Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/5582
DOI URL: http://dx.doi.org/10.21474/IJAR01/5582

RESEARCH ARTICLE

STUDY OF THE KINETICS OF FORMATION OF BIOGENIC AMINES IN THE SARDINE AND MACKEREL DEPENDING ON THE MICROBIAL FLORA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

ElyounoussiCharifa[1.2], Rachidi Abderrazzak[2] and Bekkali Mohammed[1].

1. Laboratory of Aquatic Ecology and Environment. Research Team "Biodiversity, Pollution and Water Treatment, University Hassan II Faculty of Science Ain Chock, Casablanca, Morocco).
2. ONSSA (Regional Analysis Laboratory and Research, Casablanca - Morocco).

Manuscript Info

Manuscript History

Received: 09 August 2017
Final Accepted: 11 September 2017
Published: October 2017

Key words:-
Food poisoning, Fish ,Enterobacteriaceae, Température Biogenic amine, HPLC.

Abstract

The production of biogenic amines (histamine, putrescine, cadaverine and tyramine) was tested in strains of Enterobacteriaceae (3 *Enterobacter Cloaceae* and 2 *Escherichia Coli*) isolated from sardine (Sardina pilchardus), and (2 *Raoultella Terrigena*, 3 *Citrobacter Braa*kii and 5*Serratia Fonticola*) isolated from mackerel (Scombers combrus), freshwater fish. In general, the bacterial strains have the ability to décarboxylate more amino acids. Biogenic amines have been produced in particular in the exponential phase, with maximal accumulation which occurs between 8 and 32 h, according to the biogenic amine and the microbial species considered. Accumulation amines varied between fish species and in the same species between the bacterial strains, the concentration of putrescine varied from 0 mg / kg A2 in Escherichia coli isolated from sardine, ranging to 1038 mg / kg in *Citrobacter Braakii* A5 with mackerel. The maximum accumulation of cadaverine varied less than that of putrescine and varied from 0 mg / kg in Serratia Fonticola A6 isolated mackerel to 463.73 mg / kg in *Raoultella Terigena* A8 isolated as in mackerel during storage at 20 ° C.

The results also indicate that the highest concentration of histamine in microbial strains studied is (384.92 mg / kg), which is six times more than the legal limit (50 mg / kg) suggested by the FDA (US FDA 2001), and is also much higher than the acceptable level (100 kg / mg) fixed for scombrids fish by the EU (EEC, 1991). And tyramine represents the lowest biogenic amine in treated samples, which is similar to previous results (Kim et al, 2009; Kuley, Özogul and Özogul, 2005).

Introduction:-

Biogenic amines (AB) are compounds in which one, two or three hydrogen atoms of ammonia are replaced by alkyl or aryl groups (Shalaby, 1996; Zhai et al, 2012.). Putrescine and cadaverine have an aliphatic structure while tyramine contains an aromatic structure. Heterocyclic structures are found in histamine (Silla Santos, 1996; Mohamed et al., 2009).They can also be classified into monoamines (phenylethylamine and tyramine), diamines

Corresponding Author:-Elyounoussicharifa.
Address:-Laboratory of Aquatic Ecology and Environment. Research Team "Biodiversity, Pollution and Water Treatment, University Hassan II Faculty of Science Ain Chock, Casablanca, Morocco).

(putrescine and cadaverine) or polyamines (spermine and spermidine) in function of the number of amine groups (Spano et al. 2010). The histamine, tyramine, putrescine and cadaverine, are formed from amino acids namely free histidine, tyrosine, ornithine, and lysine, respectively. Spermidine and spermine from putrescine (Zarei et al., 2011).The presence of biogenic amines in fish is source of concern for researchers, consumers, food companies and health authorities because of their toxicological effects. Biogenic amines were classified as potentially dangerous compounds, which can cause problems for the consumers (Halász et al., 1994; Santos, 1996), including facial-cervical redness, rash, facial swelling , hot flashes, burning sensation in the throat, a taste of pepper in the mouth, itching, tingling of the skin. They are usually followed by type of headache disorders, heart palpitations, dizziness. Secondary symptoms, gastrointestinal, may appear: nausea, stomach pain, vomiting, diarrhea. (McLauchlin et al., 2006; Hungerford, 2010).In general, the incubation period is short, it ranges from minutes to hours. The symptoms usually disappear spontaneously within three hours. Exceptionally, they can last several days in the most severe cases. Biogenic amines are non-volatile compounds which are present in low concentrations in fresh fish but rapidly accumulate in the flesh after alteration by the infection of bacterial flora (Fernandez-Salguero and Mackie, 1987) formed by decarboxylation of amino acids. Although many biogenic amines were found in fish, only histamine, cadaverine and putrescine were found to be significant in the safety of fish and determination of its quality. Despite widely reported an association between histamine and Scombroid food poisoning, histamine alone seems insufficient to cause food toxicity. Putrescine and cadaverine were suggested to potentiate the toxicity of histamine. As regards the other hand deterioration only cadaverine was found to be a useful index of the initial stage of the decomposition of the fish. (Al Bulushi et al., 2009; Rezaei et al., 2007).

In most studies on the formation of biogenic amines in fish, researchers have focused on histamine poisoning and concluded that families Scombridae and Scomberesocideae are commonly involved in cases of histamine poisoning they contain high levels of free histidine in their muscle. Histamine is produced by bacteria which décarboxylate histidine to histamine in the fish under the action of enzymes of bacterial origin (Lehane and Olley, 2000; Taylor, 1986). Indeed, various Scombridae, including mackerel, tuna, bonito and saury, have been implicated in cases of histamine poisoning (Taylor, 1986; McLauchlin et al., 2006). However, non-scombroid fish that also contained high levels of free histidine in the muscle, such as sardines, anchovies, herring and merlin, has also been implicated in cases of poisoning with histamine (Taylor, 1986).

The accumulation of biogenic amines usually results from the decarboxylation of the amino acids by enzymes of bacterial origin, which is associated with hygiene. Therefore, the lack of hygiene is probably the main factor involved in the formation of these compounds. (Halász et al., 1994). Bacterial contamination may be derived from post-harvest contamination board fishing vessels at the treatment plant or in the distribution system.

In freshly caught fish, bacterial contamination is primarily found on the skin and gills from there, these organisms invade the muscle of fish and grow rapidly in response to a number of factors related to processing and storage conditions such as temperature, time, etc. In this case, it is important to identify some bacteria have the amino acid decarboxylase activity in order to estimate the risk of production of biogenic amines in seafood and prevent its accumulation in the product the sea (Ruiz-Capillas and Jiménez-Colmenero, 2010).

Fresh fish can be contaminated by a mixed bacterial population consisting of psychrotrophic bacteria, Gram-negative bacteria such as Enterobacteriaceae and Gram-positive bacteria such as lactic acid bacteria. The type of bacteria present in fish determines the type and amount of biogenic amines formed. Indeed, enterobacteria were often described as producers of high concentrations of biogenic amines in fish. (Ruiz-Capillas and Jiménez-Colmenero, 2010).

The objective of this study was to study the ability of bacteria isolated to produce biogenic amines (histamine, putrescine, cadaverine and tyramine) in fresh fish available for human consumption in Casablanca, Morocco, using chromatography high performance liquid with diode array detection (HPLC-DAD) reference method in accordance with Regulation No 2073/2005 and 11441/2007 that set the health conditions for the production and placing products on the market fishing.

Materials and Methods:-

Sampling procedure:-

The study focused on freshwater fish, sardine (Sardina pilchardus) and mackerel (Scomber scombrus), these fish were collected at different Casablanca outlets. At the time of landing, once collected, the samples were collected

aseptically in a cooler and transported directly to the laboratory. Soon as they arrive at the laboratory, all samples were subjected to a moderate washing followed unbref spin cycle the filter cloth to be washed free of the superficial mucus that would have helped too quickly to their poor appearance and odor.

Bacterial isolates:-

Fish samples were taken from the part of the gills. The Enterobacteriaceae strains were isolated using the agar violet red bile glucose (VRBG) (Oxoid CM485) as described by (Pons-Sanchez et al., 2005). Production of oxidase was tested using oxidase disc (Fluka, 70439 oxidase test) and the production of catalase by suspending the cellular material in 3% hydrogen peroxide. Glucose metabolism was studied by the O / F test. All these tests were carried out for the initial classification of the strains isolated. Then, each of the selected individual bacterial colonies was extended repeatedly on agar plates using sterile loops to produce pure colonies. The isolates were identified according to the instructions of the API 20E reactions produced during the incubation period from 16h to 24 h, result in spontaneous color changes or revealed by the addition of reagents. The results were analyzed using the TM web Api Identification software API (bio Merieux). The identified strains are reported in Table 1.

Table 1:- The isolated bacterial flora of the sardine and mackerel at the Gills

species	microorganisms	The number of bacterial species%
Sardine (Sardinapilchardus)	*Enterobacter Cloaceae* *Escherichia Coli*	3 (19,8%) 2 (13,2%)
Mackerel (Scomberscombrus)	*Raoultella Terrigena* *Citrobacter Braakii* *Serratia Fonticola*	2 (13,2%) 3 (19,8%) 5 (33%)
Total		15

Chemical reagents:-

Histamine dihydrochloride, Fluka, France – Putrescine dihydrochloride, MP Biomedicams, USA – cadaverine dihydrochloride, Sigma Aldrich, Switzerland - Tyramine, Sigma Chemical, USA - Perchloric acid 70% pa Merck, Germany - 1,3 diaminopropane, Sigma Aldrich, Switzerland - sodium carbonate, Sharlau, Spain - Dansyl chloride, Sigma Aldrich, Switzerland - L-Proline, Sigma Aldrich, Switzerland – Acetone HPLC grade, Sharlau, Spain - Toluene HPLC grade, Merck, Germany.

The mobile phase consisted of acetonitrile HPLC grade, Sharlau, Spain and water.

Apparatus and column "HPLC":-

A Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan) equipped with SPD-M20A diode array detector and two binary gradient pump (Shimadzu LC-10AT), autosampler (SIL 20AC), column oven (CTO-20AC), and a bus communication module (CBM-20A) with FCV-11AL valve unit was used. For data analysis, the solution of the LC version 1.11 SP1 program (Shimadzu, Kyoto, Japan) was used. The column used was a reversed phase; Spherisorb 5 Si C18 pH-St, 250 x 4.6 mm column (Phenomenex, Macclesfield, Cheshire, UK).

Chromatographic conditions:-

Column Wacosil C18 RS 5µm, 100 Å (25 cm X 4.6 mm)
Mobile Phase (A: Water / B: acetonitrile). A gradient developed by (Duflos et al., 1999) and obtained by two pumps Shimadzu LC-20 AD.

Table 2 :-gradient of elution (duflos et al., 1999)

Time (min)	Mobile phase	
	Water A%	**Acetonitrile** B%
0	40%	60%
6	25%	75%
8	25%	75%
13	5%	95%
20	5%	95%
20,01	40%	60%
30	40%	60%

This gradient allows gating the biogenic amines with a 1ml / 1min rate. Identification of the peaks is performed using the reference chromatogram (Figure 1: Duflos et al., 1999)

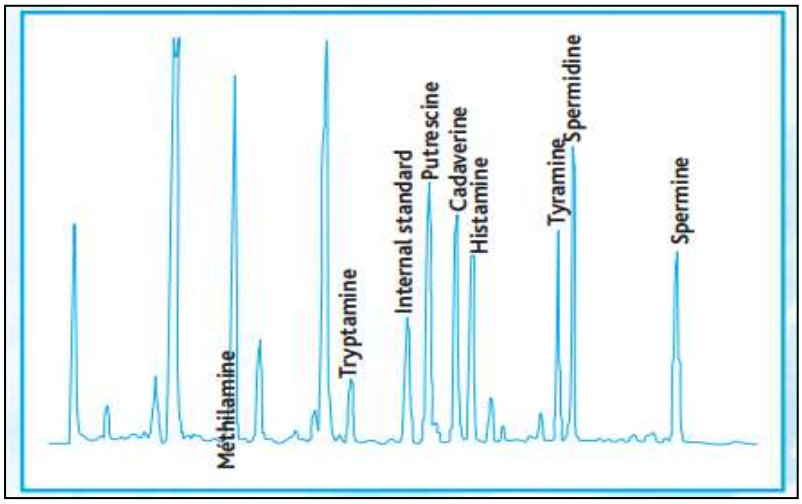

Figure 1:-Chromatogram of reference (Duflos et al., 1999)

The dosage of biogenic amines:-

Sample preparation, mothers solutions and seeding dilutions were performed according to standard NM 08.0.101 (2008), in order to follow the kinetics of formation of biogenic amines: histamine, putrescine, cadaverine and tyramine products by the strains isolated from the gills of sardines and mackerel. The extraction of these amines is done after deproteinization step by perchloric acid 0.2 M are then labeled with dansyl chloride after the L-proline is added to neutralize excess dansyl chloride.

Results:-

The microbial flora of sardines and mackerel is reported in Table 1. The microflora of both species was found belonging to Gram-negative Enterobacteriaceae.

The average concentration of histamine and other biogenic amines produced by bacterial strains isolated from sardines and mackerel were estimated from samples in triplicate.

However, the variation in the ability to produce biogenic amines in different species is extremely wide, and this change was also observed between strains of the same species.

The concentrations of biogenic amines in function of incubation time, in Casablanca fish samples, are shown in Tables 3 and 4 and illustrated in Figures 2, 3, 4, 5, 6, 7, 8 and 9.

From the tables below, it may be noteworthy that the concentrations of histamine and putrescine, encountered in mackerel, are higher than sardines.

The results showed that the maximum concentration of putrescine is 1038 mg / kg produced in *Citrobacter Braakii A5* isolated mackerel. And the minimum concentration was cadaverine is 3.51 mg/kg produced in *Enterobacter Cloaceae A1* isolated from sardines.

As shown in Tables 3 and 4, putrescine was detected in all strains, with values ranging from 4.63 mg / kg to 1038 mg / kg after 32h of incubation.
The highest concentrations of putrescine (1038 mg/kg in *Citrobacter Braakii A5*, 470.02 mg/kg in Raoultella *Terrigena* A8 and 433.27 mg/kg in *Serratia Fonticola*) have been found in the mackerel, and were greater than in the strains isolated from sardine, *Enterobacter Cloaceae* A1 (97.91 mg / kg), *Escherichia Coli* A2 (4.63 mg / kg) and *Escherichia Coli* A3 (8.93 mg / kg).

The detection level of cadaverine was similar to that of putrescine and the maximum concentration of cadaverine was also found to occur in mackerel with a maximum value of 463 mg / kg in *Raoultella terigena* A8. In addition to the high level of cadaverine and putrescine, the histamine was also found to occur in mackerel with a maximum value of 384.92 mg / kg in *Serratia Fonticola* A6. And tyramine represents the lowest biogenic amine in the treated samples (45.96 mg / kg, 39.35 mg / kg, 35.12 mg / kg, 22.65 mg / kg, 4.61 mg / kg, 17.65 mg / kg to 22.85 mg / kg) in *Raoultella Terigena* A8, *Raoultella Terigena* A7, *Serratia Fonticola* A6, *Citrobacter Braakii* A4, *Escherichia Coli* A3, *Escherichia Coli* A2 and *Enterobacter Cloaceae* A1, respectively, after 32heures incubation at 20 ° C with a maximum concentration at 201.9 by *Citrobacter Braakii* A5.

While the production of biogenic amines is a very complex phenomenon, depending on several variables, such as raw materials, processing conditions, micro-organisms of the growth kinetics and their proteolytic activities and decarboxylase, which interact with each Others (Gardini et al., 2001)

Table 3:-Changes in levels of biogenic amines in the bacterial flora of the sardine depending on the incubation time

	Enterobacter Cloaceae A1				*Escherichia Coli A2*				*Escherichia Coli A3*			
Temps	**HIS**	**PUT**	**CAD**	**TYR**	**HIS**	**PUT**	**CAD**	**TYR**	**HIS**	**PUT**	**CAD**	**TYR**
0	48,31	7,59	2,55	22,76	0	0	18,78	0,51	5,31	3,66	4,11	3,62
4	52,24	7,7	3	22,83	0,82	0,65	44,49	0,6	18,02	3,65	4,22	3,62
8	66,95	10,91	3,23	22,76	59,51	0,99	48,19	0,77	18,72	4,12	33,43	3,66
16	76,31	88,36	3,34	23,04	78,24	1,45	122,41	1,19	19,53	4,23	47,52	3,68
20	85,31	90,83	3,49	22,34	162,81	1,77	151,32	2,76	21,21	4,35	61,86	3,72
24	91,51	96,81	2,28	22,35	184,58	2,23	165,23	3,46	24,45	4,49	70,78	3,76
28	96,07	97,45	4,4	22,51	230,56	4,27	183,42	5,4	31,39	8,49	71,47	4
32	99,82	97,91	3,51	22,85	230,07	4,63	190,03	17,65	51	8,93	74,55	4,61

HIS=histamine, PUT=putrescine
CAD=cadaverine, TYR=tyramine

Table 4:-Changes in levels of biogenic amines in the bacterial flora of mackerel function of incubation time

	Citrobacter braakii A4				*Citrobacter braakii A5*				*Serratia fonticola A6*			
Temps	**HIS**	**PUT**	**CAD**	**TYR**	**HIS**	**PUT**	**CAD**	**TYR**	**HIS**	**PUT**	**CAD**	**TYR**
0	53,58	1,88	2,42	0	0,16	2,27	0,21	0,03	106,96	139,7	0	22,74
4	69,39	2,18	2,47	4,27	0,2	25,33	0,45	1,08	137,94	177,99	1,12	24,78
8	92,2	3,38	2,98	4,37	0,27	73,06	1,2	1,75	221,13	213,99	10,74	25,38
16	105,76	14,66	5,64	13,46	3,74	581,1	6,46	39,62	238,32	225,46	23,58	25,75
20	125,01	24,04	5,94	13,72	4,42	796,06	6,58	141,56	271,9	257,71	44,64	25,98
24	126,05	29,75	6,36	17,83	5,34	801,9	7,02	155,55	331,43	272,55	56,15	28,36
28	156,72	34,62	7,45	20,74	23,07	865,67	7,11	165,2	344,36	317,79	81,12	30,34
32	270,89	42,94	7,78	22,65	87,36	1038	7,69	201,9	384,92	433,27	85,35	35,12

	Raoultella terigena A7				*Raoultella terigena A8*			
Temps	**HIS**	**PUT**	**CAD**	**TYR**	**HIS**	**PUT**	**CAD**	**TYR**
0	23,41	7,49	16,34	20,61	7,57	9,18	21,22	0
4	50,16	12,32	23,01	20,33	11,87	12,3	52,11	0
8	78,03	32	46,15	22,27	35,07	259,89	155,34	2,19
16	140,31	77,43	92,57	26,04	49,55	304,94	201,54	2,27
20	167,1	82,72	192,21	27,51	74,79	327, 6	297,06	3,6
24	178,91	93,12	153,39	29,24	103,5	426,35	339,94	3,79
28	200,81	117,11	176	35,06	117,58	459,17	457,56	5,15
32	220,02	126,32	200,01	39,35	145,5	470,02	463,73	45,96

Figure 2 shows a significant increase of histamine and putrescine during 32 hours of incubation at 20 ° C.

The results of Figures 3 and 4 showed that tyramine and putrescine remained at low levels in the 2 strains of *Escherichia Coli* during incubation. However, a significant increase of histamine and cadaverine were observed in these two strains. For example, the concentrations of histamine and cadaverine in *Escherichia Coli* A2 rose sharply to 230, 07 and 190.03 mg / kg for 32 heures incubation instead of 0 and 18.78 mg/ kg at the initial time respectively.

Significant differences were also observed among strains isolated the mackerel and are illustrated in Figures 5, 6, 7, 8 and 9. According to these figures, putrescine becomes the dominant biogenic amine produced by the following strains *Citrobacter Craakii* A1, *Serratia Fonticola* A7 and *Raoultella Terrigena* A2, while histamine is the dominant biogenic amine in other strains such as *Citrobacter Braakii* A6, *Serratia Fonticola* A7 and *Raoultella Terrigena* A8.

Tyramine and cadaverine remained at low levels compared to other biogenic amines in the majority of strains isolated mackerel, except for Raoultella were observed terrigena a significant increase in the cadaverine with a concentration of 200.01 and 463.73 in *Raoultella Terrigena* A7 and *Raoultella Terrigena* A8, respectively after 32 hours of incubation at 20 ° C. Therefore histamine and putrescine are the main biogenic amines in the mackerel.

Results showed that all isolated strains are fish capable of producing at least four biogenic amines (Histamine, Putrescine, cadaverine and tyramine) (Ferencik, M 1970), and biogenic amine content was quite different in samples tested. These results confirm that the tested strains are capable of decarboxylating one or more amino acids, but the amine production differs between species. It depends not only on the species but also of the bacterial strain and environmental conditions (Lehane and Olley, 2000).

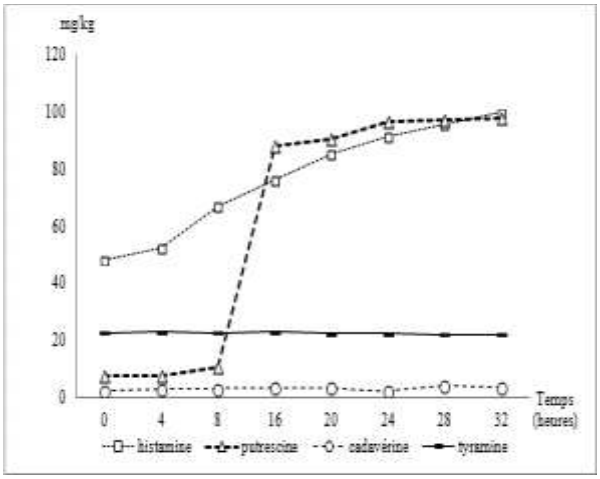

Figure 2:- The evolution of biogenic amine contents in *Enterobacter Cloaceae A4* isolated from sardine during incubation time

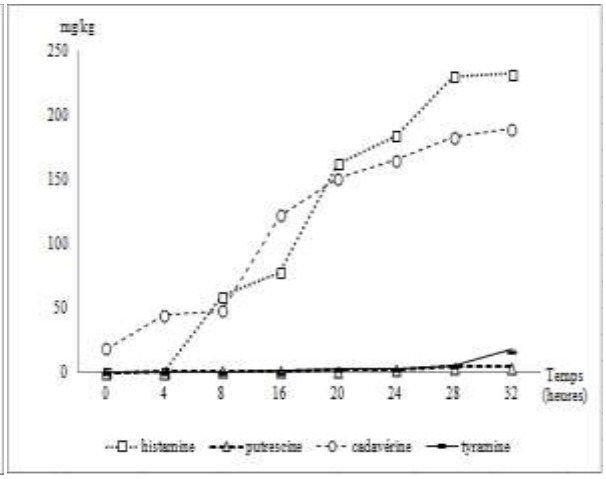

Figure 3:- The evolution of biogenic amine contents in *Escherichia Coli A5* isolated from sardine during incubation time

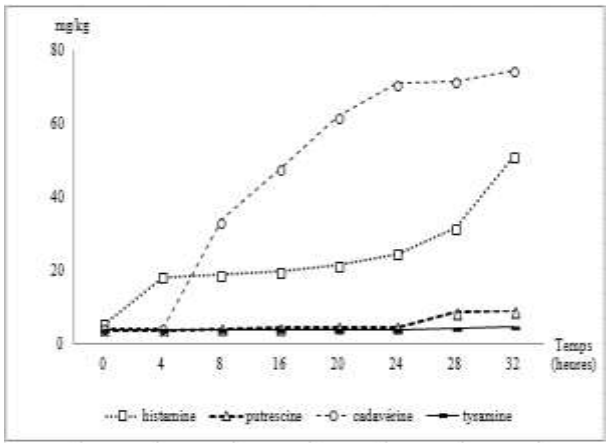

Figure 4 :- The evolution of biogenic amine contents in *Escherichia Coli A3* isolated from sardine during incubation time

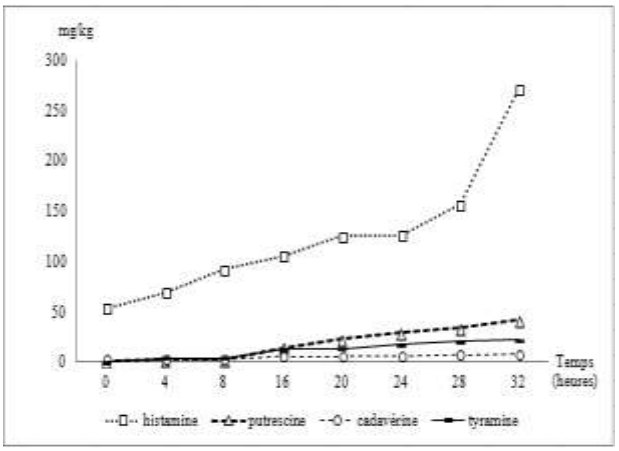

Figure 5 :- The evolution of biogenic amine contents in *Citrobacter braakii* A6 isolated from mackerel during incubation time

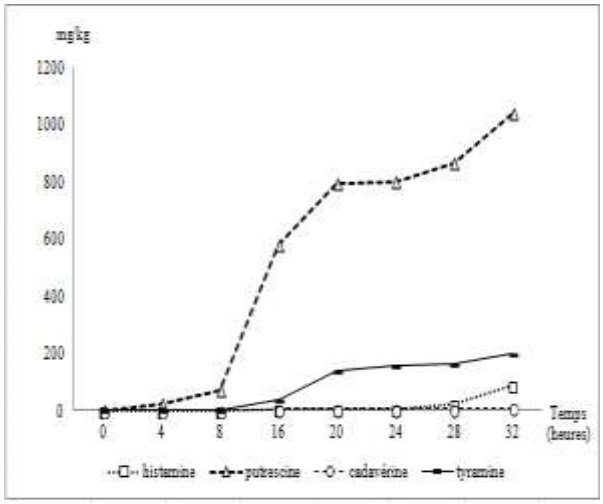

Figure 6 :- The evolution of biogenic amine contents in *Citrobacter braakii* A1 isolated from mackerel during incubation time

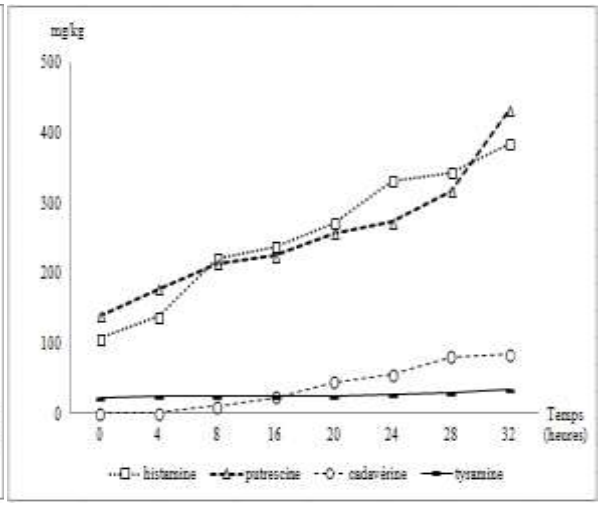

Figure 7 :- The evolution of biogenic amine contents in *Serratia Fonticola* A7 isolated from mackerel during incubation time

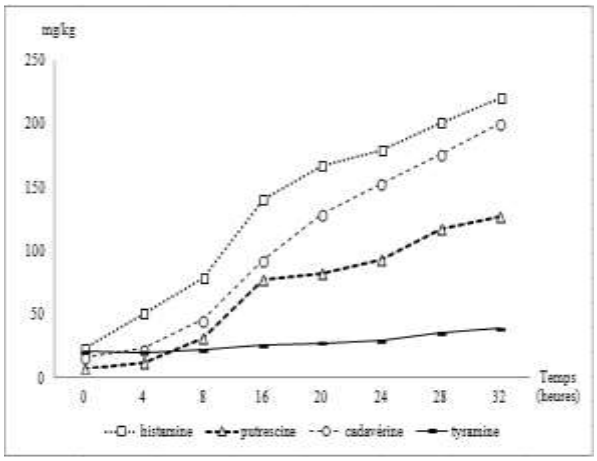

Figure 8:- The evolution of biogenic amine contents in *Raoultella terrigena* A8 isolated from mackerel during incubation time

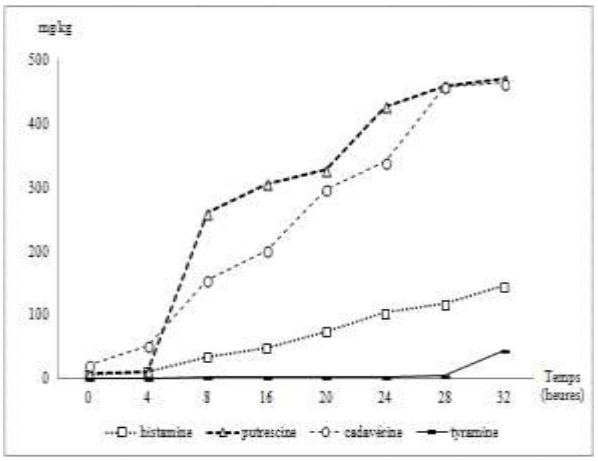

Figure 9:- The evolution of biogenic amine contents in *Raoultella Terrigena* A2 isolated from mackerel during incubation time

Discussion:-

Fish is one of the most perishable foods, mainly due to the action of microorganisms that occur on the surface of freshly caught fish. Determining the microbiological quality at a very important role in maintaining the high quality of fishery products.

A large population of microbial flora were isolated and identified to have the ability to produce biogenic amines, *Morganella morganii, Enterobacter aerogenes* and *Raoultella planticola* (Björnsdóttir-Butler et al., 2009). In this study, strains were isolated and identified, which is similar to previous findings, such as *Escherichia coli*, *Citrobacter braakii*, *Serratia fonticola*, *Raoultella terigena* and *Enterobacter cloaceae*, the microbial flora was found Gram-negative Enterobacteriaceae of the genus has Using the API 20 E galleries (Table 1).

The biogenic amine content in Casablanca fish samples are shown in (Table 2 and 3). The result showed that the fish contained at least four biogenic amines and biogenic amine content was quite different depending on the species. It depends not only on the species but also of the bacterial strain (Lehane and Olley, 2000).

Putrescine and cadaverine has no direct toxicological effects on human health. However, it has been proved that the presence of putrescine and cadaverine in aquatic products can potentially promote the toxicological effects of histamine and tyramine, inhibiting histamine metabolizing enzymes, such as monoamine or diamine oxidase and histamine methyl transferase (Smith, 1980; Stratton et al., 1991). In addition, the appearance of putrescine and cadaverine in food can react with nitrite to form heterocyclic carcinogenic nitrosamines, which are among the most important human carcinogens (Park et al, 2010.Santos, 1996).

In this study, the maximum concentrations of Putrescine and cadaverine were observed in mackerel, with 1038 values and 463 mg / kg, which were much higher than 4.63 and 3.51 mg / kg, respectively, in the sardine.

The largest quantities of histamine presented earlier in fish species were 1270 mg / kg in mackerel (Shalaby, 1996) and 399 mg / kg in amberjack (Auerswald et al., 2006). Note that in this study, however, the species of fish contained much lower levels of histamine that previous reports (Shalaby, 1996) and similar to the reports (Auerswald et al., 2006) with a maximum value of (384.92 mg / kg) in mackerel, as presented in Table 4, that is a level six times greater than the legal limit (50 mg / kg) suggested by the Food and Drug Administration for scombrids and related products (US FDA, 2001), and is also much higher than the acceptable level (100 kg / mg) fixed for scombrids fish by the EU (EEC, 1991). Knowing that levels of histamine are regulated differently in different countries, for example, 100 mg / kg in South Africa and Italy, or 200 mg / kg in Australia and Germany (Auerswald et al., 2006; Lange et al., 2002).

It is essential to pay attention to tyramine in aquatic products because of its toxic effects on the human body, the concentrations of tyramine in all studied samples were much lower than the suggested limit of 100 mg / kg (Santos, 1996; Ten Brink et al., 1990). Low levels of tyramine were also observed in the octopus and squid (Kim et al., 2009), sardines and sea bream (Sánchez and Ruiz-Capillas, 2012), and tuna (Veciana-Nogués et al. 2004). However, high levels of tyramine, ranging from 101 to 222 mg / kg have been reported in the Spanish mackerel and amberjack (Kim et al., 2009).

Low levels of tyramine were found in almost every sample studied, which is similar to previous reports for fish, such as mackerel, sardines and sea bream (Kim et al., 2009; Kuley et al., 2005) , ranging from 4.61 mg / kg to 45.96 mg / kg except for one strain of Citrobacter mackerel braakii A5, with a maximum concentration of 201.9 mg / kg, which is identical with the preceding results (Kim and al, 2009), which was a higher concentration than the standard (100 mg / kg) to human health suggested by (Brink et al., 1990;. Santos, 1996). However, this high level of tyramine in fresh fish was rarely recorded.

The formation of biogenic amines in fish depends on various factors such as the contents of free amino acids gradually increase with extended storage times due to proteolysis by both endogenous and exogenous proteases (Makarios-Laham and Lee, 1993) and the presence of bacteria producing decarboxylases. Thus, biogenic amines can be formed by microbial decarboxylation of free amino acids. However, levels of biogenic amines formed can be heavily influenced by the storage conditions.

In the present study significant increases ($p < 0.05$) of the biogenic amines histamine, putrescine and cadaverine (as shown in the figures) are observed in all samples during incubation at 20 ° C for 32heures. The results show that significant increases ($p < 0.05$) putrescine and cadaverine were held in all strains studied after incubation, and these two amines become the dominant biogenic amines in these aquatic products. Similar changes in putrescine and cadaverine were observed in slices of barramundi (Bakar et al., 2010) and Spanish mackerel (Middlebrooks et al., 1988).

The results show that the biogenic amines, including putrescine, cadaverine, histamine and tyramine are found to be strongly correlated with the presence of enteriobacteria in the fish.

Conclusion:-

The bacterial strains (Enterobacteriaceae) isolated from fish (sardine (Sardinapilchardus) and mackerel (Scomberscombrus)) constitute a group quite homogeneous with the ability to produce histamine, cadaverine, putrescine and tyramine.
The production and accumulation of biogenic amines has been registered in the exponential phase between 8 to 32 h. However, the maximum production appears to be variable and depends on the species of fish, bacterial strains of the same species and the amine considered.

However, several samples of fish showed relatively high levels of histamine (up to 384.92 mg / kg), putrescine (up to 1038 mg / kg), cadaverine (up to 457.56 mg / kg) and tyramine (up to 201.9 mg / kg). In addition, we found that the concentrations of biogenic amines can greatly increase during storage at temperatures above 4 ° C. Therefore, it must raise safety concerns, given the high levels not only for the production of histamine but also to putrescine, cadaverine, and tyramine in the species of fish.

References:-

1. Al Bulushi, I., Poole, S., Deeth, H. C., & Dykes, G. A. (2009). Biogenic amines in fish: roles in intoxication, spoilage, and nitrosamine formation. A review. Critical Reviews in Food Science and Nutrition, 49, 369-377.
2. Auerswald, L., Morrom, C., &Lopata, A. L. (2006). Histamine levels in seventeen species of fresh and processed South African seafood. Food Chemistry, 98, 231–239.
3. Bakar, J., Yassoralipour, A., Bakar, F. A., &Rahman, R. A. (2010). Biogenic amine changes in barramundi (Latescalcarifer) slices stored at 0_C and 4_C. Food Chemistry, 119(2), 467–470.
4. Björnsdóttir-Butler, K., Bolton, G. E., Jaykus, L. A., McClellan-Green, P. D., & Green, D. P. (2010). Development of molecular-based methods for determination of high histamine producing bacteria in fish. International Journal of Food Microbiology, 139, 161e167.
5. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.
6. Duflos, G., Dervin, C., Malle, P., Bouquelet, S. 1999. Relevance of matrix effect in determination of biogenic amines in plaice (Pleuronectesplatessa) and whiting (Merlangusmerlangus). J AOAC International 82: 1097–1101.
7. EEC (1991). Council directive 91/493/EEC of 22 July 1991 laying down the health conditions for the production and the placing on the market of fishery products. Official Journal of European Communities (NrL268), 15–32.
8. Ferencik M (1970) J Hygiene EpidemiolMicrobiolImmunol 14(1):52–60
9. Fernandez-Salguero, J., &Mackie, I. M. (1987). Comparative rates of spoilage of fillets whole fish during storage of haddock (Melanogrammusaeglefinus) and herring (Clupeaharengus) as determined by the formation of non-volatile and volatile amines. International Journal of Food Science and Technology, 22, 385–390.
10. Gardini F, Martuscelli M, Caruso M C, Galgano F, Crudele M A, Favati F, Guerzoni M E, Suzzi G. Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of Enterococcus faecalis. International Journal of Food Microbiology 2001; 64:105– 117.
11. Halász, A., Baráth, A., Simon-Sarkadi, L., &Holzapfel,W. (1994). Biogenic amines and their production by microorganisms in food. Trends in Food Science and Technology, 5, 42-49.
12. Hungerford, J. M. 2010. Scombroid poisoning: A review. Toxicon, 56(2), 231-243.
13. Kim, M. K., Mah, J. H., & Hwang, H. J. (2009). Biogenic amine formation and bacterial contribution in fish, squid and shellfish. Food Chemistry, 116(1), 87–95.
14. Kuley, E., Özogul, F., &Özogul, Y. (2005). Effects of aluminium foil and cling film on biogenic amines and nucleotide degradation products in gutted sea bream stored at 2 ± 1 _C. European Food Research and Technology, 221(5), 582–591.

15. Lange, J., K. Thomas, and C. Wittmann, 2002. Comparison of a capillary electrophoresis method with high-performance liquid chromatography for the determination of biogenic amines in various food samples. J. Chromatogr. B., 779: 229–239.
16. Lehane L, Olley J (2000). Histamine fish poisoning revisited. Int. J. Food Microbiol. 58:1-37.
17. Malle, P., Valle,M., Bouquelet, S. 1996. Assay of biogenic amines involved in fish decomposition. J. AOAC International 79: 43–49.
18. Makarios-Laham, I. K., & Lee, T. C. (1993). Protein hydrolysis and quality deterioration of refrigerated and frozen seafood due to obligately psychrophilic bacteria. Journal of Food Science, 58(2), 310–313.
19. McLauchlin, J., Little, C. L., Grant, K. A. and Mithani, V. 2006. Scombrotoxic fish poisoning. Journal of Public Health 28(1): 61–62.
20. Middlebrooks, B. L., Toom, P. M., Douglas, W. L., Harrison, R. E., & McDowell, S. (1988). Effects of storage time and temperature on the microfloras and amine development in Spanish mackerel (Scomberomorusmaculatus). Journal of Food Science, 53(4), 1024–1029.
21. Mohamed R., Livia S. S., Hassan S., Soher E. S., Ahmed-Adel E. B. (2009). Changes in free amino acids and biogenic amines of Egyptian salted-fermented fish (Feseekh) during ripening and storage. Food Chem. 115, 635–63810.1016/j.foodchem.2008.12.077
22. Park, J. S., Lee, C. H., Kwon, E. Y., Lee, H. J., Kim, J. Y., & Kim, S. H. (2010). Monitoring the contents of biogenic amines in fish and fish products consumed in Korea. Food Control, 21, 1219e1226.
23. Pons-Sanchez CS, Vidal-Carou MC, Marine-Font A, Veciana-Nogues MT (2005). Influence of the freshness grade of raw fish on the formation of volatile and biogenic amines during the manufacture and storage of vinegar-marinated anchovies. J. Agric. Food Chem. 53:8586-8592
24. Prester, L., Macan, J., Varnai, V. M., Orct, T., Vukusic, J., &Kipcic, D. (2009). Endotoxin and biogenic amine levels in Atlantic mackerel (Scomberscombrus), sardine (Sardinapilchardus) and Mediterranean hake (Merlucciusmerluccius) stored at 22)°C. Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment, 26(3), 355–362.
25. Rezaei, M., Jafari, H., Sahari, M. A., Hosseini, H., Montazeri, N., Parviz M. and Nazarinia, A. 2007. Relation of biogenic amines and bacterial changes in ice-stored southern caspiankutum (*Rutilusfrisiikutum*). Journal of Food Biochemistry 31(4): 541– 550.
26. Ruiz-Capillas C, Jiménez-Colmenero F (2010). Biogenic Amines in Seafood Products. In: Nollet LML, Toldora F (eds) Handbook of Seafood and Seafood Products Analysis. New York: Taylor & Francis Group, CRC Press. pp. 833-850.
27. Sánchez, J. A., & Ruiz-Capillas, C. (2012). Application of the simplex method foroptimization of chromatographic analysis of biogenic amines in fish. EuropeanFood Research and Technology, 234(2), 285–294.
28. Shalaby, A.R. 1996. Significance of biogenic amines to food safety and human health. Food Research International 29(7): 675–690.
29. Silla Santos M H. Biogenic amines. Their importance in foods. International Journal of Food Microbiology 1996; 29: 213–231. Silla, M. H. (1996). Biogenic amines: their importance in foods. International Journal of Food Microbiology, 29, 213-231.
30. Smith, T. A. (1980). Amines in food. Food Chemistry, 6, 169–200.
31. Spano, G., Russo, P., Lonvaud-Funel, A., Lucas, P., Alexandre, H. and Grandvalet, C. 2010. Biogenic amines in fermented foods. European Journal of Clinical Nutrition 64(S3): S95-S100.
32. Stratton, J. E., Hutkins, R. W., and Taylor, S. L. (1991). Biogenic amines in cheese and other fermented foods: A review. Journal of Food Protection, 54, 460–470.
33. Taylor, S. L. (1986). Histamine food poisoning: toxicity and clinical aspects. Critical Reviews in Toxicology, 17, 91-128.
34. Ten Brink, B., Damink, C., Joosten, H. M. L. J. and Huisin 't Veld, J. H. J. (1990). Occurrence and formation of biologically active amines in foods. International Journal of Food Microbiology 11(1): 73-84.
35. US FDA (US Food and Drug Administration) (2001). Scombrotoxin (histamine) formation. In Fish and fishery products hazards and controls guide, 3rd ed. (pp. 73-93). Washington, DC, USA: Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood.
36. Veciana-Nogués, M. T., Bover-Cid, S., Mariné-Font, A., & Vidal-Carou, M. C. (2004).Biogenic amine production by Morganellamorganii and Klebsiellaoxytocaintuna. European Food Research and Technology, 218(3), 284–288.
37. Zarei M., Najafzadeh H., Enayati A., Pashmforoush M. (2011). Biogenic amines content of canned tuna fish marketed in Iran. American-Eurasian J. Toxicol. Sci. 3, 190–193.
38. Zhai, H., X. Yang, L. Li, G. Xia, J. Cen, H. Huang, and S. Hao, 2012. Biogenic amines in commercial fish and fish products sold in southern china. Food. Control., 25:303–308.