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CHEMICAL, PHYSICAL AND MICROBIOLOGICAL STUDIES ON SALTED MULLET FISH (FESSEKH) USING DIFFERENT SALTING SYSTEMS

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ABSTRACT

This study concerns with salted Mullet fish (*Mugil cephalus*) produced in Egypt, which named "Fessekh". Since the demand for Fessekh is increasing every year in Egypt specially at certain occasions and feasts. Lately many cases of salted fish hazards were announced with its consumption, therefore, this investigation was designed for production safety salted cured fish "Fessekh" using three applicable salting processes. The first treatment (traditional method) a pre-fermentation process was carried out for 72 hrs., then dry salting was applied by 25% dry salt (w/w). The second treatment (T_2) was directly achieved by brining in 20% salt solution and stored at 30°C, while the third treatment (T_3), samples were directly treated with relatively low dry salting (15% salt w/w) + 0.2% $NaNO_3$). Both T_1 and T_3 were stored at 20°C. Salt penetration rate, pH values and water activity were determined for produced samples. Protein derivatives such as NPN, TVN and FAN, also TBA values were detected for all samples as parameters to define the suitable ripening degrees. Samples safety was estimated by pursuing the development of the coliform bacteria, *Clostridium* spp. and *Staphylococcus* spp. through the storage periods till consumption times corresponding such product. Also sensory evaluation for all different samples was determined.

The main established results could be summarized as following: Possibility to achieve fish salting process at rigor mortis stage without needing the pre-fermentation process used in traditional method which causes high risk to contaminate fish with pathogenic bacteria. Optimum ripening degrees of Fessekh to consumption could be done after 5, 3 and 4 week from salting storage period to T_1, T_2 and T_3, respectively. Success to use the brining method (20%) at relative high temperature (30°C), in condition that shortness the storage period (no more than 45 days). Relatively low dry salting with 15% NaCl in addition to admissible preservative (0.2% $NaNO_2$) conduced to obtain good quality and safety Fessekh at the same time. *St. aureus* microorganisms were only detected in both visena and flesh at first two weeks of salting of traditional product (T_1). The third treatment showed the highest safety rate regarding to disappearance of *Cl*. spp and *St*. spp. after 5 and 14 days of salting, respectively.

INTRODUCTION

Fish is one of the most important foods in human nutrition diet. Gray mullet or Mullet (*Mugil cephalus*) is the most famous species in Mugilidae family and proper for consumption needs all over the world. Mullet may be catch in winter (from October to January) because of it is hard to preserve (FAO Fisheries Report, 1990).

The total production of Mullet fish in Egypt gradually increased which ranged from (35-50) thousand tons (GOARD, 1999).

Mullet is considered a suitable material for fish salting industry, having a popular flesh, high nutritive value, multiple preservation and cooking ways, high ability to cultivate in farms and its consumption in private occasions. Salting is a traditional method of preservation because of its process simplicity, low production cost, ability to be combined with other methods and to satisfy consumers requirements (Clueas, 1982).

Fessekh is a kind of fermented salted fish product, salt content in salt cured fish determining its shelf life (El-Sharnouby, 1989).

El-Shehawy (2000) found that the moisture content in the final product of salted cured fish in parallel with the water activity plays an important role in development of the keeping quality in addition with the salt content. FAO (1981) reported that sodium and potassium nitrites and nitrates have been reported to lengthen the keeping time and retard the growth of microorganisms, especially in the presence of sodium chloride.

The presence of sodium nitrite in meat and fish products reduces the levels of sodium chloride which necessary to prevent toxin production. The maximum salt tolerance of *Cl. potulinum* type E is 5% (Jay, 1996).

Since, lately many cases of salted fish poisoning and infections were announced in Egypt, it may be due to not enough used salt (NaCl) to inhibit the proteolytic activity and putrefying bacteria.

Therefore this work was designed to:

- Study the changes occurred in chemical, physicochemical, microbiological, and organoleptic properties through the prefermentation and salting storage periods at three different treatments.
- Produce safety salted cured products with relative low salt concentration.
- Compare the manufactured Fessekh with prefermentation process (traditional method) with the other treatments regarding to quality, rate of spoilage and its shelf life.
- Show the correlation between the chemical changes and the product quality.

MATERIALS AND METHODS

Materials:

Fish samples:

Mullet fish (*Mugil cephalus*) used in this study was obtained from Manzala fish farm and was transferred directly after catching at the end of February, 1999 without ice to the laboratory of Food Industries Department, Faculty of Agriculture, Mansoura University.

Salt:

Sodium chloride was obtained from El-Nasr Salinas Company, Egypt. It was coarse salt, namely salt for Food Industries (for salted meat and fish). Sodium chloride is coincided with Egyptian Standardization.

Other additives:

Sodium nitrite was obtained from El-Gomhorya Medical Company, Mansoura Egypt.

Containers:

Plastic barrels (6.0 kg) were used in which fish were salted and packed tightly then salted.

Arrangements:

The fish as received in the laboratory, it was immediately washed with tap water. Then the whole fish were divided into three equal groups (5 kg). Every group was treated alone according to the salting method as followed in the next table :

Table (1): Salting treatments.

	Treatments		
	1	**2**	**3**
Technique	Fish were left for 72 hr. to swell, then dry salted with 25% (w/w) coarse salt. (Traditional method)	Fish are dipped in 20% salt solution.	Fish are dry salted with mixture of 15% dry salt (w/w) + 0.2% $NaNO_2$
Fish flesh state	At autolysis (swollen)	At rigor mortis	At rigor mortis
Additives	---	---	$NaNO_2$
Temperature	20°C	30°C	20°C

Preparation of sample for analysis:

Firstly for chemical and physico-chemical analysis fish were beheaded, eviscerated, washed with tap water to remove the blood, then minced using an electric meat chopper and homogenated.

Secondly, for bacteriological examination, fish were washed with tap water, left to remove drained water for 10 minutes, then swept by filter paper and under sterilizing conditions, two samples were taken from the viscera and the flesh, each weighting 1 g on aluminum foil.

Analytical methods:

Chemical analysis:

1. Moisture, ash, total nitrogen and salt contents were determined according to the method described by AOAC (1990).
2. The salt concentration of salted Mullet fish was calculated from the estimated moisture and salt contents using the following equation as mentioned by Zaitsev *et al*. (1969).

$$C = \frac{100 + S}{S + W}$$

Where c = is salt concentration %, S and w are the percentage of salt and water in the fish, respectively.

3. Non protein nitrogen (NPN) was determined according to the method of Durand (1982). Results were expressed as mg nitrogen per 100 g samples.
4. Total soluble nitrogen (TSN) was determined according to the method of Soloviev (1966).
5. Total volatile nitrogen (TVN) was determined according the method mentioned by Pearson (1968). Results were expressed as mg nitrogen per 100 g samples.
6. Free amino nitrogen (FAN) was determined by applying the Sorenson's method as explained in the AOAC (1990). Results were expressed as mg nitrogen per 100 g samples.
7. Thiobarbituric acid (TBA) value was calorimetrically determined according to the procedure described by Pearson (1986).
8. TBA value was expressed using the following equation:
   mg malonaldehyde/kg sample = 7.8 x O. D
   where:
   O. D = Optical density.
9. Sodium nitrite content was determined directly in samples as described by Ockerman (1976).
   Results were expressed as part $NaNO_2$ per million sample part (ppm).

Physico-chemical analysis:

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

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

Microbiological examination:

Microbial analysis was carried out on fish samples before and after salting period from both flesh and viscera.

1. Total viable bacterial counts (TVBC) were determined according to the Difco Manual (1966).
2. Coliforms, fecal coliforms were detected as described in Oxoid Manual (1962). The inoculated tubes were incubated at 37°C for 72 hr.
3. Staphylococci: *Staph*. media No. 110 was used to detect staphylococci (Difco Manual, 1974). The coagulase test to differentiate between virulent and unvirulent staphylococci was carried out where no clumping occurs in 10 to 20 sec. After human or rabbit plasma is added with a straight needle with stirring that means *Stap. aureus* is not found.
4. Anaerobic spore forming bacteria (*Clostridium* spp.): This method is based on the detection of Gram-positive bacilli with subterminal oval spores. The medium was described by Bhat and Barker (1947).

Sensory assessment:

Salted fish was subjected to a panel test of a point hedonic scale, with higher values denoting better quality according to the procedure of Fey and Regenstein (1982). Five parameters were analyzed: flavor, color, taste, flesh consistency (texture) and overall acceptability.

The evaluation sheet is shown as follows:

Spoiled	3 > 4
Onset of spoilage	4 > 5
Accepted	5 > 6
Good	6 > 7
Very good	7 > 9
Excellent	9 > 10

RESULTS AND DISCUSSION

Data in table (2) showed that the moisture content decreased from 72.4% in fresh mullet to 68.2% after 72 hr., so salt and ash contents slightly increased. This increment of both salt and ash contents may be due to release of mucus and body fluids from gills and belly. These observations are in agreement with Poulter and Nicolaides (1985).

Table (2): Approximate chemical composition of Mullet fish flesh and some bacteriological changes in flesh and viscera during fermentation period.

Components		Hyperaenia 2 h after death	Rigor mortis 24 h after death	Autolysis 72 h after death
Moisture %		72.40	71.00	68.20
Salt (NaCl) %		0.23	0.26	0.29
Ash %		1.24	1.23	1.47
TN %		3.14	3.17	3.26
TSN (mg/100 g)		1580	1620	1640
NPN (mg/100 g)		410	480	520
TVN (mg/100 g)		23.4	26.1	30.7
FAN (mg/100 g)		280	310	360
TBA		5.35	5.46	6.85
pH value		6.40	6.20	7.30
TVBC	Flesh	0.004	1.2	99
	Viscera	0.88	4.5	77
Coliform	Flesh	-	-	+
group	Viscera	+	+	+
Staph. Spp.	Flesh	-	+	+
	Viscera	+	+	+*
Clostridium	Flesh	-	-	+
spp.	Viscera	+	+	+

TN, Total Nitrogen; TSN, Total Soluble Nitrogen; NPN, Non Protein Nitrogen; TVN, Total Volatile Nitrogen; FAN, Free Amino Nitrogen; TBA, Thiobarbituric Acid (Mg Malonaldehyde/Kg sample); TVBC, Total Viable Bacterial Count (CFU x 10^6/g); *, *Staphylococcus aureus*.

In the same table, protein hydrolysis derivatives (NPN, TSN, TVN and FAN) increased from 410, 1580, 23.44 and 280 mg/100 g in fresh Mullet to 480, 1620, 26.10 and 310 mg/100 g in rigor mortis and 520, 1640, 30.70 and 360 mg/100 g in autolysed Mullet after 72 hr from primary fermentation. These gradual increases may be due to the muscle protein breakdown by the action of proteolytic enzymes and may due to the development of bacterial growth. These findings are in concordance with data obtained by El-Kardaway (1985), Khallaf (1986) and Shalaby (1990). Also TBA values increased due to lipolytic hydrolysis. On the other hand, pH value, firstly decreased from 6.4 in fresh mullet to 6.2 because of glycogen consumption by bacteria, forming lactic acid after 12 hr. from catching and then rised again to 7.3 after 72 hr., this may be due to the action of protease and lipase enzymes in the tissues to give pyridine bases (Zaitsev *et al*., 1969).

Data in the same table showed that the (TVBC) in mullet fish were 0.004×10^6 and 0.88×10^6/g in flesh and viscera after 2 hr. from catching, respectively, which sharply increased to 99×10^6/g and 77×10^6g after 72 hr. in flesh and viscera, respectively.

The TVBC was higher in flesh than viscera during autolysis stage (after 72 hr.), this may be due to the penetration of microorganisms into fish flesh which is more rich with decomposed products during the pre-fermentation period (Qyvind *et al.*, 1994). So, it could be observed that *Clostridium* spp., *Staph.* spp. and coliform groups in fish flesh after primary fermentation for 72 hr., where it was free of them in fresh fish. Also, pathogenic *Staph. aureus* was detected only in viscera after 72 hr. These findings are in concordance with data obtained by Rashad (1986).

Data presented in Table (3) indicated that (T_1) showed the higher salt content and salt concentration after 3 days of salting period (3.80% and 6.34%) while (T_3) gave the lowest salt content and salt concentration (2.66% and 4.09%) at the same time. On the other hand, after 7 days of salting period the salt concentration surpassed the preserved concentration (critical salting point, realizing 13.78, 10.15 and 10.50% for (T_1), (T_2) and (T_3), respectively. The increment of salt concentration in viscera to certain limits during few days of salting lead to delay and control the spread of microbial contamination of fish flesh conducting to the optimal balance between occurred changes and desirable ripening point. Also data showed sharply increases of sodium nitrite contents in flesh and visera reaching the maximum values (137 and 98 ppm), respectively after 7 days as a result of its penetration with salt from gills, during salting process. The penetration rate of $NaNO_2$ in flesh was higher than in viscera as was detected by Zaitsev *et al.* (1969) who found that the salt penetration rate through 1 cm^3 from flesh and viscera as a ratio of salt penetration of whole fish were 1.0 and 0.85, respectively, and to the higher content of fat in viscera. The sodium nitrite contents in flesh and viscera markedly decreased arriving to 98 and 68 ppm, respectively, after 28 days of salting, which means that the residual nitrite will be less than the permissible limits according to Egyptian Standard (1989), which defined that final salted cured products must be contained less than 125 ppm $NaNO_2$. The main reason for $NaNO_2$ decrement is its reduction and consumption by microorganisms (Knochel and Huss, 1984).

Regarding to the results illustrated in Table (4), it could be noticed that moisture diffused out from fish and attributed with salt penetration. High salt penetration rate during the first 7 days was observed, where the salt concentration being 14.48, 10.94 and 10.96% for T_1, T_2 and T_3, respectively.

There were a slight decrease in moisture content after the second week up to the end of storage period accompanied with some fluctuations. These fluctuations may be due to the increase of salt contents which consequently increase to osmotic pressure and then tissues gained water. The sucking back of brine into the tissues was pointed by Rulev (1980) and Lupin (1986).

Table (3): Changes in salt content (NaCl%), salt concentration % in viscera during salting and sodium nitrite contents (ppm) in flesh and viscera of Mullet fish (*Mugil cephalus*) during salting and storage.

Time (days	Treatments							
	T_1		T_2		T_3			
	NaCl %	Salt Conc. %	NaCl %	Salt Conc. %	NaCl %	Salt Conc. %	$NaNo_2$ in flesh	$NaNo_2$ in viscera
Zero Time	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1.52	2.29	2.03	2.93	1.28	1.90	20	25
3	3.80	6.34	3.28	4.88	2.66	4.09	68	60
5	5.22	8.72	4.85	7.40	4.32	4.91	108	76
7	7.88	13.78	6.60	10.15	6.38	10.50	137	98
21	ND	ND	ND	ND	ND	ND	118	82
28	ND	ND	ND	ND	ND	ND	98	68

ND = Not determined.

Fessekh manufactured by T_1 showed the highest reduction in moisture content which related to high salt content, these findings are confirmed with mentioned data by Sakai and Miki (1982) and Foda *et al.* (1986), while T_3 showed the highest final moisture content because of its lower salt level. At the end of the 2nd week of salting and storage period, all treatments reached the preserved salt concentration.

The salted product manufactured with brining method (T_2) showed higher salt penetration rate than that of T_3 during the prolongation of storage period. This is may be due to the high temperature used (30°C) and high salt concentration in the wet salting process. These results are in agreement with data obtained by Abo Raya (1975); Rashad (1986) and El-Sharnouby (1989).

Demonstrated data showed that the water activity values (a_w) decreased in all treatments within the first two weeks of salting storage periods. These decrements in a_w were in parallel with highly salt penetration into fish tissues and highly loss of moisture at the same period which confirm with data obtained by Awad (1999). The certain decreases occurred in a_w in Fessekh with the prolongation of storage period lead to retard the bacterial decomposition, delay the enzymatic hydrolysis which reflect an arriving the suitable ripening degree and then to increase the keeping quality and shelf

life of such products. These results are in a good agreement with Doe *et al*. (1982).

Data presented in Table (4) also indicated that Fessekh samples prepared by T_1 showed the highest pH values, not only as initial value but also during the first salting week, compared with T_2 and T_3 because of the long pre-fermentation period of such product. This conclusion agreed with data obtained by Yatsunami and Takenaka (1996).

Table (4): Changes in moisture, salt content (NaCl %), salt concentration %, water activity (a_w) and pH value in flesh of salted Mullet fish during salting and storage period.

	T_1					T_2					T_3				
Time (days)	Moisture %	NaCl %	Salt Conc. %	a_w	PH	Moisture %	NaCl %	Salt Conc. %	a_w	pH	Moisture %	NaCl %	Salt Conc. %	a_w	pH
ZT	68.20	0.29	0.42	0.99	7.3	72.40	0.23	0.32	0.99	6.4	72.40	0.23	0.32	0.99	6.4
1	64.58	1.63	2.46	0.98	6.8	67.18	2.08	3.00	0.98	6.6	65.78	1.32	1.96	0.98	6.1
3	65.12	3.74	6.24	0.96	6.4	63.92	3.49	5.17	0.96	6.3	62.35	2.85	4.37	0.97	5.8
5	54.60	6.14	10.12	0.93	6.2	60.65	5.32	8.06	0.94	6.0	58.19	4.14	6.64	0.95	5.6
7	49.28	8.35	14.48	0.90	6.0	58.42	7.18	10.94	0.93	5.8	54.33	6.69	10.96	0.92	5.6
14	50.00	12.23	19.65	0.84	5.8	59.90	10.18	14.52	0.89	6.1	58.92	8.17	12.17	0.91	5.8
21	48.88	14.35	22.69	0.80	5.6	57.14	11.76	17.06	0.87	6.3	62.24	9.13	12.79	0.91	6.0
28	49.61	15.41	23.70	0.79	5.5	55.40	12.23	18.06	0.86	6.5	60.88	9.63	13.65	0.90	6.3
35	51.28	16.30	24.00	0.78	5.6	53.22	12.50	19.00	0.84	6.6	59.00	9.98	14.46	0.89	6.4
50	49.92	17.18	25.60	0.77	5.8	52.10	12.22	18.99	0.85	6.8	56.55	10.72	15.55	0.88	6.2
65	52.83	17.80	25.20	0.77	6.1	55.66	12.00	17.73	0.86	7.0	54.22	10.95	16.86	0.97	6.4
80	51.76	18.00	25.80	0.76	6.4	56.98	11.65	16.97	0.87	7.1	55.24	11.11	16.74	0.87	6.6
95	54.30	19.20	25.10	0.77	6.6	ND	ND	ND	ND	ND	56.66	11.27	16.59	0.87	6.9
110	53.47	20.40	25.60	0.79	6.7	ND	ND	ND	ND	ND	58.78	11.40	16.24	0.88	6.8

ND = not determined.

The signs of spoilage of the salted products manufactured by T_1, T_2 and T_3 were detected after 95, 35 and 80 days of storage, respectively, which accompanied with the increasing of pH values (above pH 6.6).

Data in Table (5) showed that there is a gradual increase in TN content during the first week of salting then it gradually decreased particularly till 21st day of salting storage period for the products manufactured by T_1 and T_2, while T_3 showed a minimum content of TN after 35 days from salting storage period. This decrement in TN during salting storage period may be due to its reduction and consumption by microorganisms. After that the TN content tends to slight increase due to sucking back for a part of brine solution which contains some of salt soluble proteins into the fish flesh. Similar changes in TN content were observed by Aman (1983) and Rashad (1986).

Results in the same table showed that (NPN) contents gradually increased throughout salting and till the end of storage period in Fessekh produced by (T_1) and (T_3), while T_2 showed the highest NPN values till reached the maximum value after 28 days, then began to fluctuate until the end of storage, this fluctuation in NPN contents was due to the diffusion of NPN compounds from fish muscles into the brine or due to the growth of microorganisms. These findings are in agreement with data reported by Abo-Taleb (1993).Regarding to Fessekh produced by the traditional method (T_1) is considered ripe after 35 days of salting storage period depending upon

NPN/TN ratio (40.66), while Fessekh produced by T_3 realized the optimum ripening point at 28th day where NPN/TN ratio were 41.11. On the other hand, Fessekh produced by brining method (T_2) was considered faster than the others to reach the ripening point, where NPN/TN ratio was 42.85 after only 21 day of salting storage period taking into consideration that the optimal ripening point depending upon NPN/TN ratio (40%). The protein hydrolysis and ripening point occurred during the manufacture of salt cured fish were studied by El-Kardawy (1985) and Awad (1999).

From data in the same Table, it could be seen gradually increase in FAN and TVN contents during salting storage period for all treatments associated with slight variations at certain times within storage period, this occurrence swinging in FAN and TVN may be attributed to the diffusion of FAN and TVN compounds from fish muscles into the brine or to the growth of microorganisms using these compounds as an ideal substrate to grow and produce another matters. During storage period, manufactured Fessekh by (T_2) showed the highest protein hydrolysis and microbial decomposition regarding to FAN and TVN contents, compared with other treatments. This observation may be due to elevated moisture content, high temperature used (30°C) and the relative low salt concentration. The increase of FAN and TVN contents with elevated temperature was also studied by Deng (1981).

Generally, previous changes of TVN and FAN contents are in agreement with data obtained by El-Sharnouby (1989).

Also results in the same Table showed that the TBA values in all treatments were gradually increased during the first month of salting storage period and decreased after that till the end of storage period. This phenomenon was in agreement with Yang *et al.* (1981) who found that sodium chloride enhanced the lipid oxidation as shown in T_1. The decrement of TBA values after certain periods during storage time may be due to loss of formed malonaldehydes because of its reaction with the protein-decomposed products to produce tertiary products (Reddy and Setty, 1996). From aforementioned data, the maximum values of TBA in Fessekh samples manufactured by the three different treatments were 22.84, 13.54 and 14.81 mg malonaldehyde/kg sample, respectively, which are corresponding with the optimal ripening point (35, 21 and 28 days) for each product, respectively. Generally, foregoing data are in agreement with El-Sebaly and Metwalli (1989) and El-Shehawy (2000).

Tabulated results in Table (6), indicated that the total viable bacterial count TVBC sharply increased particularly at the first week of salting especially in (T_1) which in parallel with reaching 12% or more salt concentration, after that it decreased with some fluctuations during salting storage period for all treatments. These fluctuations in such three products may be due to the swinging which takes place in protein decomposed products, with low molecular weight, such as FAN and NPN which are considered good substrates for bacterial growth. The more low molecular weight substances (FAN and NPN) presence, the more bacterial growth was observed and vice versa. These results were also detected by Del-Valle (1976); Anonymous (1981), Rashad (1986) and Shalaby (1990).

Table (6): Effect of salting treatments on the detection of (TVBC), coliform bacteria, *Clostridium* spp., *Staphylococcus* spp (+) and coagulase positive types in flesh and viscera of salted Mullet fish during salting and storage period.

Time (days)	Treatments																							
	T_1								T_2								T_3							
	TVBC		Coliform		*Clostridium*		Staph.		TVBC		Coliform		*Clostridium*		Staph.		TVBC		Coliform		*Clostridium*		Staph.	
	F	V	F	V	F	V	F	V	F	V	F	V	F	V	F	V	F	V	F	V	F	V	F	V
ZT	99.0	77.0	+	+	+	+	+	+	0.004	0.88	-	+	-	+	-	+	0.004	0.88	-	+	-	+		+
1	8.40	18.0	+	+	+	+	+	+	0.33	1.50	-	+	-	+	+	+	2.20	6.50	-	+	+	+	+	+
3	14.0	34.0	+	+	+	+	+	+	2.40	4.60	+	+	+	+	+	+	6.40	9.80	-	-	+	+	+	+
5	26.0	42.0	-	+	+	+	+	+	4.50	6.20	-	+	+	+	+	+	7.00	12.0	-	-	-	+	+	+
7	27.0	2.60	-	-	+	+	+	+	6.00	8.20	-	-	+	+	+	+	8.50	8.50	-	-	-	-	+	+
14	10.0	2.70	-	-	+	+	+	+	1.80	3.20	-	-	+	+	+	+	2.70	1.50	-	-	-	-	+	+
21	3.20	4.40	-	-	+	-	-	+	5.60	1.70	-	-	+	+	+	+	9.30	3.60	-	-	-	-	-	-
28	1.60	2.50	-	-	-	-	-	-	9.20	3.50	-	-	+	+	+	+	1.80	5.40	-	-	-	-	-	-
35	5.50	3.40	-	-	-	-	-	-	4.40	3.20	-	-	+	-	+	+	2.40	7.60	-	-	-	-	-	-
50	1.40	8.20	-	-	-	-	-	-	2.50	7.40	-	-	-	-	+	+	3.00	7.20	-	-	-	-	-	-
65	2.60	6.10	-	-	-	-	-	-	1.60	1.80	-	-	-	-	-	-	6.80	2.00	-	-	-	-	-	-
80	0.16	0.40	-	-	-	-	-	-	2.50	3.80	-	-	-	-	-	-	0.25	0.85	-	-	-	-	-	-
95	0.52	0.60	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	0.14	0.28	-	-	-	-	-	
110	0.80	0.40	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	0.28	0.19	-	-	-	-	-	-

TVBC = Total viable bacterial count TVBC x 10^6 (CFU/g).
F = Flesh, V= Viscera.

From aforementioned data, it could be concluded that there were strong relationship between the decline of bacterial growth and the decrease in water activity values. These findings are in agreement with Nunes *et al*. (1992).

It could be cleared that coliform bacteria are salt sensitive, do not grow above 6% salt concentration, where it was only detected with all treatments in flesh and viscera for the first few days of salting. These results are in agreement with Krieg and Holl (1984). Also the disappearance of coliform within only one day from viscera in (T_3) may be due to its high sensitivity to inhibit by action of $NaNO_2$. The role of $NaNO_2$ was referred also by Knochel and Huss (1984). With respect to pathogenic *Staphylococcus aureus* which can secrete toxins, it could be detected in flesh only in between 7 to 14 days in case of traditional method of salting (T_1) where the salt concentration ranged from 14.98 to 19.65%. As was proved by Silliker and Wolfe (1980) whom found that the maximum tolerance of *Staphylococcus aureus* was detected at 18% salt concentration. This aforementioned product was full ripened at 35 days depending on NPN/TN ratio, meaning that processed Fessekh is destitute from *Staphylococcus*, including *Staphylococcus aureus* at consumption time. While the existence of *Staphylococcus* spp. in manufactured Fessekh by (T_2) continue till 50 days of salting storage period and then were not detected in both flesh and viscera. It could be concluded that *Staphylococcus* spp. in such product (T_2) continued to detect for a long time in comparison with (T_1), due to relatively lower salt concentration and relatively higher water activity. The coagulate positives type (*Staphylococcus aureus*) is not detected in salted fish flesh and viscera in both of T_2 and T_3. On the other hand, using of $NaNO_2$ led to repress the growth of *Staphylococcus* spp. in Fessekh. It was detected up to the end of second

week of salting storage period and then it was not detected until the end of storage period, from aforementioned data, it could be concluded that the produced Fessekh were in a good hygienic state at consumption time (21 and 28) days for T_2 and T_3, respectively, view point to *Staphylococcus aureus*. These findings are in agreement with Rashad (1986). Produced Fessekh using T_2 method showed that the growth of *Clostridium* spp. continued until five weeks, while in (T_1) the growth of *Clostridium* spp. repressed after 21 days of salting storage period. These results are in agreement with Rashaed (1986) who reported that the more salt concentration the more slowly growth of *Clostridium* spp. On the other hand, produced Fessekh by (T_3) gave a positive test for *Clostridium* spp. throughout 3 and 5 days in both flesh and viscera, respectively, and after that a negative test was obtained until the end of storage period. The extermination of *Clostridium* spp. at the first week of salting period was realized at 6.5% salt concentration and 108 ppm $NaNO_2$ in media. This observed extermination of *Clostridium* spp. firstly backs to the lethal action of $NaNO_2$, which is more than the effect of sodium chloride. These findings were in agreement with Knochel and Huss (1984) and FAO (1992).

This is may be considered a good indicator that using of $NaNO_2$ and salting the fish at the rigor mortis stage (without pre-fermentation) guarantees emptiness such products from toxins which may be formed only in (T_1) during pre-fermentation period.

Results in Table (7) indicated that produced Fessekh by (T_3) showed average score ranged from 8:7.2 within intervals 14:50 days giving a “very good” grade, while after 50 days was “good” until the end of storage period. This best quality was due to the role of sodium nitrite which fix a desire red colour and its bacteriostatic effect, and for the medium salt used. On the other hand, (T_1) occupies the second grade, while the lowest sensory characteristics showed with (T_2) which quickly deteriorate after 35 days and unfit after 80 days of storage period.

In case of (T_1) Fessekh was in a “very good” degree only within interval between 14:35 days, while after that such product was in a “good” degree up to the end of storage period.

On the other hand, produced Fessekh by (T_2) were markedly lower organolepticaly than other products, where it was in a “good” degree up to 28 days of storage period, then it became accepted within the intervals 35-50 days, and after that became unfit for consumption after 80 days of storage period.

Table (7): Sensory assessment during salting and storage period of salted Mullet fish

	Treatments														
	T_1					T_2					T_3				
	Time (days)														
Taste	14	28	35	50	110	14	28	35	50	80	14	28	35	50	110
Texture	8	8	7	6	6	7	7	6	5	5	7	8	8	8	7
Odor	7	8	8	7	7	7	6	6	6	4	8	8	8	7	6
Color	9	8	8	7	6	6	6	5	5	5	8	8	7	7	6
Overall	7	8	7	6	6	6	7	6	6	4	8	9	8	6	6
Quality	7	8	8	7	6	7	6	6	5	5	9	8	8	8	7
Mean	7.6	8.0	7.6	6.6	6.2	6.6	6.4	5.8	5.4	4.6	8.0	8.2	7.8	7.2	6.4

CONCLUSION

It could be concluded that, using of NaNo3 with the mixture of salting (T_3) give the best salted fish free from toxins due to $NaNO_3$ bacteriostatic effect. Moreover, the sensory evaluation also showed that this treatment (T_3) had the best with regard to its acceptability till the end of storage period.

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دراسات كيميائية وطبيعية وميكروبيولوجية على سمك البورى المملح (الفسيخ) بإستخدام نظم تمليح مختلفة .

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تهتم هذه الدراسة بالفسيخ المنتج فى مصر من تمليح أسماك البورى ، حيث يزداد الطلب على الفسيخ سنوياً خاصة فى بعض المناسبات والأعياد المصرية والتى لوحظ مؤخراً بعض المخاطر عند إستهلاكه ، لذلك ففى هذه الدراسة نحاول إنتاج فسيخ آمن وذلك بإستخدام ثلاث أنظمة تمليح مختلفة .

فى المعاملة الأولى أجريت عملية تخمر أولى للأسماك لمدة ٧٢ ساعة وذلك قبل تمليحها بالطريقة الجافة بإستخدام ٢٥% ملح جاف والتخزين على درجة حرارة ٢٠°م كما يتم بالطريقة التقليدية المتبعة فى تمليح الفسيخ فى مصر .

أما فى المعاملة الثانية فإنه تم إجراء التمليح مباشرة دون اللجوء للتخمر الأولى وذلك بإستخدام طريقة التمليح الرطب (٢٠% محلول ملحى) والتخزين على درجة حرارة ٣٠°م .

المعاملة الثالثة عوملت فيها العينات أيضاً بالتمليح المباشر (دون إجراء التخمر الأولى) وذلك بإستخدام كمية من الملح تؤدى إلى تركيز منخفض نسبياً من الملح المعامل بنتريت الصوديوم (١٥% كلوريد صوديوم + ٠,٢% نتريت صوديوم) ثم التخزين على درجة حرارة ٢٠°م . هذا وقد قدرت معدلات إنتشار الملح وقيم الأس الأيدروجينى (pH) وكذلك النشاط المائى (a_w) للعينات المنتجة كما درست أيضاً المشتقات البروتينية مثل NPN و FAN و TVN وكذلك قيم TBA لجميع العينات أثناء فترات التمليح والتخزين كمقياس لتحديد درجات النضج المناسبة .

كذلك فإن مدى آمان العينات صحياً أمكن توقعه بمتابعة تطور العدد الكلى للبكتيريا وكذلك بكتيريا الكوليفورم و الكلوستريديم والأستافيلوكوكس خلال فترات التخزين وحتى زمن الإستهلاك المناسب لعينات كل معاملة – كما أجرى كذلك التقييم الحسى لمختلف العينات .

ويمكن تلخيص أهم النتائج فيما يلى :

إمكانية إنجاز عملية التمليح للأسماك وهى فى مرحلة التيبس دون الحاجة إلى عملية التخمر الأولى (الإنتفاخ) المستخدم فى الطريق التجارية لما لها من خطورة على تلوث الأسماك بالبكتيريا الممرضة .

إن أنسب درجات النضج لإستهلاك الفسيخ الناتج من المعاملات الأولى والثانية والثالثة كان بعد ٥ و ٣ و ٤ أسابيع من التمليح والتخزين ، على الترتيب .

نجاح إستخدام طريفة التمليح الرطب (٢٠% محلول ملحى) والتخزين على درجة حرارة مرتفعة نسبياً (٣٠°م) مع قصر الفترة التخزينية بألاتتجاوز ٣٥ يوم .

إن إجراء التمليح الجاف المنخفض نسبياً بـ ١٥% كلوريد صوديوم والمعامل بـ ٠,٢% نتريت صوديوم أدى إلى الحصول على فسيخ عالى الجودة وآمن صحياً فى نفس الوقت على أن يتم إستهلاكه بعد مضى ٤ أسابيع للوصول إلى درجة النضج المناسبة .

تواجدت ميكروبات *Staphylococcus aurus* فى أحشاء وأنسجة الأسماك المملحة بالمعاملة الأولى فقط لمدة أسبوعين من بداية التمليح . وأظهرت المعاملة الثالثة أعلى معدل أمان بالنسبة لإختفاء *Clostridium* spp و *Staphylococcus* spp بعد ٥ و ١٤ يوم من بداية التمليح ، على الترتيب .

Table (5): Changes in total nitrogen % (TN), non-protein nitrogen (NPN), total volatile nitrogen (TVN), free amino nitrogen (FAN) in mg/100 g sample, and TBA values (mg malonaldehyde/kg sample) in flesh of salted Mullet fish during salting and storage period.

Treatments																		
days Time	T_1						T_2						T_3					
	TN%	NPN mg 100g	NPN/TN%	TVN mg 100g	FAN/mg 100g	TBA*	TN%	NPN mg 100g	NPN/TN %	TVN mg 100g	FAN/mg 100g	TBA*	TN%	NPN mg 100g	NPN/TN %	TVN mg 100g	FAN/mg 100g	TBA*
ZT	3.26	520	15.95	30.7	360	6.58	3.14	410	13.05	23.4	280	5.35	3.14	410	13.05	23.4	280	5.35
1	3.60	460	12.70	35.7	ND	ND	3.46	590	17.05	32.5	ND	ND	3.55	380	10.70	27.8	ND	ND
3	3.74	590	15.78	40.2	ND	ND	3.88	630	16.23	48.8	ND	ND	3.62	520	14.36	33.8	ND	ND
5	4.18	730	17.46	45.6	ND	ND	4.20	740	17.62	56.4	ND	ND	3.84	670	17.45	39.6	ND	ND
7	4.26	820	19.25	48.2	760	11.16	4.15	920	22.16	62.0	840	9.83	3.96	850	21.46	42.6	750	8.25
14	3.72	960	25.80	58.6	810	12.99	3.57	1280	35.85	80.2	1020	11.33	3.53	1040	29.46	65.6	950	9.38
21	3.21	1170	36.44	63.0	940	15.10	3.08	1320	42.85	95.8	1180	13.54	3.38	1260	37.26	80.8	1010	10.46
28	3.28	1222	37.19	81.4	1020	18.46	2.93	1600	54.60	124.8	1280	10.86	2.87	1180	41.11	104.0	1110	14.81
35	3.32	1350	40.19	94.8	1160	22.84	3.41	1370	40.18	140.4	1250	9.58	3.22	1290	40.06	116.2	1240	14.40
50	3.37	1180	35.01	106.6	920	17.48	3.38	1550	45.85	132.2	1330	8.49	3.35	1620	48.35	129.4	1320	9.66
65	3.48	1420	40.80	118.4	1160	13.63	3.05	1280	41.96	152.6	1160	7.19	3.66	1840	50.27	134.2	1360	8.00
80	3.59	1580	44.00	116.8	1150	8.04	2.95	1420	48.13	163.6	1240	6.10	3.80	2240	58.94	128.6	1660	7.58
95	3.65	1750	47.90	128.6	1280	9.25	ND	ND	ND	ND	ND	ND	3.68	2370	64.40	132.4	1570	6.34
110	3.71	1620	43.66	134.4	1230	11.40	ND	ND	ND	ND	ND	ND	3.62	2530	69.88	143.2	1450	8.54

ND = Not determined.