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ASSESSMENT OF HISTAMINE IN FISH AND FISH PRODUCTS THIN LAYER CHROMATOGRAPHIC METHODS

(With One Table)

By

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تقدير للهستامين في الأسماك ومصنعات الاسماك باستخدام التحليل الكروماتوجرافى بالطبقه الرقيقه

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يعتبر تواجد مادة الهيستامين فى الأسماك ومصنعاتها من دلائل بدء حدوث الفساد الغير ظاهر والناتج عن النشاط البكتيرى الذى يؤدى الى انخفاض درجة الجوده .

فى تلك الدراسه تم اجراء التقدير النوعى والكمى لمادة الهيستامين فى عدد ٢٠٠ عينه عشوائيه تم جمعها من الأسماك ومصنعاتها التى كانت متداوله فى الأسواق ، وقد أظهرت نتائج التقدير الكمى للهيستامين أن جميع عينات التجربه قد تجاوزت الحد المسموح به طبقاً للمواصفات القياسيه المصريه .

وقد تم التوصل لتلك النتيجه بعد اجراء دراسة مقارنه باستخدام نظامين للفصل الكروماتوجرافى ، النظام الأول مكون من (بيوتانول:ن - حمض الخليك - الماء) مع تعريض الألواح للأبخره المتصاعده من اليود المعدنى للاظهار وكانت نتائجه أفضل من النظام الثانى المكـــون من (أسيتون - ايدروكسيد الامونيا).

لذلك تم استخدام النظام الأول فى التقدير النوعى والكمى لمادة الهيستامين فى عينات التجربه .

أوضح التحليل الاحصائى وجود فروق معنويه بين النتائج التى تم التوصل اليها . وقد تمت مناقشة سمية مادة الهيستامين وأهميتها للصحه العامه وفى مجال صحة الأغذيه .

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SUMMARY

Higher histamine levels had been recorded in 200 samples of fish and fish products, the achived results proved the microbial spoilage of all the samples which graded as inferior quality. Comparative study of two systms for detection of histamine in fish and fish products was applied. The n-butanol-acetic acid-water system was found to be superior to acetone-ammonium hydroxide system. It was used for quantitave determination of histamine in 200 collected samples of fishes and fish products. The method is simple, rapid and sensitive enough to be used for routine screening in determining which fish samples may contain unsual histamine levels. This method can be used for histamine level as law as 1.658 mg % in the standard solution. The mean values of histamine levels in naturally spoiled fish and fish products were recorded. By statistical analysis significant difference was found in between the examined types of fish and fish products. The hygienic and toxicological significance of histamine were discussed.

Keywords: Comparative, assessment, histamine, fish, fish products, Suez Canal locality, TLC

INTRODUCTION

The assessment of histamine in foods is interesting from both the hygienic and toxicological viewpoint. *(VIDAL-CAROU et al. 1990).* Histamine is a pharmacologically important compound which is associated with the development of allergic reactions; including some forms of food poisoning.

The hygienic significance of histamine in fish, especially scombroid fish which had undergone some microbial decomposition, had been discussed by several authors *(IENISTEA, 1973; SCHUTZ et al., 1976; FOO, 1977; TAYLOR et al., 1978; PEARSON & DUTSON, 1986 and VIDAL-CAROU et al., 1990).*

Histamine is formed in food by the bacterial decarboxylation of histidine through the action of histidine-decarboxylase enzyme, such histamine is a normal constituent of food and food products exposed to microbial degradation, *(TAYLOR et al., 1978).*

Mossel, (1977) recorded that amino acid decarboxylation potentialities encountered among several species of bacteria including *Bacillus cereus, Colstridium Perfringens;*

Enterobacter aerogenes; Ent. cloacae; Escherichia coli; Proteus morganii, P. vulgaris; Pseudomonas aeruginosa; Ps. reptilivora; Serratia marcescens; Streptococcus faecalis, Str. faecium, Citrobacter spp.; Ristela spp.; Salmonella spp. and Shigella spp. *Proteus* morganii is the most active species in producing histamine poisoning *(ICMSF, 1978a).*

Histamine is either N-methylated in the liver to form 3-methyl histamine for urinary excretion or deaminated by histaminase and pyrdoxal phosphate in plasma and kidney to give B-imidazoleacetaldehyde and ammonia. Some undegraded histamine is excreted in the urine as N-acetyl and as N-methyl derivatives; the latter is the major metabolite of histamine in humans *(SMITH & HILL, 1983 and DAS, 1984).*

Scombroid poisoning results from the consumption of fish which have undergone some microbial decomposition; frequently without overt signs of spoilage. The analytical methods now in use for detecting histamine in fish products are time consuming and/or costly *(CODE & MELNTIRE; 1954 and SHORE; 1971).* Such work was planned for the monitoring histamine in fish and fish products which were sold as inferior grade at fish market in Suez Canal locality using a convenient and sensitive enough method; to be used in the routine analysis of large numbers of samples.

MATERIAL AND METHODS

200 samples of fish and fish products were collected at random; 20 each of frozen mackerel (Scomber scombrus); frozen palamita (Sarda spp.); Sardine (small Sardina pilchardus); Mulle spp. (Mullus surmuletus); Solea (Solea solea); boltti (Tilapia nilotica); salted fish "fesiekh" (Mugil spp); smoked fish "aringa" (Clupea harengus); canned fish "pilchard" (Sardina pilchardus) and canned tuna (Thunnus spp.) after *WATERMAN, (1982).* The samples were bought from the fishmongers who exhibit inferior quality fish and fish products. The samples were directly transferred to the laboratory in an ice box container. The techinques recommended by *A.O.A.C., (1975); SCHUTZ* et al. *(1976); VOIGET* et al. *(1977) and HUI and TAYLOR (1983)* were applied.

1- Samples preparation:

1.1 Fish Flesh:

100 grams of muscles free from skin were taken from each individual sample and triturated and homogenized with 100ml. of distilled water (Homogenizer, Type MPW-302, Poland); the completely clear filtrate was taken to be used for TLC.

1.2 Plichard packed in brine:

The juice had been letted to ran from the opened can, the lid pressed into fish with moderate pressure. Aqueous and oil phases were separated using a separatory funnel. The used aqueous phase must be clear.

1.3 Tuna packed in oil:

The afore-mentioned technique was applied.

2- Apparatus and reagents

2.1 Thin layer chromatographic plates

20cm X 20cm, pre-coated with 0.1mm silica gel (ADWIC-Prolabo).

2.2 Devloping solvent systems for TLC

2.2.1. n-Butanol-Acetic acid-water (4-1-5) "upper layer". The chromatograms were viewed on day light when sprayed with Pauly's reagent or exposed to vapour of metallic iodine.

2.2.2. Acetone-ammonium hydroxide system (28 wt% ammonia) (95+5) with Ninhydrin aerosol.

2.3. Histamine standard solution 165.8mg histamine dihydrochloride (Sigma) were dissolved in 100.0ml distilled water.

2.4. Pippets: Disposable, capillary, 5ul capacity.

2.5. Pauly's spary reagent, which immediately before use, combine with aqueous 12% (W/V) Na_2CO_3, (2+5 V/V). The reagent solution lightly sprayed onto plates *(HAIS & MACEK, 1963).*

2.6. Ninhydrin reagent, was freshly prepared by dissolving 0.20g ninhydrin (BDH) in 100ml Cadmium stock solution *(FOO, 1977).*

3- Quantitative assessment of histamine:

The spot's area of histamine on silica gell layer was scraped in a test tubes containing 8ml. methanol, then silica gell was removed by filtration.

The optical density of colour of the spot was determined spectrophotometrically (Spokel, Jenoptik, Carlzeiss, Jena) at wave length 570nm. The concentrations of the histamine were calculated as mg/100 grams sample using a previously prepared standard curve *(HUI & TAYLOR, 1983).*

RESULTS

Are presented in Table 1

DISCUSSION

It was evident from the present investigation that histamine was detected in naturally spoiled samples of fish and fish products. As the migration distances of histamine on TLC are generally fixed, histamine elutes with an Rf value of 0.54 in the n-butanol-acetic acid-water system "upper layer" was described. It rapidly produces permanent orange spots when sparyed with Pauly's reagent or clear reddish-brown spots if exposed to the vapour of metallic iodine.

The acetone-ammonium hydroxide system induced faint purple spots when sprayed with ninhydrin. It recorded Rf 0.52. Furthermore, the elution properties of such system changes slowly with time with a resultant decrease in the Rf of histamine. Thus Rf of histamine decreased from 0.52 to 0.21. This change may refer to volatilization of ammonia from aged solution as the process of histamine determination were performed on successive days but with the same reagents and apparatus.

Other visible fish components had very low mobility (Rf < 0.1) with n-butanol-acetic acid-water system were clearly separated from histamine and easily seen.

The results obtained were in agreement with *SCUTZ* et al. *(1976)* who recommended TLC method for histamine; the recorded Rf value was 0.54 decreased to 0.33 as the eluant became aged by time.

In the present study, histamine level as law as 1.658mg% in histamine standard solution was readily detected by n-butanol-acetic acid-water system "upper layer" and the chromatograms were viwed on day light after exposure to the vapours of metallic iodine. Table (1) summarized the results obtained about the assessment of histamine content in 200 samples of fish and fish products. By analysis of variance it was verified that there were significant statistical differences (P=0.01) in values of histamine content of the different type of fish. There was significant differences between salted fish (fesiekh), sardine, mullus and tuna in comparison with other types of fish. There is also significant differences between frozen mackerel, canned pilchard, smoked fish "aringa" and frozen palamita "bonito" with boltti and solea.

The recorded levels of histamine were higher than the level pointed out by *EGYPTIAN STANDARDS (1989, 1990 a,b)* which

recommended histamine level 10mg% not more. *ICMSF (1978)* reported that, spoiled scombroid fish was toxic to humans at histamine level 100mg% or more, although an outbreak strongly suspected to be scombroid poisoning, no histamine was detected in the uneaten remnants of the incriminated food.

As compared with obtained data lower results were registered by *SCHUTZ* et al. *(1976); FOO (1977) and VIDAL-CAROU* et al. *(1990)*; they recorded histamine levels in naturally spoiled fish ranged from 22mg/kg. up to 200mg/kg.

Concerning canned fish-products and the smoked fish, the mean values of histamine levels were 26.20 ± 0.45mg%, 23.80 ± 0.43mg% and 23.50 ± 0.32mg% in each of canned tuna, canned pilchard and smoked fish "aringa" respectively. *FOO (1976)* stated that the histamine is very thermostable, such record illustrated it's occurrence in canned fish and other heated fishery products. Furthermore, *ICMSF (1978 a)* added that the toxic canned tuna undoubtedly had became toxic prior to canning, probably as a result of inadequate refrigration on the fishing vessels.

As regards the public health viewpoint; *MOSSEL (1977)* assured that the food-borne pressor amine (histamine) syndrome is much complicated by the fact that individual sensitivty to orally administered histamine varies widely. He added, this in turn, seems to be due to great variations in the levels of monoamine oxidase enzymes occurrung in the gut in different persons, moreover in the same person under varying conditions. Such enzyme inactivates histamine in the intestinal tract. On the other hand, *ICMSF (1978 b)* stated that histamine has a physiological effect when introduced parenterally in small amount, but large doses of ingested histamine may not, because they were detoxified rapidly in the gastrointestinal tract. Possibly the detoxifying mechanism is inactivated by some material co-produced with histamine during microbial activity on scombroid fish.

VIDAL- CAROU et al. *(1990)* recorded that, histamine content in fish had been used as an index of microbial spoilage, low quality of a product or as an indicator of defective processing. In addition, high levels of this amine in foods can cause toxicological effects "histamine poisoning" and other indirectly related problems such as interaction with monoamine oxidase inhibiting drugs.

However, individuals and agencies concerned safety of seafoods should be aware of the potential hazard of histamine, moreover the maximum tolerable histamine content for certain foods- especially fish and marine products must be implemented besides the legal regulations.

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Table (1): Histamine levels estimated in mg % in naturally spoiled fish and fish products.

Samples	Histamine content mg/100g sample			
	Min.	Max.	$\bar{X}$	SE.
Fish products				
Salted fish "fesiekh"	36	43	39.20	±0.46
Tuna packed in oil	33	30	26.20	±0.45
Pilchard packed in brine	19	26	23.80	±0.43
Smoked fish "aringa"	21	25	23.50	± 0.32
Fish				
Sardien	27	39	33.45	±0.75
Mullus	24	36	30.35	±0.65
Frozen Mackerel	20	29	24.15	±0.65
Frozen Palamita "bonito"	19	25	22.05	± 0.4
Solea	16	20	18.10	±0.35
Boltti	13	23	17.05	± 0.6

Least significant difference (L.S.D) = 2.4