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Assessment of the freshness state of preserved *Sarpa salpa* under ice

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Abstract

In this study we estimated by four appreciation methods the freshness state of *Sarpa salpa* species immediately captured. The TVB-N content is a tool of choice in the evaluation of fish species deterioration. The TBV-N content made possible to establish a narrow relation (R = 0.98) with the organoleptic examination (*Freshness indexes*). According to our results we suggest a value (28 mg N/100 g) as a limiting value of acceptance for the human consumption. Our results show that the bacterial development is the origin of the deterioration of freshness in the species studies, where *Pseudomonas* genus is the prevailing germ implied in the deterioration process. The storage time of *Sarpa salpa* should be limited to less than 6 days.

Introduction

The salema, *Sarpa salpa* (L.), is a marine sparid fish that lives in shallow water. It is an eurytherm species widely distributed throughout the Mediterranean and the Eastern Atlantic coast (round South Africa to South of Mozambique including the Azores and Canary Islands) (Bauchot and Hureau, 1990) and some zones of the Black Sea. This species represents a significant part of the fish fauna (40-70% in biomass) (Francour, 1997; 2000). In the last 15 years, this poorly studied species in the Mediterranean sea has attracted research interest because of first, its role as macro-grazer of sea grass (Velimirov, 1984; Havelange *et al.*, 1997; Jadot *et al*., 2000; Jadot *et al*., 2002), second, its biology in order to develop a management strategy (Méndez-Villamil *et al.*, 2001; Méndez-Villamil *et al*., 2002), and finally for its toxicity. (Spanier, 1988; Spanier *et al.*, 1989; Chevaldonne, 1990). Bellassoued *et al.* (2012) studied the toxicity assessment of dreamfish Sarpa salpa from the Gulf of Gabes (Tunisia, Eastern Mediterranean sea); they conclude that the toxin present in organs of dreamfish had a cytoxin effect on liner, kidney and especially on the brain of treated rats. In Tunisia, gold line is commonly consumed as fresh fish for its desirable aroma and quality and is an important species. In Tunisia, statistical data revealed that the production of fish has increased between 1980 and 2001 about 70,3% where in 2001, the production has reached 1024 tons, which constitute 57% of the total catch that was taken in Mediterranean Sea (Fishbase, 2001). Thus, the ministerial order on fish hygiene specifies that unprocessed fishery products shall be regarded as unfit for human consumption where organoleptic assessment has raised doubts as to in the particular case of salema. However, quality parameters of Mediterranean fish species have not been extensively studied as either freshwater or marine fish species from tropical or cold waters. (Simeonidou *et al.*, 1998). Currently, there are no data available about *Sarpa salpa* on its alteration at refrigerated temperature and no author has established TVB-N limits. Accordingly, this paper has been justified. Therefore, the main objectives of the present study were to investigate quality changes of *Sarpa salpa* during storage at 0°C simulating retail handling conditions in the fish markets and establish the TVB-N limits for this species. This study includes chemical criteria (Total Volatile Basic Nitrogen, pH), organoleptic criteria (Freshness Indexes) and microbial criteria (Aerobic Plate Count).

Materials and methods

Materials

Fish samples: averages: weight (150 g ± 4 g) and length (20 cm ± 2 cm). Ninety whole specimens of salema (Sarpa salpa) stored in flaked ice were purchased at the docks of Monastir and immediately

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transported in an isotherm containers to the food laboratory of the Institute of Biotechnology twenty-minutes later. Immediately, fishes are divided in three lots of thirty pieces. To evaluate the quality of the fresh fish, thirty specimens were removed from ice at day0 and tested for chemical, sensory and microbiological analysis. The remaining sixty fish were divided into two groups of thirty samples and stored in ice and under refrigeration condition (0°C) until analysis at days 3 and 6 of storage time for each group.

Sensory evaluation

This exam is carried out at regular intervals by the determination of the index of freshness. A subjective appreciation of the aspect, state and odour are carried out according to the grid of quotation of regulation the EEC n°103/76. This examination was complemented by the determination of the arithmetic average of the coasts appreciation.

Protein content

The protein content was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

TVB-N content

TVB-N was determined, following the official method proposed by the ministry of Agriculture (JORT, 1998). All fish were eviscerated, washed thoroughly of blood and filleted. 10 g of fish muscle were blended with 90 ml of perchloric acid 6%. The blend was minced by passing through an ULTRA-TURRAX T 25 homogenizer. The mixture was filtered on WHATMAN N° 2 filter paper. 50 ml of filtrate were put into the distillation tube followed by 6.5 ml of the NaOH solution (20 g/100 ml). Steam distillation was performed using a kjeldahl distiller (BüCHI-323) and the distillate (volume of 200 ml in 10 minutes of distillation) was collected in a beaker containing 100 ml of boric acid (3 g/100 ml) and few drops of Tashiro indicator for the titration of ammonia. The boric acid solution turned green when alkalinised by the distillate, which was titrated with 0,01 N hydrochloric acid solution. The TVB-N content (mg N/100 g flesh) was calculated as follows:

$TVB\text{-}N = (V_1 - V_0) \times 0{,}14 \times 2 \times 100 / M$

Where:

- V_1 : ml HCl used in the titration
- V_0 : ml HCl used for the white essay.
- M : g of sample.

Bacteria count (total flora)

The skin from the dorsal anterior area was aseptically removed using sterilised materials. A quantity of 10 g from the underneath flesh was removed, chopped in pieces and aseptically homogenized with 90 ml of tryptone salt (TS). Further dilutions (from 10^{-2} up to 10^{-7}) were prepared with 9 ml of tryptone salt. Duplicate plate counts were incubated at 30°C for 3 days.The boxes containing between 30 and 300 colonies are counted. The counting of the colonies was followed of biochemical identification, on API, of the various isolated bacterial species.

pH measurements

Three grams of fish muscle were homogenized in five volumes of distilled water, and then the pH values were measured with a pH with special food electrode (electrode 202 DCF).

Statistical analysis

All measurements (pH, TVB-N, FI) were carried out in triplicates (n = 3). For microbial counts (n = 5). Only the proteins data were statistical analysis by ANOVA.

Results and Discussion

In order to evaluate the quality of fish and propose a limit acceptability value for human specific consumption of saupe (*Sarpa salpa*) species. We evaluated the freshness state of *Sarpa salpa* preserved at 0°C under ice during 6 days by four different quality indicators such as chemical indexes (TVB-N, pH), freshness indexes (FI) and microbiological index (total flora). TVB-N and TMA are most useful indices for spoilage in fish and lightly preserved sea food (Dalgaard, 2000). TVB-N Values is more useful in assessing the degree of fish freshness than in evaluating the changes occurring during the first stages of storage (El Marrackchi *et al*., 1990). According to the results, the initial TVB-N content expressed in mg N/100 g is 17.50 (day 0) (day of the captured). This content level is probably the result of the endogenous enzymes and not the result of the bacterial activities. Normally, the flesh of healthy fish is sterile (Shewan, 1962). No production of TVA and TVB was noted in sterile fish (Lobben and Lee, 1968). However, initial values of 13.2 mg N/100 g for sardine (*Sardine pilchardus*) at 4°C have been reported (Gökodlu *et al*., 1998). In Brazilian freshwater fish (Maia *et al*., 1983). In *Tuna albacore* (Perèz-Villarreal and Pozo, 1990) and in silver carp during frozen storage (Siddaiah et al., 2001). In our study, after 6 days storage TVB-N was 28 mg/100 g. As you can see in Figure 1, the increase of TVB-N depending to the storage time is linearly ($R^2 = 0.98$). According to Haard (1992) after harvest there is a progressive decline in the initial quality of the fish due to two major causes where the first is

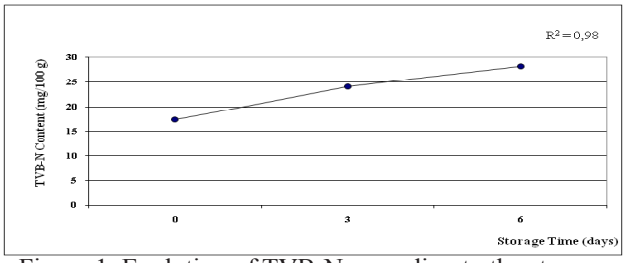

Figure 1. Evolution of TVB-N according to the storage time

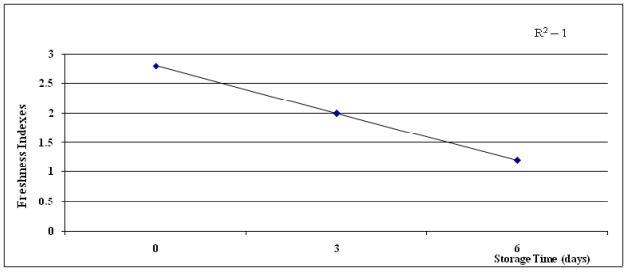

Figure 2. Evolution of the Freshness Indexes according to the storage time

sometimes called autolysis and the second resulting from bacterial growth and metabolism. It has been reported that for several fish species, TVB-N values increase curvilin early or linearly with time (Perèz-Villarreal and Pozo, 1990; Gökodlu *et al*., 1998). Gökodlu *et al.* (1998) found that the TVB-N value in fresh sardine increased from 13.2 to 64.8 mg/100 g during refrigerated storage. The TVB-N values of sea breams (Sparus aurata) stored in ice increases from 15.24 to reach after 15 days 21.81 mg/100 g (Özogul *et al*., 2007). Yet, a similar pattern of the increase in TVB-N values for the control has been reported during cold storage of sea salmon (Hozbor *et al*., 2006). A level of 30 mg/100 g has been considered the upper limit above which fishery products are considered unfit for human consumption (Sikorski *et al*., 1989). According to Vidya-Sagar-Reddy *et al.* (1995), the acceptability limit of TVB-N was between 18 and 24 mg/100 g meat for frozen stored pink perch mince. Bennour *et al.* (1991) reported that the TVB-N values of several types of mackerel fish varied from 22.2 to 23.13 g/100 g at different rejection times. According to the Commission Decision 95/149/EC (1995), TVB-N limits were in the range of 25-35 N/100 g muscle considering the limits of acceptability for some fish species. However, the TVB-N levels are dependent of the species of fish and the storage temperature of the product (Park *et al.*, 1981). Also, it is suggested that the TVB-N value is affected by species, catching season and region, age and sex of fish.

The freshness of fresh fish is the single most important attribute when assessing fish quality (Reineccius, 1990). Sensory assessment has always played a key role in quality and freshness evaluation in fish industry (Abbas *et al*., 2008). Sensory

Table 1. *Salpa sarpa* evaluation of freshness quality indexes

Days	Indexes of freshness	T-VBN content (mg N/100 g)	pH	Total Flora (UFC/g)
Day_0	2.80	17.50 ± 0.010	6.52 ± 0.02	$1.5\,10^3$
Day_3	2.00	24.07 ± 0.020	6.60 ± 0.03	$3.5\,10^3$
Day_6	1.20	28.13 ± 0.025	6.70 ± 0.04	2.10^7

(TVB-N) Content = average of 3 replications, Index of freshness (N = 30), Total Flora (N = 5 replications), pH (N=3).

evaluation is the most important method today for freshness evaluation of the seafood (Fatma-Hassan, 2011). As you can see in Figure 2, the freshness indexes (FI) passed from 2,8 at day 0 to 1,2 at day 6 which is the limit value of acceptability according to E.E.C N°103/76. Massa *et al.* (2005) studied the freshness state of ice stored Flounder (*Paralichthys patagonicus*). For the sensory assessment, they noted that the quality index (total QIM score) increased linearly from a score of 1 to 26. The score of 18, reached after 7 days of storage, is noted by the panel as the limit of fish acceptability and in this period, according to the same authors, some alterations of general organoleptic characteristics were observed. According to Gram and Huss (1996) enzymatic and chemical reactions are usually responsible for the initial loss of freshness whereas microbial activity is responsible for the secondly alteration process. In another report, the initial quality loss in fish is first caused by post-mortem autolytic changes and is unrelated to the microbiological activities.

Microbial activity is the main factor limiting the shelf life of fresh fish (Mazorra–Manzonna *et al*., 2000). According to Jayasinghe and Rajakaruna (2005), the number and the nature of bacteria on fish are affected by many factors such as method of capture, handling practices. Thus, microbial safety criterion is normally applied for determining storage life of fresh fish seafood (IFST, 1999). In this study the initial quality of fish used was good, as indicated by a low initial number of bacteria at day 0 (<105 CFU/g) (see Table) according the microbiological criterion recommended by the French regulation (Ministerial decree of December 21, 1979). The initial number of bacteria is 1.5 x 10^3 CFU/g at day 0 and it increases gradually to reach 2 x 10^7 CFU/g at day 6 (see Table). This number of colonies (2.10^7 CFU/g) is largely higher than the microbiological criterion recommended by the French regulation (Ministerial decree of December 1979) which is fixed to 10^5 CFU/g and higher than the maximal recommended limit criterion by ICMSF (1986) which is fixed to 10^7. According to Shewan (1977), the bacteria develop in an exponential way to reach populations from 10^8 to 10^9/g of muscle, after 8 to 10 days at 0°C. According to Fevolden and Eidsa (1981)

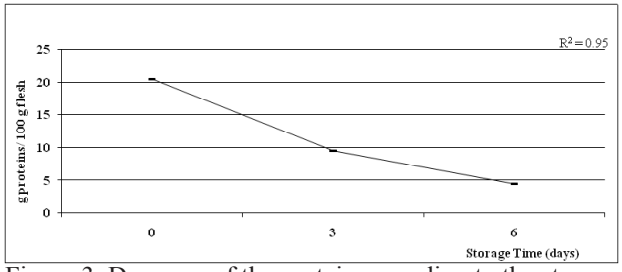

Figure 3. Decrease of the protein according to the storage time

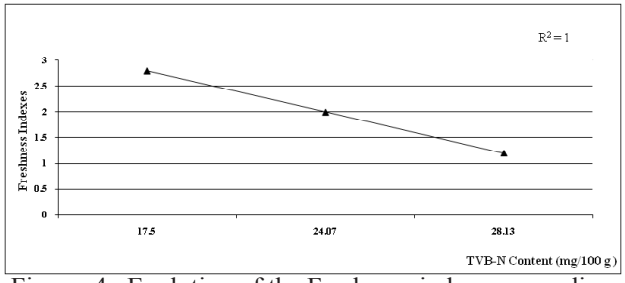

Figure 4. Evolution of the Freshness indexes according to the TVB-N content

that the total viable bacterial count of krill stored at 0°C remains very low during the first week of incubation, and subsequently shows a steep increase with time. Bacteria growth is the main cause of fish spoilage (Suvanich *et al*., 2000).

The pH of live fish muscle tissue is close to neutrality (Huss, 1995). pH depend on fish species and others factors (Church, 1998; Simeonidou *et al*., 1998). pH value has been employed often as a complementary analysis to fish spoilage detection. In this study, we note a slowly increase of the pH during storage time where the initial pH is 6.52 ± 0.02 (day 0), 6.60 ± 0.03 (day 3) and 6.70 ± 0.05 (day 6) (see Table). This increase may be explained by autolytic changes such as denaturation or breakdown of protein which provide an optimum condition for growth and reproduction of spoilage microflora (Parkin and Brown, 1983; Pedrosa-Menabrito and Regenstein, 1988). The decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh (Shenderyuk and Bykowski, 1989). A high degree of association was observed between pH of flesh and log CFU/g (R^2 = 0.99) in the spoiled fish (Kyrana *et al*., 2002). In addition, in the literature, the increase in pH indicates the loss of the quality.

Figure 3 reports the decrease of protein depending storage time (days). The protein content of fish muscle normally ranges from 18 to 22%. As you can see in Figure 3, the decrease of total protein is continuously during the storage time (6 days). It has been reported that the changes of protein solubility during frozen storage showed a two successive change of two first order rate process (Ohnishi *et al.*, 1978). The total protein of muscle of *Sarpa salpa* decrease continuously from 20 g/100 g (day 0) to 4 g/100 g (day 6) where R^2 = 0.95. Significant difference between the protein content following decreases ($P \leq 0.05$). In the post-mortem muscle, the action of spoilage-causing micro organisms eventually leads to the accumulation of a number of volatile basic nitrogen (TVB-N) compounds including ammonia by the action of bacterial decarboxylases on amino acids and other nitrogenous compounds (Liston, 1980; Alur *et al*., 1995). According to Bonnal *et al.* (2001) and Munasinghe *et al.* (2005); the degradation and/or digestion of proteins by proteolysis as a consequence of post-mortem changes have been monitored SDS-PAGE. The rate of proteolysis varies among species (Papa *et al*., 1996; Tejada *et al*., 2002). It is appropriate to note that the TVB-N is being defined like the bacterial degradation of proteins and the non protein substances (Olafsdottir *et al*., 2003).

In order to establish the relations which could exist between the various parameters, we used straight regression line function the "Microsof" Excel version 2003. Figure 4 reports the freshness indexes depending to the TVB-N content. Also, we note that the values obtained are near to the theoretical values represented by the straight regression line. A good negative narrow correlation R^2 = 1 was obtained between FI and TVB-N content. Similar results were found by Ruiz-Capillas and Moral (2001) with hake stored under ice. A good correlation was noted between TVB-N and odor intensity (r = 0.84) in Mahi-Mahi (*Coryphanea hippurus*) refrigerated at 7°C (Antoine *et al.*, 2002).

Conclusion

The appreciation of the freshness of *Sarpa salpa* species preserved 6 days under ice shows clearly that there are a positive correlations (R^2 = 0.98) between TVB-N and storage time (days) (Figure 1) and between freshness indexes and TVB-N with the narrow correlations (R^2 = 1) (Figure 4). On an another hand, as shown in the Table, Pseudomonas ceracia and *Klebsiella rhinoscleromatis* are the germs responsible of the spoilage of *Sarpa salpa*, where the evolution of the total flora is characterized by the predominance of *Pseudomonas*. In conclusion and according to the obtained results in this study, TVB-N (28 mg), freshness index (1.2) and microbiological criterion (2.10^7 CFU/g) at 6 days authorize to us to propose the value of 28 mg N/100 g for this species as a shrinkage limit value of human consumption and the shelf life or the storage time should be limited to less than 6 days. However, it is very interest to follow the evolution of the TMA in order to draw up

TMA/TVB-N which constitutes a complementary criterion of the TVB-N. Yet, this study indicates clearly the importance of taking measures to improve the facilities fish sales outlets formulation of a management process for proper handling of fish from capture to sold for the handling practices. Thus, a great effort will be realized by the government which should be arranged to educate the fish vendors on proper handling of fish. Besides, recognizing that the storage time and storage conditions have a great impact on the quality of sea foods, and the stability of fish depends on the chemical composition (Ashie *et al*., 1996; Esaiassen *et al*., 2004). Further studies are needed about *Sarpa salpa.*

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