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The Effect of Stacking of Narrow-Barred Spanish Mackerel (*Scomberoides commersonnianus*) on Protein Composition and Amino Acid Profile

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ABSTRACT: Salt curing (Kencing) is a method used for preserving food products, including fish, that is believed to influence the quality and chemical composition of the finial product. The present study is focused on the alteration of protein quality and amino acid profile of *Scomberoides commersonnianus* after stacking during 190 days of shelf life in ambient temperature. A12 assorted of specimens were collected from Gheshm Island, Hormozgan Provience, North-West of the Persian Gulf and were transferred to the laboratory for processing. The samples were divided into six batches and cured with common salt. The samples were used for quality control at six intervals, including day 0(before salt curing),15, 30, 90, 150 and 190 (after salt curing); and TVN, crude protein, ash, moisture and salt contents were measured. Moreover, the amino acid profiles of the product were also being evaluated at three intervals including day 0(before saltcuring), 90 and 190 (after saltcuring). The increment of crude protein, ash, salt, moisture and TVN contents were significant after 190 days of preservation ($p<0.05$).18 amino acids were distinguished in the fresh sample, including 8 and 10 essential and non essential amino acids, respectively. The total amino acid content decreased significantly after 190 days curing with salt ($p<0.05$). The results indicated that the salt curing of *Scomberoides commersonnianus* could be a helpful method in preserving the product for just 90 days after processing, and the quality of the product was decreased significantly after 90 days of preservation.

Keywords: *Amino Acid Profile, Approximate Analysis, Crude Protein, Fisheries Product, Salt Curing, Scomberoides commersonnianus, Total Volatile Nitrogen.*

Introduction

Salt curing is one of the old preserving methods used for increasing the shelf life of foods and food products, including fisheries' products through dehydration and decreasing the water activity of the product (Mujaffar and Sankat, 2005; Andres *et al.,* 2005; Rodrigues *et al.,* 2003). Curing fish products with salt was traditionally carried out in two general ways; pickling/brining and stacking (Boeri *et al.,* 1982). During salt curing liquid is released from the muscle, and salt begins to be uptaken and this will continued until the osmotic pressure becomes equal between the tissue and the surrounding area (Moini, 1391). Many factors are expected to affect the quality of the products. These are distinguished by variation of raw materials, salting methods and the concentration of salt and the amount which is penetrated into the fish muscle. The amount of salt penetration is varied among the applied methods that results in producing safe products with different acceptable tastes (Espe *et al*., 2004; Fuentes *et al.,* 2008; Mujaffar and Sankat, 2005). Salt curing has an influence on the

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structural and mechanical properties of the fish muscle. The salt is diffused into the muscle, and the water and soluble proteins are extracted depending on the salt concentration. Some protein bonding are affected that resulted in denaturation of proteins (Thorarinsdottir *et al.,* 2004). All of these alterations not only affects the taste and the texture of the final product (Ismail and Wootton, 1992; Thorarins dottir *et al.,* 2004), but also affect the shelf life through inhibiting bacterial and enzymatic degradation procedures (Ismail and Wootton, 1982; Beraquet *et al.,* 1975, Del valle and Gonzales-Inigo, 1968). Salted products might be classified into two groups, the deeply and lightly salted products. The former need to be desalted before consumption and the value of water activity (a_w) should be close to 0.75 since the liquid phase is saturated with NaCl. Cod and *Tasajo* (the traditional dry meat product popular in some Latin-American countries) are among the deeply salted products. To reach the optimum moisture content and a_w, products are equilibrated with dry salt or submitted to sun or air drying. The lightly salted products (ham, cheese, sausages, olives, pickles, etc) are directly consumed. These are also fermented or ripened to enhance the preservation process and promote particular characteristics (Chiralt *et al*., 2001). The salt curing methods were used to preserve many species in fish industry, including Cod (Barat *et al.,* 2003), Horse mackerel (Mol *et al.,* 2010), Common carp (Mahmoud *et al.,* 2007), Anchovies (Siriskar *et al*., 2011), Saradine (Bellagha *et al.,* 2007), Sea perch and Sea bream (Fuentes *et al.,* 2008).

The aim of this study was to evaluate the effect of stacking on chemical composition, alteration of crude protein content of muscle and amino acid profile of processed Narrow-barred Spanish Mackerel during 190 days in ambient temperature.

Materials and Methods

- *Sample preparation*

Twelve Narrow–barred Spanish Mackerels (*Scomberoides commersonnianus*) were caught from Gheshm Island, Hormozgan province, North-West of the Persian Gulf by a fishing boat. Immediately, after gutting and filleting, the samples were divided into six batches. One batch was transferred to the laboratory for chemical analysis without salting. The other five batches were used for stack preservation. Split fish were piled into a plastic vat, containing common salt (1 cm). A layer of salt (0.5 cm) was added on the first layer of fish, and this was repeated until the height of one meter. The last layer of fish was covered with a thin layer of salt and kept at 5±1˚C. After the first five days, the upper layers were replaced by the lower layers each three days to have salt penetration and water extraction (Moini, 1391). Approximate analyses were carried out in different intervals consisting of days 0, 15, 30, 90 and 190 after preservation.

- *Approximate analysis*

Moisture content was determined by weight loss after drying the samples in an oven at 105˚C (AOAC, 1990). Salt concentration was determined by volhard method (AOAC, 1990). The crude protein content of the fish muscle was determined using Kjeldahl apparatus (AOAC, 1990). The ash content was calculated through burning the organic matter in the muffle furnace at 550˚C for 24 hours (AOAC, 1990). All the determinations were performed in triplicate order.

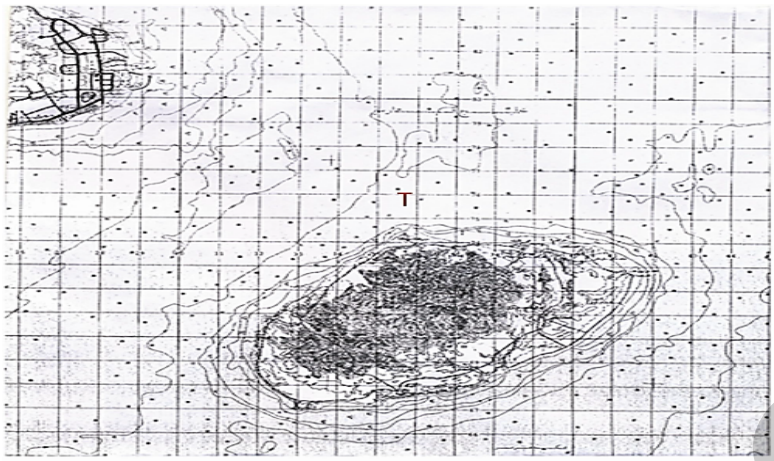

Fig. 1. The location of catching samples (T), Gheshm Island, Hormozgan province, North- West of the Persian Gulf

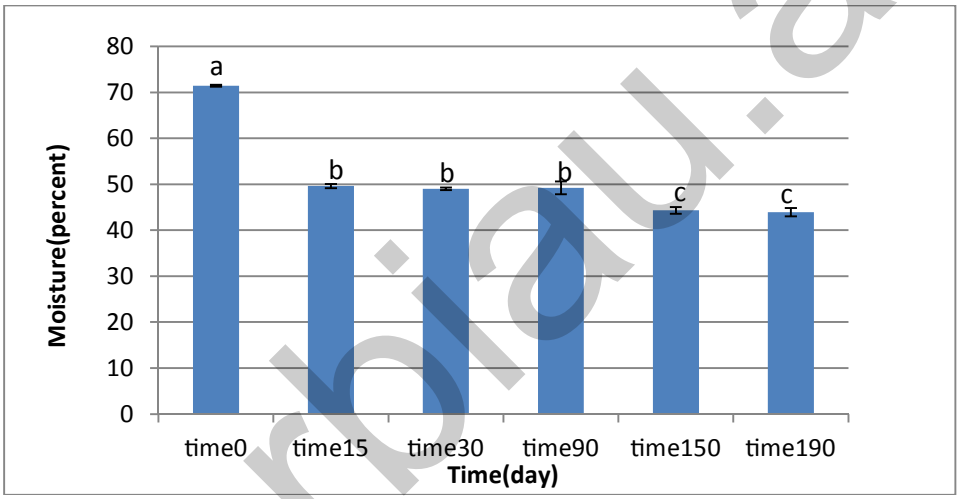

Fig. 2. Moisture content (±SE) of fresh and salted Narrow – barred Spanish Mackerel during 190-day storage at ambient temperature (Different superscripts have shown significant differences between the variables ($p<0.05$))

- *Determination of amino acid profile*

Amino acid contents of the samples were measured according to British pharmacopoeia-2011 for fresh and salted samples at days 90 and 190 after preservation. Samples were hydrolyzed at 110ºC for 24 hours with HCl. The buffered solution of sodium citrate was added to the hydrolyzed sample and then the contents of amino acids were determined by application of Amino acid analyzer (HEWLETT 1100).

- *Statistical analysis*

The obtained data were analysed using Spss13. The data was subjected to one – sample kolmogorov-smirnov test to determine the form of distribution. Ash, salt and moisture contents of the samples did not have a normal distribution and were subjected to kruskal- Walis test, and the remained data were subjected to One-way ANOVA to test the significance *($p<0.05$)* of differences. For evaluating the differences between amino acid profiles through the storage time, T-Test was used.

Results and Discussion

The moisture content of the fresh fillet (without out salt curing) was 71.44±0.19 percent, that significantly has decreased to

43.9± 0.91 percent *(p<0.05)* after salt curing and storage at the ambient temperature.

The salt concentration of the fresh fillet (without out salt curing) was 0.5 ±0.08 percent. This value has increased to 15.43±0.04 percent after preservation with common salt and storage at the ambient temperature.

The ash content of the sample composed of minerals was 2.17±0.2 percent. This value increased significantly to 12.6±0.1 percent due to curing with salt.

Crude protein content of fresh sample (without salt curing) was 20.07 ±0.85 percent and after preservation with salt the figure is increased that is due to the reduction of moisture content after the sample was cured in salt and stored at ambient temperature for 190 days *(p<0.05)*.

The amino acid profile of the fresh sample (without out salt curing) and preserved product after 90 and 190 days, were examined respectively. In the fresh sample, 18 amino acids, including eight essential amino acids (EAA) and 10 non-essential amino acids (n-EAA) were detected. Glutamic acid was found in the highest concentration (5.9 mg/g wet fillet) and aspargine was at the lowest level among the detected amino acids (0.15 mg/g wet fillet).

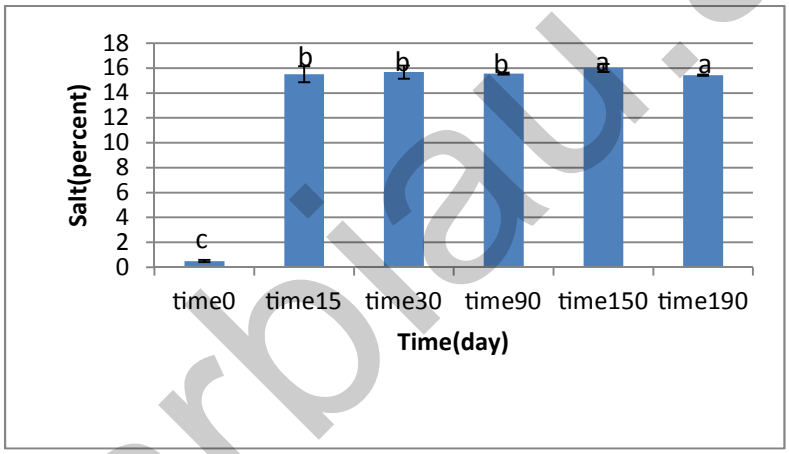

Fig. 3. Salt content (±SE) of fresh and salted Narrow – barred Spanish Mackerel during 190-day storage at ambient temperature (Different superscripts have shown significant differences between the variables (p<0.05))

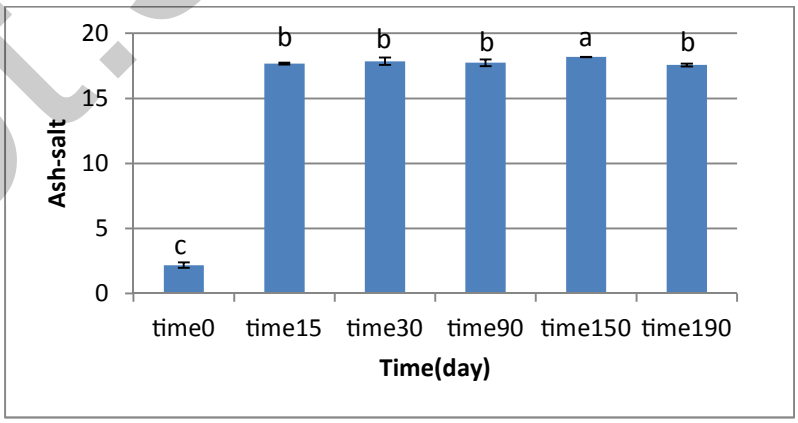

Fig. 4. Ash- salt content (±SE) of fresh and salted Narrow – barred Spanish Mackerel during 190-day storage at ambient temperature (Different superscripts have shown significant differences between the variables (p<0.05))

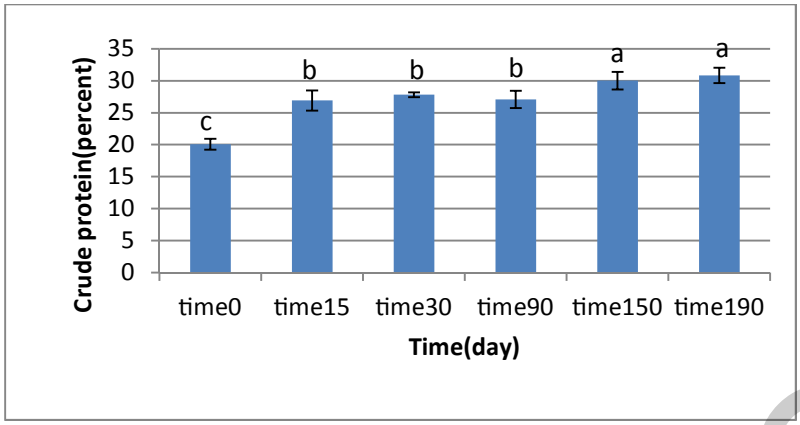

Fig. 5. Crude protein content (±SE) of fresh and salted Narrow – barred Spanish Mackerel during 190-day storage at ambient temperature (Different superscripts have shown significant differences between the variables ($p<0.05$))

Table 1. Amino acid profile of fresh and salt curing fillet of Narrow-barred Spanish Mackerel

Amino acids	Fresh sample (time 0) (mg/g)	After 90 days (mg/g)	After 190 days (mg/g)
Threonine	0.94	0.79	0.66
Valine	3.6	2.9	2.73
Phenylalanine	1.21	0.89	0.78
Isoleucine	1.73	1.39	1.2
Leucine	4.88	4.28	3.7
Lysine	5.8	5.74	5.65
Metionine	0.4	0.35	0.28
Tryptophan	0.28	0.27	-
The total content of EAA*	**18.84**	**16.61**	**15**
Aspartic acid	4.6	3.9	3.48
Glutamic acid	5.9	5.72	5.55
Aspargine	0.15	-	-
Serine	1.39	1.42	1.3
Glutamine+Histidine	0.42	0.38	0.37
Glycine	1.22	1.72	0.93
Alanine	2.05	2.1	2
Tyrosine	1.13	1.05	0.74
Arginine	0.94	0.79	0.66
The total content of NEAA*	**17.80**	**17.08**	**15.03**

*EAA: Essential Amino Acid
*NEAA: Non Essential Amino Acid

The essential amino acids lysine and lucine had the highest level with 5.80 and 4.88 mg/g in wet fillet respectively while tryptophan was at the lowest level (0.28 mg/g wet fillet) as compared to other EAAs. Among the non-essential amino acids glutamic acid and aspartic acid with 5.9 and 4.6 mg/g in wet fillet that constituted the highest concentrations respectively.

The total content of essential amino acids in the fresh sample of *Scombroides c.* was 18.84 mg/g in wet fillet, that decreased to 15.00 mg/g after salt curing and storing for 190 days at the ambient temperature. The total content of non-essential amino acids in fresh fillet was 17.80 mg/g, that decreased to 15.05 mg/g in wet fillet after preservation. Data analysis showed that aspartic acid, glutamine, argimine, glycine, alanine,

tyrosine, metionine, valine, phenylalanine, isolucine, lucine and lysine contents decreased significantly *(p<0.05)* but these reductions were not significant for aspargine and tryptophan *(p>0.05)*.

TVN content of fresh fillet (without salt curing) was 16.39±0.38mg/100gsample that, increased significantly *(p<0.05)* to 57.76±0.64 mg/100g sample after stacking and storage at the ambient temperature.

Salt curing is one of the oldest techniques of food preservation such as meat, fish and plant products (Mulvihill and Fox, 1980; Delacroix-Buchet and Trossat, 1991). The salted fish products are being more acceptable in recent years throughout the world (Basti *et al.,* 2006; Lakshmanan *et al.,* 2002; Shimosaka *et al.,* 1990; Turan *et al.,* 2007; Vreites *et al.,* 1997). The main object of preserving the food products by salting is to decrease the water activity in order to inhibits the microbial development and autolysis the enzyme activity (Ashie *et al.,* 1996; Doe and Olley, 1990; Horner, 1997; Mujaffar and Sakant, 2005; Anderes *et al.,* 2005; Rodringes *et al.,* 2003). Salt curing also affects the organoleptic charachteristics of the final product (Belitz and Crosch, 1988; Anderes *et al.,* 2005). Fish species are preserved by salt curing, but this procedure is not usually applied for *Scombroides Commersoninus*.

Moisture might be considered as one of the most important factors regarding the microbial growth and spoilage of food products. Moisture detection is regarded as a critical quality index used for quality control of fish products during storage time (Namulema *et al.,* 1999; Ben-Gigirey *et al.,* 1999). The main reason for reduction of spoilage procedure in salt curing products is the reduction of water activity due to salt penetration. The reduction of moisture affects the weight and solubility of proteins (Namulema *et al.,* 1999); and causes the increase of rancidity activities, denaturation of proteins (Barat *et al.,* 2003; Duerr and Dyer 1952; Ismail and Wootton 1982), and color alteration during the salt preservation the resulted in a decrease in quality of the final product (Ben-Gigirey *et al.,*1999). As mentioned earlier, the salt curing is effective in deduction of water activity, that is very important in inhibiting microbial growth and spoilage. The effective level of water activity for preserving fish product is equal to 0.75 or lower (Anderes *et al.,* 2005; Doe and Olley, 1990). In the present study, the moisture content after salt curing and storage was decreased significantly. Similar results were also observed in salt and dry curing of Trachurus fillet (Mol *et al.,* 2010) and Sardine fillet (Arkoudelos *et al.,* 2003). Generally, the amount of moisture has an adverse relation with the salt content.

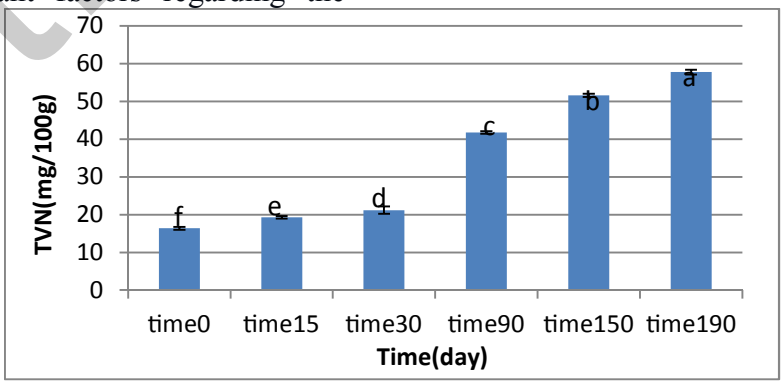

Fig. 6. TVN content (±SE) of fresh and salted Narrow – barred Spanish Mackerel during 190-day storage at ambient temperature (Different superscripts have shown significant differences between the variables (p<0.05))

The increase in the salt concentration in old techniques of food preservation resulted in a decrease in water activity and also increase of organoleptic properties which the former has an adverse effect on blood pressure (Amerin *et al.,* 1965). In the present study the salt concentration increased after stacking and storage. Same results were observed in salt curing of *Stolephorus sp*. (siriskar *et al.,* 2011), Dicentrarchus labrax L. (Fuentes *et al.,* 2008), Gadus morhua (Andres *et al.,* 2005), Trachurus mcullochi (Berhimpon *et al.,* 1990), and Trachurus trachurus (Mol *et al.,* 2010), which is due to the salt penetration and reduction of the moisture content of tissue. Lin *et al.,* 2012 have shown that salt content in stacked fish products (14.1%) increased significantly as compared to the stacked mussel products (9.49%) and stacked shrimp products (8.43%), respectively. The results of the present study showed that the salt level was similar to the salt concentration in stacked fermented fish products in Japan (9.1-16.5%) (kada *et al.,* 2007), and higher as compared to fermented fish product containing rice (kuda *et al .,* 2009). The relationship between the salt and water content is linear, and this is supported by the obtained results in the present study.

The ash content of salted cured products is increased from 2 to 2.5 percent based on the original weight of the product. This is similar to the increment of percent ash in the stacked *Stolephorus sp.* (siriskar *et al.,* 2011).

According to Mahmoud *et al.* (2007) short term salt curing of the common carp fillet (15 min) by the use of NaCl electrolyte solution did not affect the ash content of the product. As it is shown in the present study the ash content increased significantly in long term salt curing procedures, due to the salt absorption and loss of water content.

Crude protein content of *Scombroides c.* fillet after stacking and storing at the ambient temperature for 190 days, increased significantly. The increment was also reported by Gudjonsdottir *et al.* 2011 in salt curing of the cod fillet. The rate of changes in crude protein content of the fillet is a function of salt concentration and the method of salt curing (Lawrie, 1998; Thorarinsdottir *et al.,* 2004; Lauritzsen *et al.,* 2004a; Duerr and Dyer, 1952). According to Hamm (1960) in lower concentration of salt, Cl^- - protein bonding occurred and resulted in muscle swelling, but at higher concentrations of salt, protein bonding of muscle was affected and resulted in structural changes and denaturation and thereby the water-holding capacity of muscle decreased and ultimately, the crude protein content increased in the final product. As it is mentioned earlier the methods of salt curing also affected the protein content of the final product. Some studies have shown that salt curing with brine increased the rate of salt penetration in muscle as compare to dry salting that resulted in promoting the rate of protein extraction. This could be observed in the stacking procedure of Gadus morhua by Ferraro *et al.* (2011), where the muscle proteins were found in the drain off.

The amino acid profile of a protein is an important factor in evaluation of the nutritional value of the food (Okland *et al.,* 2005). The presence of some amino acids are being a quality Index for introducing different species of fish as a valuable food source. On the other hand, some amino acids including tyrosine, argenine, and lysine are important factors in quality control of aquatic food products (Ruiz-Capillas and Moral, 2001). Some amino acids are responsible for organoleptic properties of fish products such as glutamic acid, aspartic acid, alanine, and glycine (Ruizcapillas and Moral, 2004). According to Oladapa *et al.* (1984), aspartic acid, glutamic acid and lysine are the most important amino acids in the aquatic food products (Oladapa *et al.,* 1984). Aspartic acid and glutamic acid have

an important role in enzyme active cores, and maintain the solubility properties of proteins (Sikorski *et al.,* 1990; Belitz *et al.,* 2001). Rosa and Nunes (2004) reported that arginine, lysine and lucine are the most important amino acids among the others and consequently, aquatic food products are good sources for obtaining qualitative proteins. Deficiency of lysine might results in retardation as it is important for glutamate synthesize, the most important neurotransmitter in central nervous system of mammalian (papes *et al.,* 2001).

In the present study, stacking and storing the product for 190 days at the ambient temperature, resulted in significant decrease of aspartic acid, glutamic acid, serine, glutamine, argenine, glycine, tyrosine, metionine, valine, phenylalanine, isolucine, lucine, and lysine. These reductions could be due to the salt penetration, water extraction and loss of soluble proteins. These results are also supported by Ferraro *et al.* (2011) findings since they have reported that during salt curing of cod, 10 amino acids (i.e. aspartic acid, glutamic acid, argenine, keratin, glycine, lysine, metionine, phenylalanine, tuarine and tryptophan) were found in the drain off. It should be mentioned that aspargine and tryptophon did not decrease significantly in the present study.

Usydus *et al.* (2009) reported that aspartic acid, and glutamic acid were the principal amino acids among the non-essential AAs and lysine, and lucine were the most abundant amino acids among the EAAs. The findings are in accordance with the results of the present study and also the findings of El and kavas (1996), kim and Lall (2000) and Wilson and Coway (1985) who studied on salmon, rainbow trout, and flat fish.

Iwasaki *et al.* (1985) studied the egg and fillet belonging to 13 aquatic species, and reported that the contents of essential amino acids exceed the non- essential amino acids. This is supported by the present study. The ratio of EAA/non-EAA is an important Index in evaluating the nutritional value of food products. The values reported for *Otolites rubber* and *Rutilus frisi kutum* were 1.06 and 1.01 mg/g, respectively. In the present study, this Index was calculated as 1.05 mg/g.

TVN measurement is one of the important factor for determining the quality of aquatic food products (Olafsdottir *et al.,* 2000). In this measurement, volatile nitrogen compounds (i.e. trimethylamine, dimethylamine, and ammonia) that might result in spoilage of products were being studied (Huss, 1995). Microbial activities and enzymatic digestion are the main reasons of spoilage and alteration of the products. Proteolytic enzymes caused some decomposition of proteins and production of a significant amount of Voltile Nitrogenous compounds and ammonia (Conell, 1995). In the present study, the amount of TVN was measured for the stacked product during the storage time that increased significantly during 190 days. Siriskar *et al.* (2011) has reported that TVN increased from 3.8mgN/100g to 27.1mgN/100g in stacked anchory during five-week storage. This increment could be due to microbial and enzymatic activities (Watanabe, 1982; Okazak, 1983).

According to Connel (1995) and Sernapesca (1996) the acceptable limits of TVN is 30-35mgN/100g. Low increment of TVN during the preliminary phase of storage could be due to amino acid and nucleotide degradation, while the termination increment is due to microbial degradation of trimethylanine oxide to trimethyl amine (Sallam *et al.,* 2006). In the present study, the final product after 190days of storage did not present a good-quality regarding the TVN increment and it might be stated that the product is spoiled over the storage time.

Lin *et al.* (2012) reported that TVN increment of stacked mussel (55.6 mgN/100g) was lower than stacked fish and

shrimp (99.0, 102 mg/100g) respectively. According to Sotelo and Rehbein (2000) crustaceans have a medium level of trimethylamine, and trimethylamineoxide as compared to the lower levels in edible mollusca, and Bivalvia.

Conclusion

In the present study, salt curing of *scombroides commersianus* fillets and storing at the ambient temperature for 190 days resulted in spoilage and decreased the quality indices of the final product. It was concluded that the best shelf-time for consuming the final product preserved by stacking method is 90 days regarding the TVN level and the nutritional value.

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