

## CHEMICAL AND PHYSICAL CHANGES DURING SALTED SARDINE MATURATION PROCESS

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### ABSTRACT

Ripened Semi-preserved Sardine fish was prepared from *Sardinella spp.* by salting and ripening process. This process goes back to ancient times and considered as a common traditional method in some Mediterranean countries.

The goal of present study was directed to follow up chemical and physical changes during ripening of salted Sardine process at room temperature ( $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) focusing on protein fraction released during this operation. The obtained results revealed that protein fractions (TSN, NPN, FAN and TVN) play an vital role in ripening process of salted Sardine and there are number of protein derivatives released in the brine during this process. Where, total nitrogen, total soluble nitrogen, non-protein nitrogen, free amino nitrogen and total volatile nitrogen reached 3.44%, 1.70% (represented 50.83% of TN), 1.19% (represented 35.58% of TN), 0.90% and 108.5 mg/100 g at the end of ripening period, respectively.

### INTRODUCTION

Seafood is one of the most important food constituents in human nutrition and has an important economic value. In addition, seafood have received considerable attention due to reasons concerning health (*Pigott and Tucker, 1990*).

Salting is one of the earliest and most widespread preservation technique practiced by man (*Wheaton and Lawson, 1985*), it is used as commonly traditional method in some Mediterranean areas and tropical countries because of simplicity of the process, low production cost and its ability to be combined with other methods in order to satisfy consumer's habit and requirements (*Berhimpon et al., 1991*).

It is well known that fish salting is a process which aims to reach the saline equilibrium between fish muscle and the surrounding salt solution in a specific time (*Zugarramurdi and Lupin, 1980*).

Suez gulf is considered to be a good source for clupadea fishes (Sardine, Moza etc.) which are being caught through the period from September to April (*FAO fisheries report, 1990*). The annual world production of Sardine fish got to one million metric ton according to *FAO(2006)* which represent the 11<sup>th</sup> rank in the largest fish caught species.

Maturing process is taking at least two months for *E. encrasicolus* of Mediterranean Sea (*Compello, 1985 and Hernandez - Herrero, 1997*). It is considered a complex sequence of reactions that depends on different parameters related to the physicochemical conditions of maturity (temperature; pH; ionic strength and water activity) and total biology fish (fat content; enzymes; bacteria,) (*Durand, 1982*). Because of these reactions, the finished product acquires a soft consistency of fish flesh along with the development of a pink color and a strong characteristic flavor.

Ripening based on proteolysis plays an important role in the production of salted fish. During proteolysis the typical flavor and aroma substances are enzymatically formed and suitable consistency developed (Wheaton and Lawson 1985).

The proteolytic products affecting the taste are principally soluble nitrogen containing compounds: amino acids, peptide nucleotides and their decomposition fragments (Shenderyuk and Shumarova 1989).

Hernandez-Herrero (1997) found that percentage of non-protein nitrogen of anchovy muscle decreased from 0.68 to 0.53 % during the first week of the ripening process. Thereafter, it markedly increased up to the end of ripening. He concluded that 75.4% of total nitrogen of the fish muscles released in the brine as non-protein nitrogen during the first week of ripening. Also, he found that the total volatile basic nitrogen (TVBN) of anchovy decreased from 24.0 to 19.9 mg/100g of fish muscle during the first 7 days of ripening, which increased gradually in both anchovy and brine until the end of ripening (9 weeks). In this respect, TVBN content of thawed frozen fillets of sardine was decreased from 10.3 mg/100 g to 6.53 mg/100 g due to leach out nitrogen compounds from fillets via acetic acid and salt during marinating process (Berna and Sukran, 2004).

El-Shehawy (2000) stated that TSN% of 9% salted Moza packed in soybean oil stored at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  gradually increased from 1.97% to 2.79% after 30 days of storage period. Then it gradually decreased until the end of storage period. TSN% in 12% and 15% salted Moza packed in soybean oil stored at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  had the same trend.

Rabie et al., (2009) investigated changes in the formation of amino acids and biogenic amines in Egyptian salted-fermented fish (Feseekh) during ripening (20 days) and storage (40–60 days). They found that total concentration of free amino acids increased from 8 to 72 g/kg (DW) after 60 days of storage. The predominant free amino acids were leucine, glutamic, lysine, alanine, valine, aspartic, isoleucine and citrulline. Their concentrations accounted for 68% of the total concentration of amino acids after 60 days.

So, the main goal of such work was directed to follow up chemical and physical changes happen during salted Sardine maturation process at room temperature ( $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) focusing on protein fraction released during this operation.

## **MATERIALS AND METHODS**

### **Materials:**

Sardine fish (*Sardinella spp.*) caught from Suez gulf (Red Sea) were obtained from Kafr El-Sheik fish market, Egypt.

Two types of sodium chloride were used namely ordinary edible salt (fine salt) and salt used for food industries (for salted meat and fish), produced by El-Nasr Company, Egypt.

Refined and bleached soybean oil was kindly obtained from Misr Soap and Oil Company, Sandoub, Mansoura, Egypt.

Glass jars (0.5 Kg) and plastic boxes (1.0 Kg) were purchased from the local market of Mansoura City.

**Methods:**

**Salting methods:**

Fish was salted in metallic boxes (about 25 Kg) whereas, the inner walls coated with plastic layer for 65 days at room temperature, according to the method of special salting of anchovies as intermediate product using 30% dry salt (w/w) (*Borgstrom, 1965*). After that, salted fish kept in curing mixture (salt and spices) for 60 days. The final product was divided into three parts, the first part was filleted and packed in glass jars with soybean oil, the second part was beheaded and packed in glass jars with soybean oil and the third part was manually beheaded and kept with dry fine salt in plastic boxes. The packed Sardines were stored at room temperature ( $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) for 2 months.

**Preparation of samples for analysis:**

Fish packed with dry fine salt was picked up from the boxes. The salt above fish surface was completely removed. Then the fish was minced using an electric meat chopper. While fish packed in soybean oil was picked up from jar, swept by filter paper to remove the excess of oil and minced as mentioned above.

**Analytical methods:**

**Chemical analysis:**

Moisture, ash and sodium chloride contents were determined according to the method described by *A.O.A.C. (1990)*. Total nitrogen content (TN) was determined on about 0.5g fish muscle using micro-Kjeldahl method as described by *A.O.A.C. (1990)*. Where, non-protein nitrogen (NPN) was determined as described by *Durand (1982)*. Total soluble nitrogen (TSN) was estimated following the method of *Soloviev (1966)*. Total volatile nitrogen (TVN) was determined according to the method mentioned by *Pearson (1968)*. Results were expressed as mg nitrogen per 100g sample. Free amino nitrogen (FAN) was determined by applying the Sorenson's method as explained in the *A.O.A.C. (1990)*.

**Physicochemical and physical properties:**

pH value was measured according to the method of *Lima Dos Santos et al., (1981)*.

The bound water percentage and plasticity of fish samples was measured using modified method using filter paper press as described by *Volovinskaia and Kelman (1962)*, the two formed zones were measured by digital planimeter system. The bound water percentage was calculated by the

following equation:

$$\text{Bound water (\%)} = \{[(a \times m) - 8.4 \times (b-c)] / m\} \times 100$$

Where a: moisture content.

m: weight of sample (mg).

b: the outer zone area (cm<sup>2</sup>).

c: the internal zone area (cm<sup>2</sup>).

Plasticity (as an indicator for tenderness) was estimated as the area of internal zone in cm<sup>2</sup>.

The water activity of Sardine was calculated from the estimated moisture and salt content using the following equation as mentioned by Demeyer (1979).

If  $X < 0.1775$ ,  $a_w = 1.0014 - 0.6039 x$ .

If  $X > 0.1775$ ,  $a_w = 1.0288 - 0.7614 x$ .

Where:  $X = \text{NaCl}\% / \text{H}_2\text{O}\%$ .

## RESULTS AND DISCUSSION

Moisture content (parallel with the water activity) and salt percentage play an important role in the shelf life and keeping quality of salted cured fish products (El-Sharnouby, 1989).

As shown in Fig. (1) moisture content of whole Sardines packed with dry fine salt decreased from 54.11% to 47.88% after 60 days of storage. The same trend was observed either in case of whole Sardine immersed in soybean oil (from 54.11% to 51.10%) or Sardine fillet in soybean oil (from 54.11% to 48.48%). It could be noticed that reduction in moisture content was lower in case of whole Sardine in soybean oil than that Sardine fillet because of presence of fish skin which prevent water loss.

The decrease in moisture content may be attributed to the loss in the water holding capacity of fish protein as a result of proteolysis. (Foda et al., 1986). Zaitsev et al., (1969) reported that the loss of water from fish depends mainly on the strength of salt, the temperature and salting time. These results are in a good agreement with those obtained by Hernandez-Herrero, (1997).

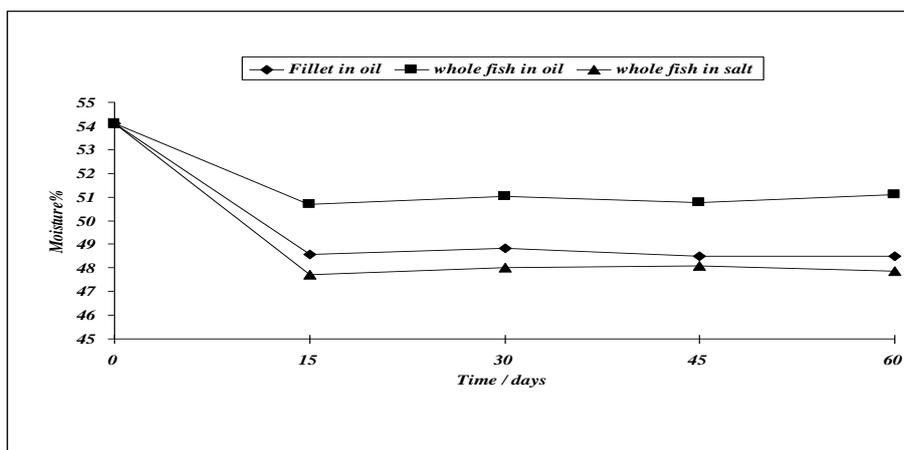
From the illustrated data in Fig. (2) it could be observed that ash content of whole Sardines packed with dry fine salt gradually increased from 25.90% to 28.80% after 60 days storage. On the other hand, ash content of whole Sardines packed in soybean oil gradually decreased from 25.90% to the minimum content (23.90%) under the same conditions. Also, ash content of Sardine fillet packed in soybean oil decreased from 25.90% to 23.50% after 60 days of storage. The decrease in ash content during storage may be related to the decrease in salt content and loss of some minerals in the separated drip. The increment of ash content from 25.90% to 28.80% could be explained by loss of moisture content and the diffusion of salt in the fish fillet during salting and ripening process (Awad, 1999).

These results are in agreement with those obtained by Hernandez-Herrero, (1997) who reported that after salting process of anchovy, salt and ash contents increased from 0.32% to 19.30% and from 1.60% to 20.74%, respectively.

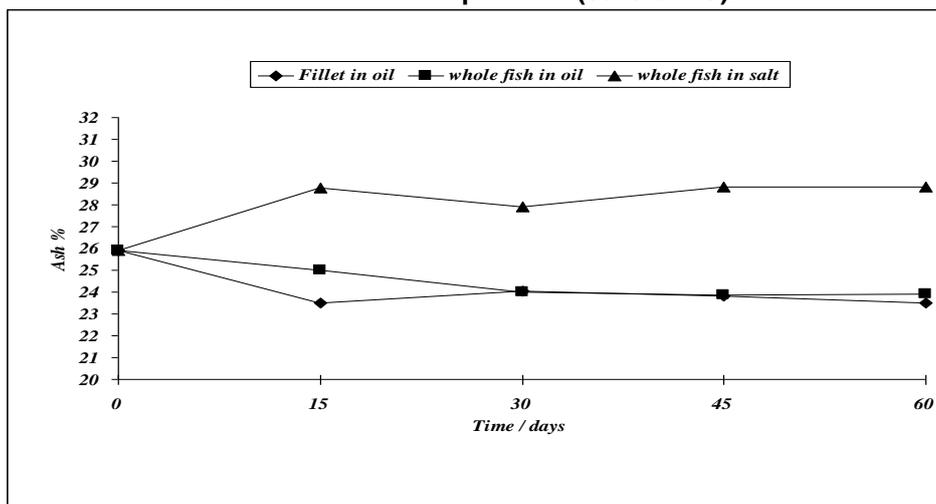
Fig. (3) indicated that salt content in the muscle tissues of whole Sardines packed with dry fine salt was constant (25%) during 60 days of storage. In the opposite, salt content of stored whole Sardines packed in soybean oil was gradually decreased until reached minimum content (23.30%) after 45 days of storage.

The same trend was also noticed for Sardine fillet packed in soybean oil. Since, it decreased from 25.86% to 22.90 after 60 days, of storage.

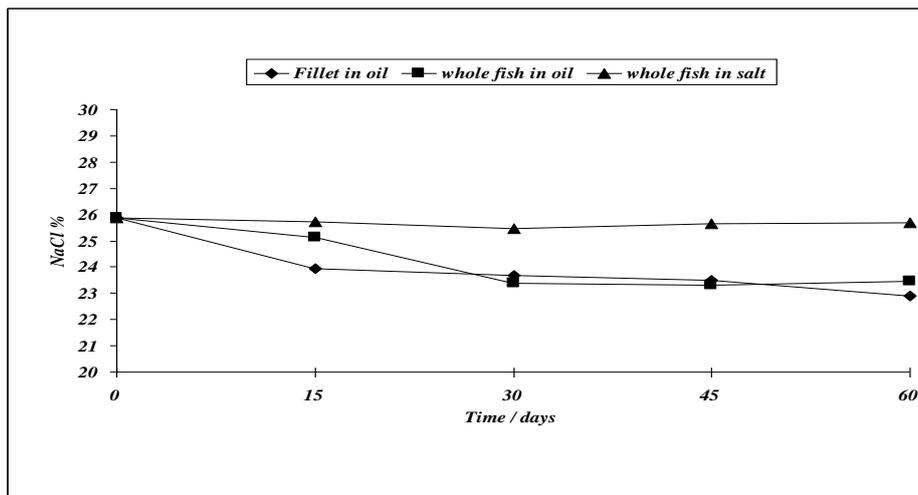
The decrement in salt content of Sardine meat with increasing salt in fish brine may be attributed to the reduction in water holding capacity of fish muscle protein. Thus, salt content of Sardine meat gradually decreased with the prolongation period of storage. Consequently, salt content of Sardine packed with dry fine salt retained the highest among the other studied samples. On contrast, salt content of Sardine fillet stored in oil had the lowest value. This may be related to the nature of packing media (*Zaitsev et al., 1969*).



**Fig.(1): Effect of maturation period on moisture content (%) in salted Sardine stored at room temperature (30°C ± 1°C).**



**Fig.(2): Effect of maturation period on ash content (%) in salted Sardine stored at room temperature (30°C ± 1°C).**



**Fig.(3): Effect of maturation period on salt content (NaCl%) in salted Sardine stored at room temperature (30°C ± 1°C).**

It was recommended that water activity ( $a_w$ ) is an important factor in fish processing and the keeping quality of cured fish products (Lupin, 1986). It could be observed from Fig. (4) that water activity ( $a_w$ ) of Sardines decreased as a result of salting and ripening process from 0.66 at zero time to 0.59 for whole Sardine with dry fine salt after 30 days of storage. Meanwhile, water activity ( $a_w$ ) of salted Sardine stored in soybean oil was constant (around 0.67) during storage under the same conditions.

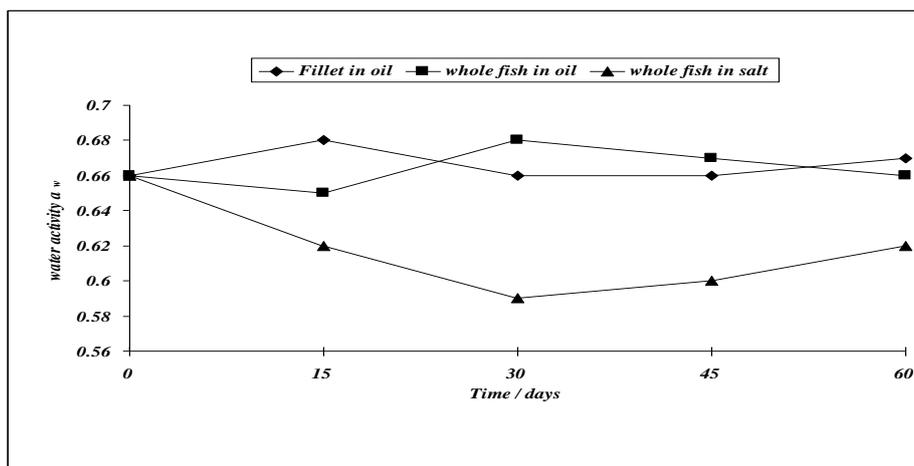
The fluctuation in water activity ( $a_w$ ) with the prolongation of storage may be due to the decrease in salt content of Sardine meat during storage (Awad, 1999). Lupin *et al.*, (1981) found a linear relationship between water activity and sodium chloride concentration. These results are in agreement with those reported by Hernandez-Herrero, (1997) who found water activity dramatically decreased in the ripened anchovies immediately after salting from 0.998 to 0.811.

Salting and ripening process caused a decrease in pH value of salted Sardines (Fig. 5). The pH value at zero time decreased from 6.0 to about 5.8, 5.9 in the salted Sardines packed with dry fine salt after 15 and 60 days of storage, respectively. But in case of whole Sardines packed in soybean oil, pH value ranged from 5.7 – 6.0 during storage. Meanwhile, Sardine fillet in soybean oil had constant pH values during the same time. These variations in pH values may be attributed to the lipid and protein decomposition and the ratio between both those formed products which affect on the final pH value, (Awad, 1999).

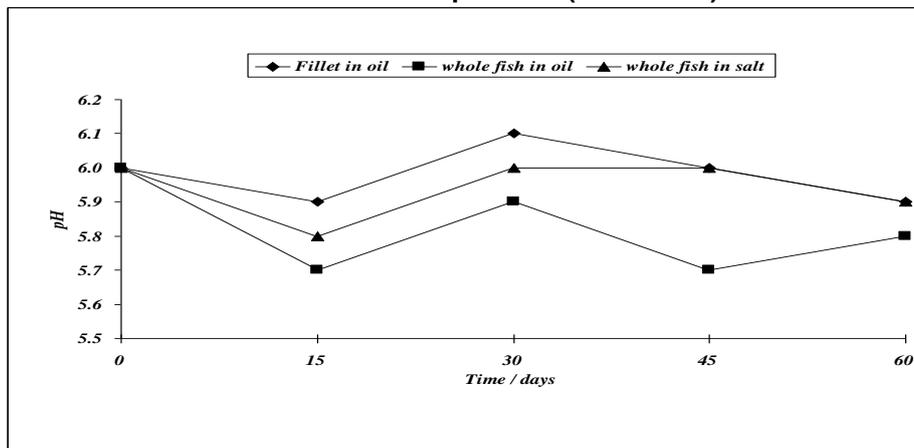
From the aforementioned data it could be noticed that the lowest pH value was found in the Sardine packed in oil. This may be attributed to higher rate of proteolysis because of its lower salt content, especially at the later stage from storage while, a sharp rise in pH value may be related to the remarkable formation of volatile basic compounds according to Yatsunami

and Takenaka, (1996). These results are in agreement with those obtained by Cha and Lee (1985).

From the demonstrated data in Fig. (6), it could be seen that salting and ripening process caused a slight decrease in the plasticity of salted Sardines. Since, it decreased from 9.10 (cm<sup>2</sup>) at zero time to 8.90, 8.10 and 8.50 (cm<sup>2</sup>) in salted Sardines packed with dry fine salt, in soybean oil and Sardine fillets in soybean oil, respectively. It could be noticed that the lowest plasticity was showed in Sardine packed in fine dry salt, this may be due to the higher salt content and less degradation of protein. From the foregoing results, it should be born in mind that the texture was significantly affected, using warm conditions of salting and ripening, resulting more tenderness ripened fish. These obtained results agree with those reported by Borgstrom, 1965.



**Fig.(4): Effect of maturation period on water activity (a<sub>w</sub>) in salted Sardine stored at room temperature (30°C ± 1°C).**



**Fig.(5): Effect of maturation period on pH value in salted Sardine stored at room temperature (30°C ± 1°C).**

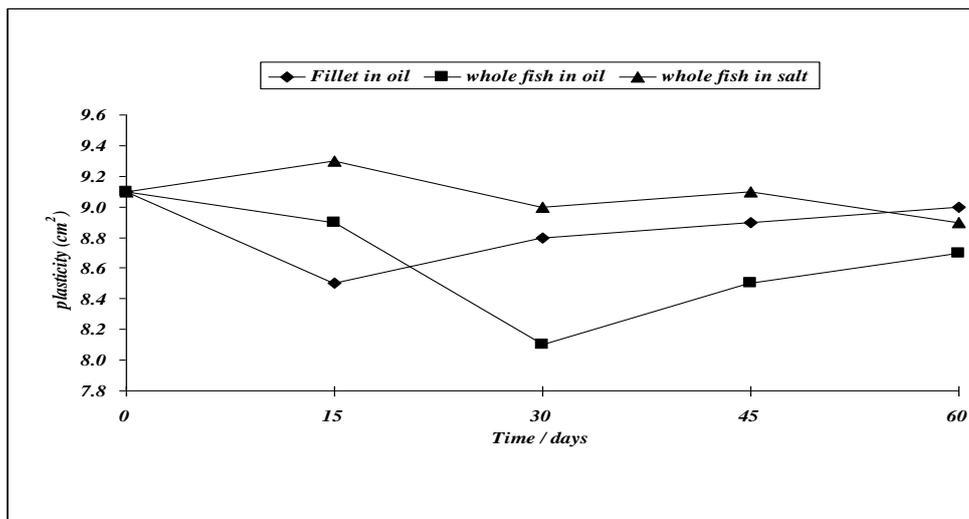


Fig.(6): Effect of maturation period on plasticity (cm<sup>2</sup>) in salted Sardine stored at room temperature (30°C ± 1°C).

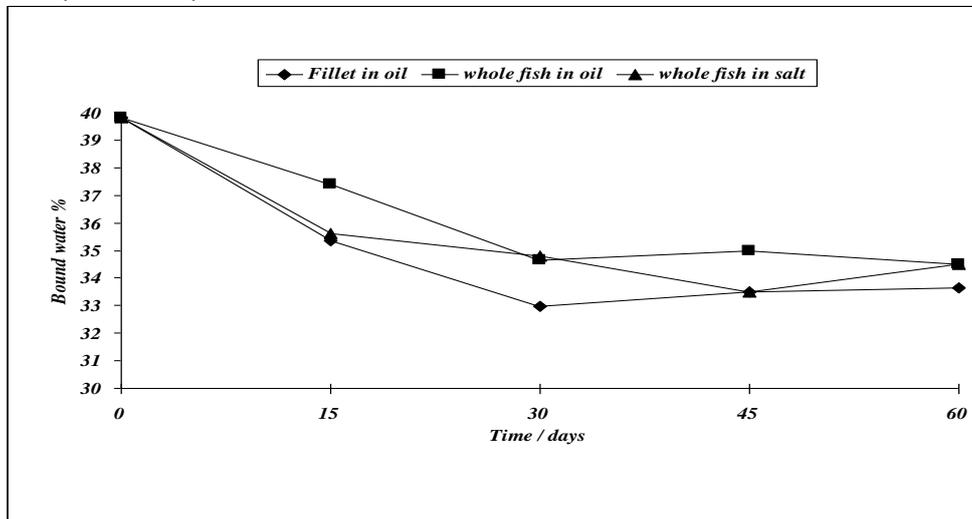
As shown in Fig. (7) salting and ripening process caused a sharp decrease in bound water % of salted Sardines. It decreased from 39.83% at zero time to 33.50% in salted Sardine packed with dry fine salt after 45 days of storage. The percentage of bound water of salted whole sardine packed in soybean oil slightly decreased until reached to 34.50% after 60 days of storage. Meanwhile, in the case of salted Sardine fillet packed in soybean oil, it decreased to 32.96% and 33.65% after 30 and 60 days of storage, respectively.

The lowest bound water of Sardine packed with dry fine salt, may be attributed to the higher salt content and storage temperature which affected on proteolysis rate. Thus, high temperature, significantly affected the final content of moisture, hence, could be reflected on the percentage of bound water in relation with denaturation occurred in fish proteins. According to the cited literature the more high temperature of salting and ripening, the less water holding capacity was obtained and thus, there was a loss in bound water values. (Borgstrom, 1965).

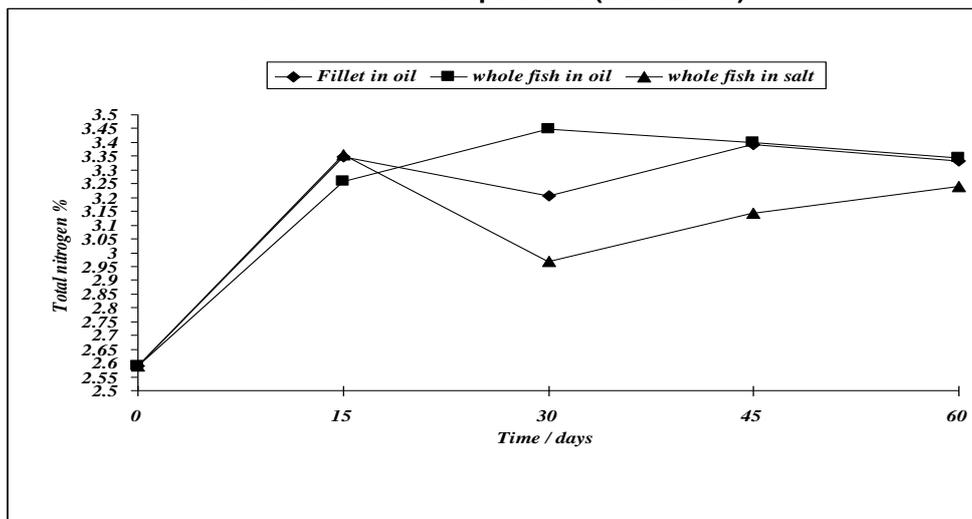
Total nitrogen content of fish meat in all treatments was slightly increased during storage as shown in Fig. (8). Since, it varied between 2.58% to 3.35% in the case of ripened Sardines packed with dry fine salt, 2.58% to 3.44% in the case of whole Sardines packed in soybean oil. While it was between 2.58% to 3.39% in the case of Sardine fillets packed in soybean oil stored at 30°C ± 1°C.

From the foregoing results, It could be noticed some loss in total nitrogen content at the end of storage period. This finding can be explained as a result of denaturation of both sarcoplasmic and myofibrillar proteins within the heavy salting of fish muscle (Awad, 1999) who found a marked decrease in sarcoplasmic (about 36% of the initial) and myofibrillar (about

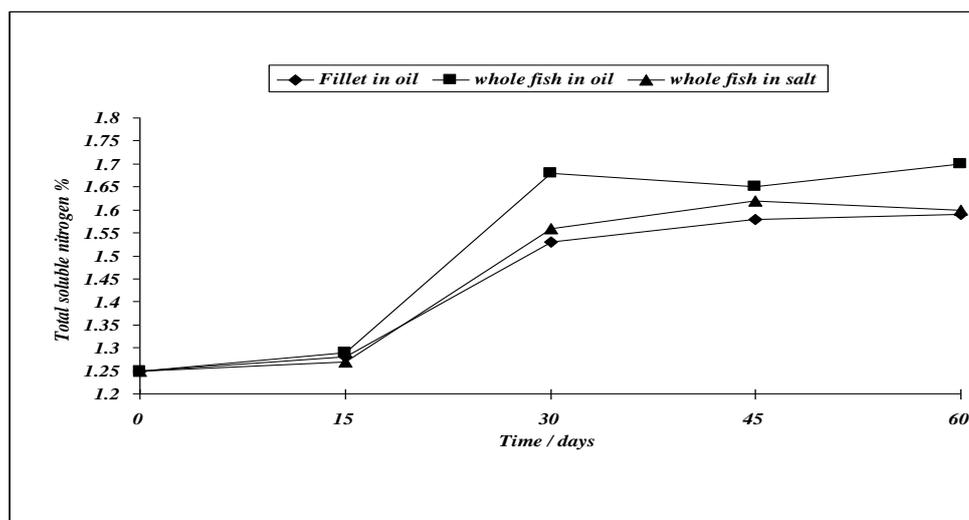
62% of the initial) proteins after 6 weeks of salting and curing process for mullet fish. This finding may be attributed to protein hydrolysis by the action of proteolytic enzymes. *Abo-Taleb (1993)* concluded that the decrease in protein content during storage might be due to protein hydrolysis by enzymes, which enhanced the loss of water soluble nitrogen with the separated drip.



**Fig.(7): Effect of maturation period on bound water % in salted Sardine stored at room temperature (30°C ± 1°C).**



**Fig.(8): Effect of maturation period on total nitrogen content (TN%) in salted Sardine stored at room temperature (30°C ± 1°C).**



**Fig.(9): Effect of maturation period on total soluble nitrogen content (TSN%) in salted Sardine stored at room temperature ( $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ).**

Data of Fig. (9) show that TSN of salted cured fish meat was augmented during storage. For example, TSN of whole Sardine packed with fine dry salt reached to the maximum value (1.62%) after 45 days of storage. While TSN of both whole Sardine and Sardine fillet stored in oil reached to the maximum values (1.70 and 1.59%, respectively) after 60 days of storage under the same conditions. This increase in TSN during storage could be related to the denaturation and hydrolysis of protein especially sarcoplasm and myofibrillar by action of proteolytic enzymes which enhanced the loss of water soluble nitrogen and other different nitrogenous compounds out side fish muscle into soybean oil (Awad, 1999 and Abo-Taleb, 1993). This results are in agreement with that obtained by Voskresensky (1965).

The NPN content of Sardine meat, generally, increased during storage (Fig. 10). Whereas, NPN of Sardine packed with fine dry salt, salted Sardine stored in soybean oil and Sardine fillet stored in oil were 1.06%, 1.19% and 0.94%, respectively after 60 days of storage at  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The increase in NPN content may be due to muscle protein breakdown by the action of proteolytic enzymes (Narayanaswamy et al., 1980). Aman (1983) found that the percentage of Non-protein nitrogen of salt-cured mullet fish was increased by about twice after 6 weeks due to the muscle protein breakdown by the action of proteolytic enzymes. Finally, from the same results, it could be noticed some loss in the Non-protein nitrogen content and this may be attributed to the diffusion of Non-protein nitrogen compounds from fish muscle into the brine or lost in the separated drip in case of salted Sardine packed in soybean oil (Awad, 1999). Also, it may be due to the growth of microorganisms, which use these compounds to grow and produce any matters. These findings were in a good agreement with that obtained by Voskresensky (1965), who noticed that during storage period the nitrogenous

substances, mainly of low molecular weight (Non-protein nitrogen), diffuse from the fish into the brine.

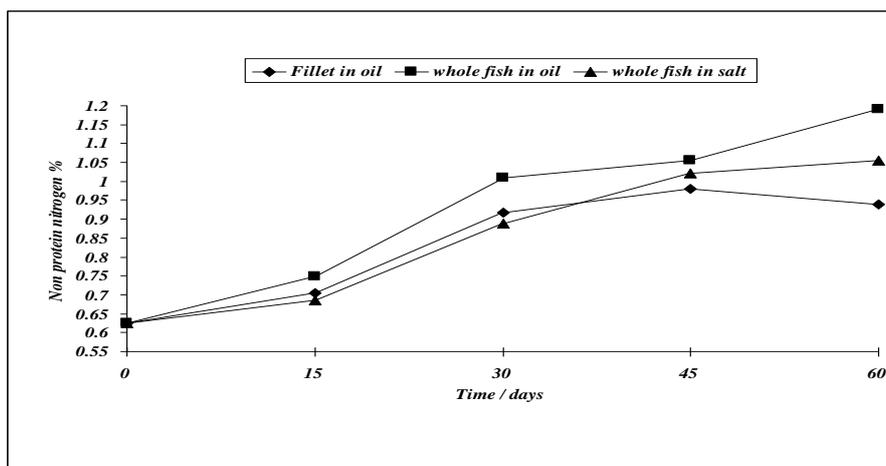
As for free amino nitrogen (FAN) content of Sardine meat (Fig. 11), it was clear that FAN of both salted Sardine packed with fine dry salt and whole Sardine stored in soybean oil gradually decreased as a result of storage. Since, FAN of ripened Sardine packed with fine dry salt reached to 0.62% after 60 days of storage. Similarly, FAN content of whole Sardine and Sardine fillet stored in soybean oil after 30 days of storage at  $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$  were 0.54% and 0.59%, respectively.

The loss of free amino nitrogen content may be due to the diffusion of free amino nitrogen compounds from fish muscle into the brine or oil and/or the growth of microorganisms, which use these compounds as an ideal substrate to grow and produce another matters.

Total volatile nitrogen (TVN) of Sardine meat gradually decreased as a function of storage (Fig. 12). The TVN of whole ripened Sardine packed with fine dry salt decreased from 106.4 mg/100g (at zero time) to 90.5 mg/100g after 60 days of storage at  $30^{\circ}\text{C}$ . At the same time, it reached to 87.0 mg/100g and 90.4 mg/100g for whole salted Sardine and Sardine fillet stored in soybean oil for 60 days, respectively, under the same conditions.

The loss of TVN during storage may be attributed the diffusion of TVN into Sardine brine or in the soybean oil (*Hernandez-Herrero, 1997*) or to the interaction of the decomposition products of protein with the compounds produced via lipid oxidation forming tertiary products, (*Reddy and Setty, 1996*).

Finally, it could be concluded that protein fractions (TSN, NPN, FAN and TVN) play an vital role in ripening process of salted Sardine and there are number of protein derivatives released in the brine during this process.



**Fig.(10): Effect of maturation period on non protein nitrogen content (NPN%) in salted Sardine stored at room temperature ( $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ).**

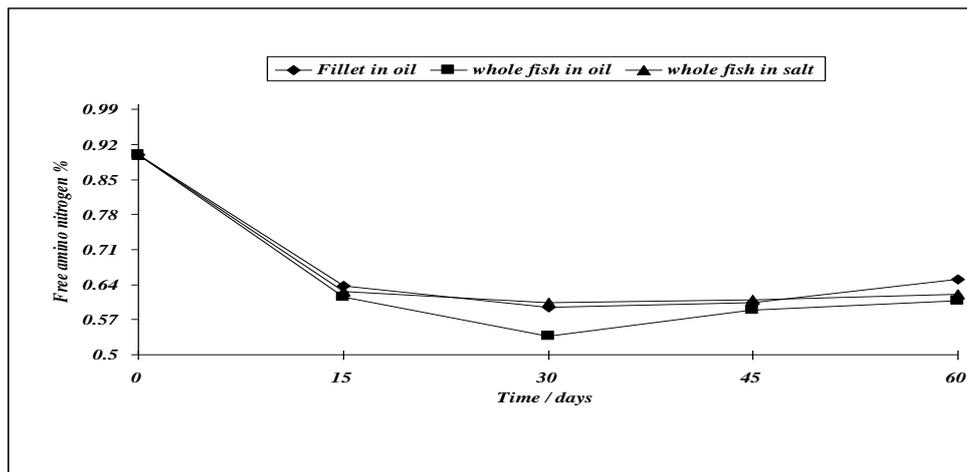


Fig.(11): Effect of maturation period on free amino nitrogen content (FAN%) in salted Sardine stored at room temperature ( $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ).

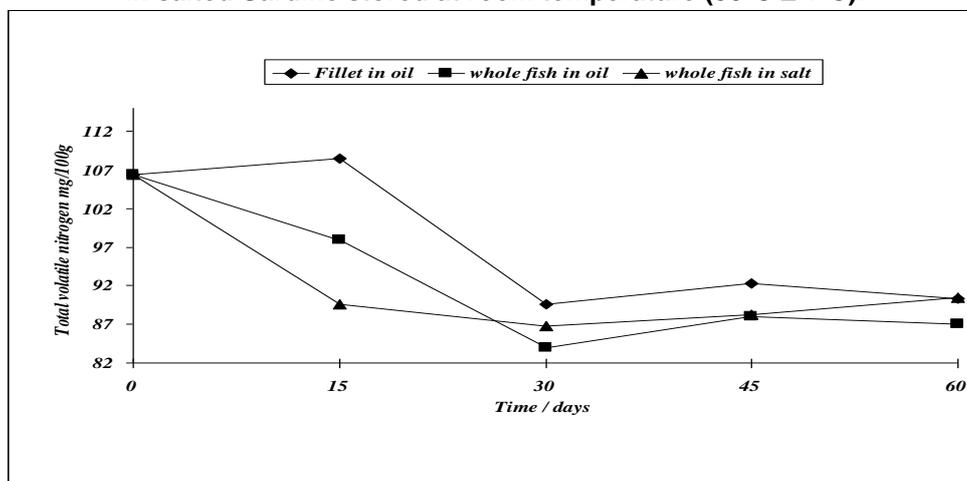


Fig.(12): Effect of maturation period on total volatile nitrogen content (mg/100g) (TVN) in salted Sardine stored at room temperature ( $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ).

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### **التغيرات الكيميائية و الطبيعية أثناء عملية نضج السردين المملح**

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تم تحضير سردين مملح و معتق من سمك الـ *Sardinella spp.* بالطريقة التقليدية و المتبعة في دول حوض البحر الأبيض المتوسط لدراسة و تتبع التغيرات الطبيعية و الكيماوية في لحم السردين و خاصة البروتين و مشتقاته أثناء عمليات التملح و الإنضاج و التخزين على درجة حرارة الغرفة ( $30 \pm 1^\circ\text{C}$ ) لمدة ستة شهور. و قد أظهرت النتائج المتحصل عليها الدور الحيوي الذي تلعبه مشتقات البروتين (النيتروجين الذائب الكلي ، النيتروجين اللابروتيني ، النيتروجين الأميني الحر و النيتروجين المتطاير الكلي) أثناء عملية إنضاج السردين المملح. حيث وصل تركيز النيتروجين الكلي إلى 3,44% ، النيتروجين الذائب الكلي 1,70% حيث مثل 50,83% من النيتروجين الكلي ، النيتروجين اللابروتيني 1,19% مثل 35,58% من النيتروجين الكلي ، النيتروجين الأميني الحر 0,90% و أخيراً النيتروجين المتطاير الكلي 108,5 مجم/100جم.