic, USA) which is a combination of a ventilated humidity and temperature probe (AWVC) with a micro processor based indicator (AW Quick).

Chemical assessments:

1- Proximate Analysis

Moisture, protein, fat and ash contents of marine fish meat at 0 and 5 days of storage period were determined according to the AOAC procedures (AOAC, 1995).

2- TMA Determination

The method of Muray and Gibson (1972) used to determine the TMA content of aquacultured and marine fish species.

Sensory evaluation

A 10-member sensory panel, consisting of the staff members and students of Food Science and Technology Department, College of Agricultural and Food Sciences, king Faisal University, semitrained according to the procedure of Cross et al., *(*1978), evaluated the fresh fish meat odor intensity by means of 9-point hedonic scale (9 = intense fresh fish odor, 1 = devoid of fresh fish odor).

Statistical Analysis

The data collected were subjected to analysis of variance and whenever appropriate the mean separation procedure of Duncan was employed (Steel and Torrie, 1980). The SAS program (SAS, 1988) was used to perform the GLM analysis.

3. Results and Discussion

Physical analysis:

1- Torry meter scores

Generally, torry meter scores for marine fish species decreased to varying degrees with storage period. On the average, marine fish species had ($p <.05$) torry meter score (13.2). Initially, among marine fish species Shaeri had higher values than Hamour. In the first three days of storage changes in torry meter values of Shaeri and Hamour were very minimal during this period. Torry meter values of these species started to decrease till the 7th day of storage. The gradual changes (decrease) in torry meter readings in the first few days of storage agree with the observation of Aldakheel (1984). (Fig.1).

2- pH values:

Initially, marine fish species under investigation had pH value higher than 6.8. Generally, Shaeri showed little or no change throughout storage period. This slight or no change in the pH values of Shaeri with increase in the storage period (day 3 to day 7) may have resulted from the production of both alkaline (pseudomonas) and acidic (lactic acid bacteria). Also white muscles especially those of fish and shellfish have a relatively high content of amino acids and peptides that probably serve to buffer changes in pH (Haard, 1992). On the other hand hamour pH decreased slightly through day 3, then showed little change through day 5, thereafter it tended to increase. On day 3 and till the end of storage period Hamour had higher pH values than Shaeri. Simeonidou et al., *(*1998) and Church (1998) indicated that postmortem pH can vary from 6.0 to 7.1 depending on season, species and other factors like handling conditions. On the other hand several investigators (Pacheo–Aguilar et al., *(*2000), Barret *et al., (*1965) reported pH values below 6.0 for several fish species e. g halibut, tuna, sardine and mackerel. In the current study, pH of Hamour varied from 6.9 to 7.4 showing an increment of 0.5 unit. Riaz and Quadri (1985) indicated that a pH increment of more than 0.2 unit means that the sample had deteriorated. Based on this parameter Hamour had an acceptable quality for 3 days on ice (Fig.2).

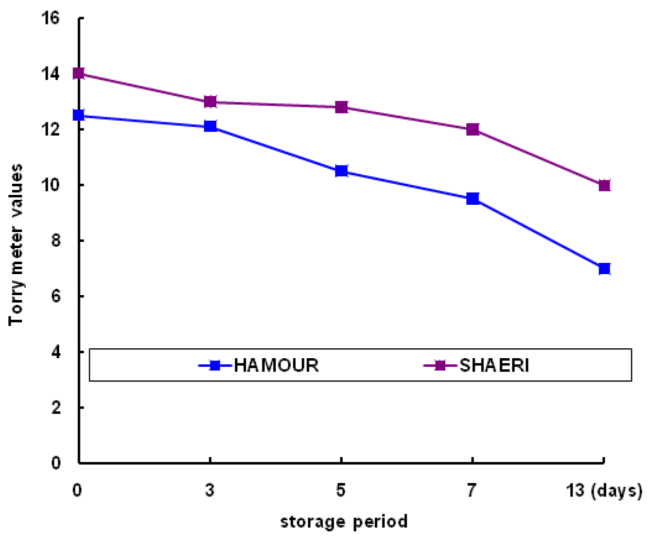

Figure 1: Changes in the torry meter values of marine fishes with storage period

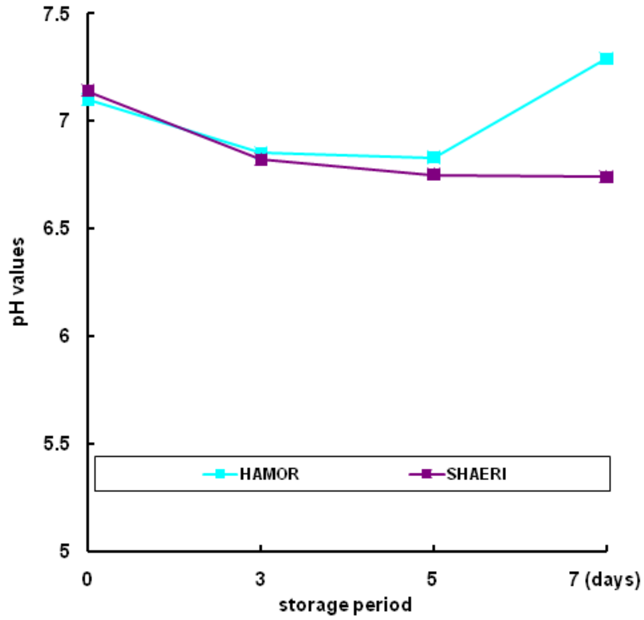

Figure 2: Changes in the pH values of marine fishes with storage period

3- Color measurement

It is well known that the naturally occurring colors of many food items undergo undesirable changes during storage. Fish meat is no exception. Fish meat pigment; usually deteriorate with postmortem time leading to visual changes in the color appearance. Changes in the Hunter color values L*, a* and b* were followed for up to 7 days of storage. Generally, the hunter color values were high (P <.05) L*throughout the storage period in tested marine fishes. Marine species. Interestingly, upon storage became darker i. e their a* values increased with storage time to reach their peaks 8.69 and 10.67 for Hamour and Shaeri respectively, andon the 5th day of storage before decreasing on the 7th day of storage. However, Shaeri, and Hamour had similar b* values (P >.05) through day 5 of storage. Mmarine species, Shaeri had higher L* values through day 3 than Hamour. While the two species had similar (P >.05) L* on the 5th day, on the 7th day the trend was reversed where Hamour had higher (P <.05) L* values. As for the a* values the two species had similar (P >.05) values through day 3 but on day 5 Shaeri had higher (P <.05) a* values than Hamour. Then again the two species had similar a* values on the 7th day of storage. Shaeri had higher b* values initially and through day 5, only on the 7th day of storage the two species had similar (P >.05) b* values. In conjunction with Hunter color L*, a* and b* values, Saturation Index and Hue angle can improve our understanding to the changes in the color of the different fish species throughout the storage period. The saturation index is the attribute of color perception that expresses the degree of departure from the grey of the same lightness. While Hue angle is the attribute of color perception by means of which an object is judged to red, yellow, green, blue or purple. Hamour and Shaeri had their maximum SI and Hue angle on the 7and 5th day of storage respectively. Changes in the Saturation Index (SI) and Hue angles (Hue) of the different fish species stored in ice for up to 7 days are shown on (table1) and (table 2).

4- Water activity (a_w)

Fish meat is a hygroscopic product that may absorb water in different ways, like sorption with formation of a hydrate, binding by surface energy, diffusion of water molecules in their structure etc. Accordingly water is bound to it with more or less strength. It is well known that moisture content of fish meat can include both an immobilized part e. g. water of hydration and an active part which, under normal circumstances, can be exchanged between fish meat and its environment. Water activity of fish meat product reflects the active part of its moisture content as opposed to its entire moisture content. The water activity of marine fish declined with storage period till day 5, however, the trend was reversed where marine fish had again increased. In general, water activity of marine fish species decreased with storage period. Leistner and Rodel (1975) concluded that for a product to be shelf stable, its a_w must be < 0.95 in combination with other parameters. Lowering the water activity to < 0.90 reduces risk of microbial spoilage; however none of the marine fish species under investigation achieved that level (Fig.3)

Table 1: Changes in hunter values of marine fishes with storage period

St. days	Hunter values	Hamour	Shaeri
0	L*	53.16^{b}_{1}	56.23^{c}_{1}
	a*	6.10^{b}_{1}	5.88^{b}_{1}
	b*	13.93^{b}_{1}	17.05^{c}_{1}
3	L*	53.96^{b}_{1}	59.27^{c}_{2}
	a*	7.49^{a}_{2}	7.54^{a}_{2}
	b*	13.75^{a}_{1}	20.42^{b}_{2}
5	L*	51.92^{d}_{2}	52.58^{d}_{3}
	a*	8.68^{a}_{3}	10.67^{c}_{3}
	b*	15.06^{a}_{2}	21.19^{b}_{2}
7	L*	55.51^{d}_{3}	54.04^{e}_{4}
	a*	6.80^{c}_{1}	7.16^{c}_{2}
	b*	16.72^{b}_{3}	17.69^{b}_{1}

Means in the same row bearing different superscripts letters differ significantly (p <.05). Means in the same column bearing different numerical for the same parameter are significantly different (p <.05)

Table 2: Changes in saturation index (SI) and Hue angles (Hue) of the different fish species stored in ice for up to 7 days

Fish species	SI and Hue values	Storage period (days)			
		0	3	5	7
Hamour	SI	15.21^{a}_{1}	15.66^{a}_{1}	17.38^{b}_{1}	18.05^{c}_{2}
	Hue	66.35^{a}_{3}	61.42^{b}_{3}	60.04^{c}_{2}	67.87^{d}_{3}
Shaeri	SI	18.04^{a}_{3}	21.77^{b}_{2}	23.73^{c}_{3}	19.09^{d}_{3}
	Hue	70.97^{a}_{4}	69.73^{b}_{4}	89.97^{c}_{4}	67.97^{d}_{3}

Means in the same row bearing different superscripts letters differ significantly (p <.05). Means in the same column bearing different numerical for the same parameter are significantly different (p <.05)

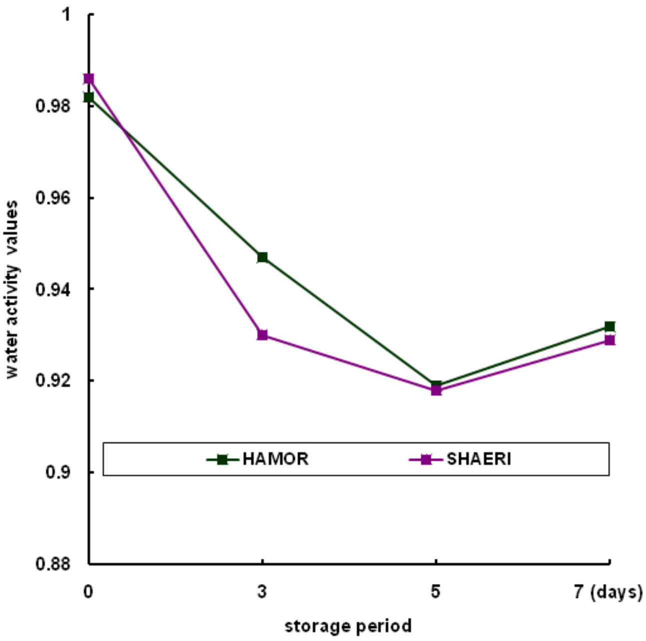

Figure 3: Changes in the water activity (a_w) values of marine fishes with storage period

Chemical analysis:

1- Proximate analysis

However, throughout the storage period studied, no significant differences (P >.05) in moisture content were noticed in marine fishes. Within marine fish species, the initial protein content of Shaeri was slightly (P >.05) higher than the protein content of Hamour. Storage period had no effect (P >.05) on the protein content of either Shaeri or Hamour. Fat content within marine fish species, initially Hamour was observed to be superior (P <.05) to Shaeri. It had 52% more fat than Shaeri. Generally, storage time had no effect on the fat content of marine fish species. Also, the storage time had no effect (P >.05) on the ash content. Hamour and Shaeri showed no significant differences (P >.05) in their M/P either initially or after 5 days of storage. Iron and copper contents of marine fish species are noted in (table 7). Transition metal ions, particularly iron and copper are considered to be major catalysts for oxidation. At very low concentrations < 0.1 ppm, they can decrease the induction period and increase the rate of oxidation. Such metal ions, either in free and bound form occur naturally in fish tissues, membrane and enzymes. The iron content of marine fish species (Hamour and Shaeri) had initially similar (P >.05) iron content. Upon storage (day 5) and showed substantial increases (P <.05) of 44% and 48% for Hamour and Shaeri respectively. With regard to copper content, the effect of storage on the copper content was similar to that of iron i. e. a decrease in content with storage except for Shaeri where a 45% increase was encountered (table 3) and (table 4).

2- Trimethylamine (TMA)

Bacterial growth of marine fishes on surface tissue is accompanied by the production of volatile amines, notably trimethylamine (TMA) is produced in fish meat by the reduction of trimethylamine oxide (TMAO). Thus, the measurement of TMA can be used as an indicator of the degree of spoilage and protein putrefaction (Dyer and Mownsey, 1945). Generally, the level of trimethylamine (TMA) was significantly much increased ($p < 0.05$) in marine fish. This was expected and the possible explanation for such higher levels of TMA in marine fish could be due to the fact that marine fish had high trimethylamine oxide (TMAO). TMAO is an osmoregulator accordingly its concentration observed to be high in marine fish species. Certain types of bacteria in fish reduce TMAO to TMA which is associated with a fish odor (Hebard*et el.*, 1982). Also, low levels of TMA were observed in European catfish (*silurusglanis*), which was stored in ice for 30 days. Manthey *et al., (*1988). Irrespective of species the TMA had steadily increased with time of storage in marine fish. In the first 5 days of storage changes in TMA were observed to be slow in Hamour and Shaeri, thereafter the tone of changes increased to reach ahigh of 22.13 mg TMA/100g in Hamour and 19.21 mg TMA/100g in shaeri on day 13. The differences observed here in the amount of TMA in Hamour and Shaeri could reflect, differences in the amount of TMAO in the two species. TMAO (a tasteless NPN compound) content varies with season, size and age of the fish (Hebard, *et el.*, 1982). The increase in TMA with storage time, particularly for Hamour and Shaeri was found to parallel bacterial growth and population. Low temperature storage might have slowed down TMA production (Ishida et al., 1976), consequently. Altering the TMA level at which fish are considered spoiled. Hamour fillet in this study was found to be sensoryly spoiled by day 5 of storage at which time TMA concentration was 2.55 mg/100gm. A wide range of TMA levels has been reported for various spoiled fish species. A TMA content of> 26 mg/100gm for cod and haddock indicated that the fish were unfit for processing (Castell and Greenough, 1958; Castell et al., 1958); whereas TMA levels of 3-8 mg/100gm were obtained from indian fish species (SenGupta *et al.*, 1972). TMA levels vary with fish species, season and type of storage (Herbard, *et el.*, 1982). (table 5)

Table 3: Proximate chemical analysis of marine fish with storage period

Fish species	St. (days)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	M/P
Shaeri	0	74.8^{b}_{1}	20.3^{b}_{1}	2.1^{b}_{1}	1.1^{a}_{1}	3.68^{b}_{1}
	5	74.3^{d}_{1}	20.4^{d}_{1}	2.0^{d}_{1}	0.99^{c}_{1}	3.64^{d}_{1}
Hamour	0	75.6^{b}_{1}	19.5^{b}_{1}	3.2^{a}_{1}	1.0^{a}_{1}	3.88^{b}_{1}
	5	75.2^{d}_{1}	19.55^{d}_{1}	3.1^{c}_{1}	1.01^{c}_{1}	3.85^{d}_{1}

Means in the same column for the same storage period bearing similar superscripts letters are not significantly different (p >.05). Means in the same column for the same fish species bearing different subscripts numerical are significantly different (p <.05). (n = 5)

Table 4: Changes in iron and copper contents of marine fish with storage period. (mg/100g)

Fish species	Iron		Copper	
	Storage period (days)			
	0	5	0	5
Hamour	0.180^{c}_{1}	0.26^{b}_{2}	0.085^{c}_{1}	0.054^{ac}_{2}
Shaeri	0.182^{c}_{1}	0.27^{b}_{2}	0.055^{d}_{1}	0.080^{d}_{2}

Means in the same row for iron or copper bearing different superscripts numerical differ significantly (p <.05).
Means in the same column bearing different letters are significantly different (p <.05)

Sensory scores:

The sensory evaluations of marine fish species for up to 13 days of storage period. Generally, sensory scores of fish species under investigation tended to decrease (P <.05) with storage period. In case of Liston et al., (1961), the panel member agreed beforehand that a score of 2 on a 5-point scale corresponded to the lower limit of edibility. Other investigators (Bottaand Show, 1975, 1976; Botta *et al.*, 1978; Show and Botta, 1975) had taken the time when the maximum scores for the various sensory parameters had been reduced by 60% as the limit of acceptability. In the current study we decided beforehand that a score of 4.5 on a 9-point scale correspond to the lower limit of acceptability. Applying this criterion i. e 50% reduction of the maximum sensory score, the keeping times of the different fish species in this study would be around 6.33 and 10.9 days for Hamour and Shaeri respectively. Within marine fish species, from day 2 through day 10 Hamour had way less (P <.05) sensory scores than Shaeri. Apparently, Hamour spoiled faster than Shaeri and had a shelf life that was about 5 days less than Shaeri. According to Disney et al., (1974) limited available evidence suggests that many tropical fish species have longer shelf life in ice than do temperate or cold water fishes (Fig.4).

Table 5: Changes in trimethylamine (TMA) values of marine fish with storage period. (mg /100g)

St. (days)	Shaeri	Hamour
0	0.15^{b}_{1}	0.21^{c}_{1}
3	0.35^{b}_{2}	0.43^{c}_{2}
5	1.50^{c}_{3}	2.55^{d}_{3}
7	5.68^{c}_{4}	7.74^{d}_{4}
10	14.05^{b}_{5}	16.15^{c}_{5}
13	19.21^{c}_{6}	22.13^{d}_{6}

Means in the same row bearing different superscripts letters differ significantly (p <. o5). Means in the same column bearing different numerical are significantly (p <. o5) different. n = 5

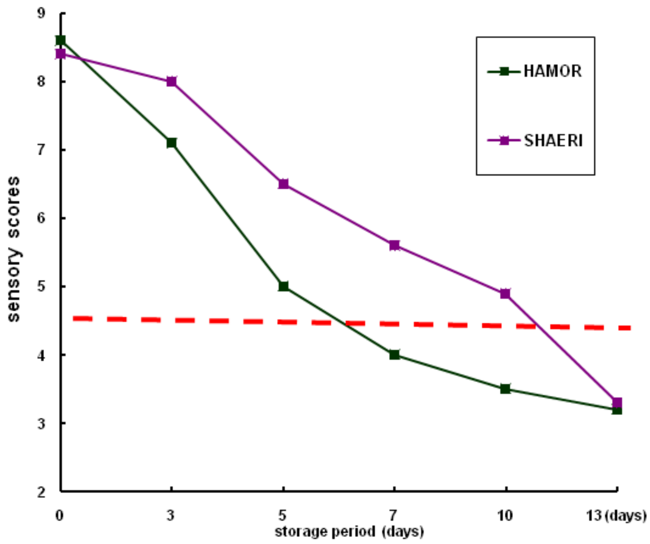

Figure 4: Changes in the sensory evaluation values of marine fish with storage period

4. Conclusions

A number of shelf life indices namely (TMA, Torrymeter, a_w, pH, color and sensory) could be used to monitor changes in the quality of marine fish species with postmortem time. However, none of the fish species under investigation had a TMA greater than 30 mg TMA-N/100g fish, which is the rejection limit, at any one of the storage period tested. Sensory evaluation indicated that marine fish species had approximately, shelf life of 8 - 9. Additional studies are needed to fully assess the applicability of the changes in some of the chemical as well as physical shelf life indices that could supplement sensory evaluation tests in predicting quality deterioration of fresh fish seawater in Saudi Arabia and ultimately determine their shelf life.

Acknowledgement

The author would like to thank The King Abdulaziz City for Science and Technology for supporting this work.

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