A STUDY OF THE EFFECTS OF HANDLING, PROCESSING AND STORAGE ON THE HISTAMINE CONTENT IN SALTED, FERMENTED TELAPIA *(Oreochromis nilotica)*

BY

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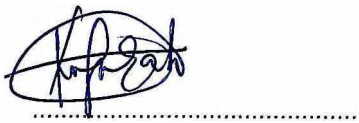
A THESIS
SUBMITTED TO THE DEPARTMENT OF NUTRITION AND FOOD SCIENCE,
UNIVERSITY OF GHANA, LEGON,

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF AN
M.Phil. DEGREE IN FOOD SCIENCE

DECEMBER, 1999

DECLARATION

I, Kingsley Ekow Gurah-Sey declare that this work was done by me under the supervision of Prof. G. S. Ayemor



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DEDICATION

To the glory of God and all the victims of Scombroid poisoning!

ABSTRACT

Salted and dried Tilapia, *Koobi,* obtained from retail outlets and that processed in the laboratory (under various treatments) were analyzed for their histamine content. These were done with a view to establishing baseline data on histamine levels in *Koobi* as well as investigate the effect of handling, processing and storage on histamine evolution in *Koobi.* Sodium chloride levels, moisture contents, water activity and microbial counts were also determined for the *Koobi* samples.

The histamine levels in salted and dried Tilapia, *Koobi* were generally found to be low. *Koobi* samples procured from retail outlets had histamine levels in the range of 7.06|ig/g to 32.15jag/g. Salting seemed to check histamine production in Koobi. The high salt content (above 15 percent NaCl) did not favour microbial growth and may hamper autolysis. Consequently histamine production through microbial activity as well as autolysis was reduced and microbial counts were low (2.54×10^3- 1.28×10^6). Salting and drying reduced the water activity of the *Koobi* which in turn might have inhibited microbial decarboxylase activity. Conversion of histidine to histamine appeared inhibited. Unsalted Tilapia, however, produced elevated levels of histamine

Temperature also did play a role in histamine production. *Koobi* samples kept in the freezer did not differ significantly from those kept in the refrigerator in terms of histamine production. The *Koobi* samples dried in a solar dryer and at ambient temperatures also did not show significant differences in their histamine content; but differed significantly from those kept under cold temperature (4°C and below). Histamine levels were very low in fresh tilapia indicating that

elevated levels of histamine observed in the processed Tilapia *(Koobi)* could be attributed to improper handling, processing and storage. Indeed Tilapia iced immediately after catch showed lower levels of histamine (11,49ug/g) than those in which icing was delayed for 24 hours(33.17 ug/g). This confirms the importance of proper handling of Tilapia as a measure to reduce histamine levels in *Koobi.*

ACKNOWLEDGEMENT

I express my profound gratitude to Prof. G.S. Ayemor, my supervisor, for his expert guidance. Indeed a man of infinite knowledge and a paragon of excellence.

My special thanks also go to Mr. Addokwei Allotey of the Food Research Institute for the immense contribution he made towards the completion of this work. I wish also to thank Messrs Edward Quansah and Stephen Nyamekye both of the Department of Nutrition and Food Science for the various assistance they gave me.

I am greatly indebted to Mrs. Kafui Kpodo of the Food Research Institute for allowing me to use the Institute's laboratory even when she had some laboratory work to do herself. You are a blessing!

I want to thank Dr. Wisdom Kofi Amoa-Awua of the Food Research Institute (FRI) for his invaluable contribution to the development of HACCP in *Koobi* processing.

Finally, I thank all my colleagues for the support they gave me.

To all those who contributed in diverse ways to the completion of this work but whose names could not be mentioned, I say a big thank you.

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1.0 INTRODUCTION

1.1 IMPORTANCE OF SALTED AND FERMENTED FISH

Salted fermented fish plays an important role in the diets of most people in Africa, Asia and other parts of the world. They are mostly used as condiments in meals where they impart a characteristic flavour and help give a palatable meal. In certain under-previleged areas, salted and fermented fish is a major source of dietary protein.

Salted and fermented Tilapia locally called *koobi* is used extensively in Ghanaian diets and is such a delicacy.

1.2 HANDLING, PROCESSING AND STORAGE OF FRESH FISH

It has been reported by various authors (Dibbs, 1961; Kagan 1970; Amu 1973; Bonsu, 1976) that fresh fish is not well handled and stored in Ghana. The environment in which artisanal fishermen cure fish is generally unhygienic paving the way for microbial contaminations and production of food toxicants such as histamine.

1.3 OCCURENCE OF HISTAMINE IN FOODS

Histamine (above lOOmg/lOOg) is a food toxicant and belongs to a group of chemical compounds known as biogenic amines. The histamine content of various foods has been widely studied because of their potential toxicity. Histamine has been observed in a variety of foods such as fish, meat, chocolate, cheese, wine, soy sauce, sauerkraut, beer and other fermented foods.

Scombroid fish(tuna, mackerel, sardine) is the most prolific source of his-

famine production. High levels of histamine in food is of grave public health concern due to the health hazards involved with ingestion of such compounds (Halasz *et al.,* 1994). Histamine is generally low in fresh foods and is often produced in high amounts during spoilage of food. Food in fresh state can thus be considered as low-risk while food in various stages of deterioration can be labelled as high-risk in terms of histamine poisoning. Histamine poisoning is a chemical intoxication resulting from the ingestion of high amounts of histamine. Histamine has been identified as the causative agent in several food poisoning outbreaks.

1.4 SYMPTOMS OF HISTAMINE POISONING

Histamine poisoning could be wrongly diagnosed as an allergic disorder, since histamine is released in response to allergic reaction.

The primary symptoms of histamine poisoning are cutaneous(rash, urticaria, oedema, localized inflammation), gastrointestinal (nausea, vomiting, diarrhoea) haemodynamic (hypotension) and neurological (headache, tingling burning itching), (Merson *et al.,* 1974; Murray, Hobbs and Gilbert, 1982). More serious complications including cardiac palpitations rarely occur in association with the illness (Norysiewicz and Krikler, 1981). Some victims also detect that the implicated food has a sharp or peppery taste.

1.5 PRODUCTION OF HISTAMINE

Histamine is produced through enzymatic decarboxylation of histidine present in the food. Histamine formation in fish is well known to be associated with the growth of bacteria possessing the enzyme histidine decarboxylase. In fish, sev-

eral histamine-producing bacteria have been implicated as primary contributors to histamine formation.

Proteus morganii appears to be the most prolific histamine producer. Histamine can also be produced by the endogenous enzymes present in the food.

1.6 IMPACT OF HISTAMINE POISONING

As a result of the worldwide network for harvesting, processing and distributing fishery products, the impact of food poisoning by histamine is not limited to specific geographical areas or consumption patterns. Histamine survives thermal processing and can therefore be present even though the product may be commercially sterile. Additionally, only one or several fish in a given lot may have high histamine levels so that the frequency of sampling of the processed product for quality control purposes must be high. The histamine levels can vary greatly throughout a single fish(Lerke *et al.,* 1978). To complicate matters even further, fish having high levels of histamine may not appear to be spoilt. It is therefore imperative that the production of histamine in foods is curbed to ensure that potentially deleterious food substances do not find their way into the retail food system.

1.7 PROJECT RATIONALE

Fish has been incriminated in an overwhelming majority' of the histamine poisoning, with scombroid species being the most commonly implicated. These include the various species of tuna, bonito, albacore, mackerel, spanish mackerel, blue fish, saury and butterfly kingfish (Arnold and Brown, 1978).

Histamine in foods is a concern due to the health hazards involved with ingestion of large amounts of such compounds (Halasz *et al,* 1994). Histamine

could be an antinutritional factor and a serious health hazard to those in need of fish protein but who consume the lowest quality products (Pan and Janies, 1985) In Ghana, *koobi* is a special delicacy for many people. *Koobi,* which is salted fermented tilapia is used in stews and soups. It is thus a source of dietary protein. Processing of *koobi* is however done under very unhygienic conditions paving the way for possible microbial contamination.

There is, therefore, a potential for more than sporadic amine poisoning in Ghana. Histamine is considered the most important of these amines since it is found in nearly all cases of amine poisoning. It is thus often used as an index of biogenic amine poisoning. It is therefore important to establish baseline data on histamine levels in *koobi* and also investigate the mechanism of histamine production in *koobi.*

1.8 AIM/OVERALL OBJECTIVE

To produce baseline data on the effects of handling, processing and storage on the production of histamine in salted and fermented tilapia *koobi.* This baseline data can form the basis for control and regulation of fermented fish product.

1.9 SPECIFIC OBJECTIVES

i) Determine the histamine content of fresh tilapia.

ii) Determine the histamine content of fresh skipjack and yellow fin tuna as a means of comparing histamine content of various fishes.

iii) Determine the histamine content of salted and fermented Tilapia, *koobi.* from retail outlets.

iv) Determine the effect of post-catch handling conditions prior to processing

on production of histamine in:

a) Bruised Tilapia iced immediately after purchase from fishermen

b) Unbruised Tilapia but delayed icing for one hour after purchase from fishermen.

c) Unbruised Tilapia but delayed icing for 24 hours after purchase from fishermen.

d) Bruised Tilapia and delayed icing for one hour after purchase from fishermen.

e) Bruised Tilapia and delayed icing for 24hours.

f) Unbruised Tilapia iced immediately after purchase from fishermen.

v) Determine the histamine content in *koobi* under the following processing conditions.

a) gutted Tilapia

b) ungutted Tilapia

c) scaled Tilapia

d) unsealed Tilapia

e) unsalted Tilapia

f) salted Tilapia

g) steamed Tilapia

vi) Determine the histamine content in *koobi* under the following storage conditions.

a) frozen storage for 12 weeks.

b) storage at ambient temperatures(in a basket) for 8 weeks.

vii) Determine the histamine content in *koobi* under the following conditions,

a) Tilapia soaked in 0.4 per cent sodium benzoate for one hour and

(1) Salted at varying levels: high 50 per cent; intermediate 25 per cent and no salt.

(2) Dried at different temperatures: ambient (28-30°C); solar dryer temperature (38-42°C), refigerator temperature (4—7°C) and freezing temperatures (-4 to -2°C) for one week.

b) Tilapia soaked in 0.4 per cent sodium benzoate for two hours and the treatments in (1) and (2) above repeated,

viii) Develop the HACCP System in *Koobi* processing.

1.10 HYPOTHESIS

The evolution of histamine in *koobi* is independent of the handling processing and storage methods used.

2.0 LITERATURE REVIEW

2.1 BIOGENIC AMINES IN FOOD

Biogenic amines such as histamine, tyramine, tryptamine, cadaverine, putrescine, spermine and spermidine etc. have been found in a wide range of foods including fish products, meat products, dairy products, wine, beer, vegetables, fruits, nuts and chocolate (Askar and Treptow, 1986).

Biologically active amines (biogenic amines) have been defined as aliphatic, alicyclic or heterocyclic organic bases of low molecular weight which arise as a consequence of metabolic processes in animals, plants and microorganisms (Rice, Eiten Miller and Koehler, 1976).

The detection of biogenic amines in foods dates back as early as 1877 when Nencki identified amylamine in putrefying meat. Van Slyke and Hart observed tyramine and probably putrescine in ripened cheddar cheese in 1903. In the next several years the presence of amines in cheeses were widely reported. Likewise, many amines were observed in putrefying meat. The finding of Waalkes *et. al.* in 1958 that bananas contain relatively large quantities of both serotonin and norepinephrine was of considerable interest, since in this case the amines were undoubtedly endogenous. Shortly thereafter, West (1959) reported the presence of tryptamine in the tomato and Udenfriend *et al.,* (1959) surveyed a number of fruits and vegetables and found amines to be rather widespread in normal food substances. Since that time several other edible plants have been reported to contain various amines and the presence of amines in microbially contaminated and fermented foods has been reestablished. The amine content of some food substances are shown in Table 2.1.

Table 2.1
Histamine and tyramine contents of foods

FOOD SUBSTANCE	HISTAMINE(|ag/g)	TYRAMEME(|j,g/g)
Beer and ale		1.8-11.2
Wines	0 -.22	0-.25
Yeast extracts	210 -2830	0 -2.256
Fish		
Tuna	2.040-5.000	
Salted dried fish	-	0-470
Pickled herring		3.000
Meat		
Meat extracts	-	95-304
Beef hver	-	274
Chicken liver	-	100
Sausage	0.740-410	0-1237
Miscellaneous		
Soya sauce	-	1.76
Sauerkraut	7-200	20-95

Source: Lovenberg (1973)

(A dash means that the food was not tested for this amine, and zero (0) indicates that the level of the amine was below the detection threshold. Values represent quantities present in selected samples and should not be interpreted as averages.)

Cheeses and some sausages contain much higher concentrations of tyramine than do fruits and vegetables and are considered more dangerous to the tyramine-susceptible individual.

In non-ferraented foods, the presence of biogenic amine is indicative of undesired microbial activity. The amounts of histamine, putrescine and cadaverine usually increase during spoilage of fish and meat whereas the amount of spermine and spermidine(originaly present in fairly high concentrations) decrease during this process. These characteristics are reckoned with in the Biogenic Amine Index(BAI) defined by Karmas (1981).

BAI = (histamine+putrescine + cadaverine) : (1 + spermine + spermidine) where the amine concentrations are expressed in mg/kg. Fish or meat with a BAI value below 1 is considered to be of first quality, whereas BAI values above 10 indicate a very poor microbiological quality. (Karmas 1981). Positive correlations between the BAI value and organoleptic acceptability or microbiological quality have been reported, both for fish (Karmas, 1981) and for meat (Sayem-El-Daher *et al.,* 1984).

Fermented foods are incubated for days, weeks or even months for the food to attain the desired level of fermentation and maturation. As a result of the fermentation process, microorganisms tend to be prevalent in fermented foods and one should therefore expect the presence of biogenic amines in some fermented foods. In yeast-fermented alcoholic beverages levels of biogenic amines are very low. In contrast all products in which a lactic acid fermentation has taken place (except yorghurt) may contain considerable amounts of putrescine, cadaverine, histamine and tyramine. (Brink, *et al.,* 1990). The biogenic amines tyramine, putrescine, cadaverine, 2-phenylethylamine, histamine and tryptamine were estimated in 13 kinds of alcoholic beverages produced in Taiwan. The total amine content of the 13 alcoholic beverages was 0.23 to 11.4 mug/ml with “charing cuen liquer” having the highest value and “mei kwei lu” the lowest. The concentrations of these amines were so low that

they seem unlikely to have adverse effects on human health. (Yen and Chandra, 1988) . It was reported by Izquierdo-Pulido *et al* (1995) that *Saccharomyces cerevisiae* var *Uvarum,* a bottom yeast, did not produce histamine or tyramine during beer fermentation. Yeast recycling also did not influence the formation of biogenic amines.

High levels of biogenic amines were however, reported in fermented sausages from retail markets (Rice *et al.,* 1976; Vandekerckhove 1977; Pechanek *etal.,* 1983; Pfannhaauser and Perchanek, 1984, Bauer *etal.,* 1989, Tschabrun *etal.,* 1990; Vidal-carou *et al.,* 1990).

High levels of biogenic amines(Histamine level >100mg/g) were also detected in the flesh of Sailfish, *Istiophorusplatypterus* implicable in a 1994 food poisoning outbreak in Western Taiwan. Reports by the victims indicate that the Sailfish fillets that caused the intoxication did not appear unpleasant or have a spoiled flavour at the time of purchase (Hwang *at al* 1995).

The average concentrations of amines in wholesome tuna were (mg percent) putrescine, 0.35; cadaverine, 1.05; histamine, 2.74; spermidine, 3.26 and spermine, 1.23. Amine levels in canned tuna implicated in an outbreak of scombroid poisoning in humans ranged from (mg percent) 1.53 and 1.16 for putrescine and spermine respectively to 2.37 for spermidine, 12.8 for cadaverine, and 116 for histamine (Kim and Bjeldanes, 1979). The high levels of histamine recorded for the latter reflect the dangers involved in consuming spoiled fish. The content of biogenic amines in fresh fish is very low and their appearance is related to spoilage(Frank *etal,* 1981,Nagayame*a/., 1985;Femandez-Salguero and Mackil, 1987). For this reason, aside from toxicological implications, histamine, tyramine, putrescine and in general all biogenic amines, have been pro-

posed as indicators of the quality and/or index of microbial spoilage of food, and in particular of fish and fish products(Mietz and Karmas, 1977 ; Hui and Taylor, 1983). Due to the thermally stable condition of these amines, they could be used as indicative parameters of the quality of raw materials used in preserved and canned fish products.

Baronowski and Brust (1984) reported that very slight microbial decomposition of the tissue makes the protein more available for utilization, but these effects can be offset by elevated histamine levels.

2.2 HISTAMINE IN FISH

Fish has been incriminated in an overwhelming majority of histamine poisoning, with scombroid species being the most commonly implicated. These include the various species of tuna, bonito, albacore, mackerel, bluefish, saury and butterfly kingfish (Arnold and Brown, 1978). The non-scombroid fish types involved in incidents of histamine poisoning include mahi-mahi *(Coryphaena hippurus*) also known as dolphin or dorado which is the most commonly implicated species in the USA, black marlin, sardines, anchovies, pilchards, herring, trumpeter fish, red snapper and goat fish(Schulze, Reusse and Tellack, 1979; Anon, 1980; Murray, Hobbs and Gilbert, 1982; Taylor, 1983). With some fish species, domestic sources seem to be the most common sources for incriminated fish. Thus, domestic supplies have accounted for the majority of histamine poisoning outbreaks in Japan and New Zealand. The same can be reported about mackerel in the United Kingdom and tuna in the USA. However, imported supplies of fish can play a major role in histamine poisoning with certain fish species in certain countries (Taylor, 1983).

Histamine poisoning is often referred to as scombroid poisoning due to the frequent association of the illness with consumption of spoiled scombroid fish. Scombroid poisoning caused by ingestion of sea food is one of the major causes of all food poisoning. Hughs *et al.,* (1977) reported that nearly half of all food borne illnesses of chemical origin which occurred between 1970 and 1974 were caused by either fish or shell fish poisoning. Almost half of these, 29 of 68 outbreaks, were due to scombroid poisoning.

Work by Plahar *et al.,* (1996) established that certain fermented mackerel species contain very high levels of histamine. The histamine content of three fermented mackerel species are:

Spanish mackerel (momone)	137.6-156.06mg/100g
Mackerel scad	127.59-165.58mg/100g
Jack mackerel	105.78-145.Omg/lOOg

These levels exceed the hazardous limit of lOOmg/lOOg stipulated by the US Food and Drug Administration. Histamine concentration were determined in 94 samples of 13 selected fish species with grey flesh (tuna, mackerel, sardine, sprat) and white flesh (Cod, Norway haddock, hake and 6 minor spp) and 9 canned fish products. The concentration of histamine in the grey flesh in the range 100-700 mg/lOOg exceeded the critical value of lOOmg/lOOg which can lead to the development of scombrod poisoning. The level of histamine in the white flesh was in the range 1.9-7.7 mg/lOOg. In the canned fish, the histamine levels(40-51.6mg/100g) exceeded the acceptable level (of20mg/100g ; indicative of mishandling).

It is apparent that extremely high levels of histamine can be produced in fish. The amount of histamine may even differ from one portion of the fish to the other.

In studies on the distribution of histamine in decomposed tuna(Lerke *et al.,* 1978; Frank *et al.,* 1981) the highest levels of histamine were reported in the anterior muscle sections adjacent to the gills, presumably because of the presence of histamine-forming organisms in the microbial population of the gill cavity which colonized the anterior sections (Middle brooks *et al.,* 1988).

Different sections cut from the same fish differed in histamine content. Histamine was found to be highest in specimens taken from the cross section at the mid portion near the gut cavity of a yellow fin tuna that caused histamine poisoning outbreak (Lerke *et al.,* 1978). A histamine gradient was observed in spoiled skipjack tuna being highest near the gut cavity of the anterior section and decreased in sections approaching the tail (Frank, Yoshinaga and Nip, 1981; Yoshinaga and Frank, 1982). During 15-days iced storage of skipjack tuna, the tail section showed the lowest histamine content throughout the period (Orejana *et al.,* 1983) .

2.3 PRODUCTION OF BIOGENIC AMINES

Biogenic amines are basic nitrogenous compounds which are produced in food as a result of decarboxylation of amino acids or transamination of aldehydes and ketones (Askar and Treptow, 1986). In most amine-containing foods the majority of the amines are generated by decarboxylation of the corresponding amino acids through substrate-specific enzymes derived from microorganisms present

in the food. Indeed, in virtually all foods containing proteins and are subject to conditions enabling microbial or biochemical activity biogenic amines can be expected.

The production of biogenic amines in food requires the presence of bacteria capable of producing decarboxylase and a suitable cofactor and/or inducer for decarboxylation. Availability of an adequate concentration of amino acids, environmental factors favouring bacterial growth and conditions conducive to decarboxylase synthesis and subsequent decarboxylation are also required(Ferencik, 1970; Arnold and Brown, 1978; Edwards and Sandine, 1981, Rraner *et al* 1991). Decarboxylase - positive microorganisms may be part of the association flora of the food or can be introduced by contamination before, during or after processing of the food. In the case of fermented foods and beverages, the introduction of starter cultures can affect the production of biogenic amines, either directly or indirectly through interaction with the association/contamination flora. (Brink *et al,,* 1990).

Free amino acids may occur as such in the food but may also be liberated from proteins as a result of proteolytic activity. Dierick *et al.* (1974) observed a general increase in free amino acids with little change in the concentration of free tyrosine during sausage ripening. However, these workers reported an increase in the presence of tyramine during sausage ripening indicating that although free tyrosine is produced, it is decarboxylated to form tyramine

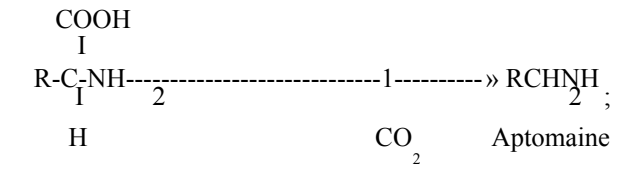
Table 2.2

Some important biogenic amines and their amino acid precursor

Biogenic amine	Precursor
Aliphatic amines	
Putrescine	Ornithine
Cadaverine	Lysine
Aromatic amines	
Tyramine	Tyrosine
Phenylethylamine	Phyenylalanine
Heterocyclic amines	
Histamine	Histidine
Tryptamine	Tryptophane

Source: Brink *et al,* 1990

The decarboxylation reaction is a common putrefactive mechanism in the spoilage of food protein resulting in the formation of ptomaines. Many amino acids undergo decarboxylation as a result of the action of intestinal bacteria to produce toxic amines (ptomaines). This is illustrated below:



These reactions are catalyzed by specific pyridoxal-dependent bacterial decarboxylases (Dasgupta, 1978).

Although amino acid decarboxylases are not widely distributed among bacteria, species of many genera (for instance *Bacillus, Citrobacter, Clostridium, Escherichia, Klebsiella, Lactobaccillus, Pediococus, Photobacterium, Proteus, Pseudomonas, Salmonella, Shigella, Streptococcus*) are capable of

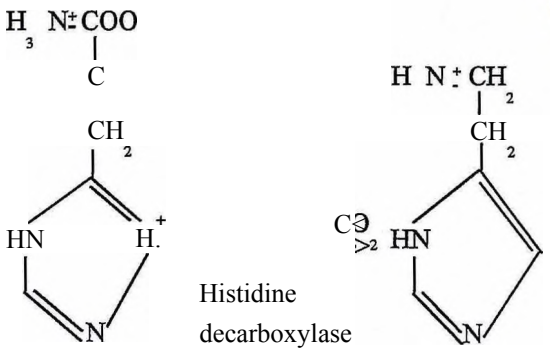
decarboxylating one or more amino acids (Rice *et al.,* 1976). Sometimes many different species within a group of related genera possess a specific decarboxylase: For instance, most enterobacteriaceae are capable of producing putrescine and/ or cadaverine. On the other hand, large species variation may occur within one genus; only a few lactobacillus species are histidine decarboxylase positive. Most amines are heat stable and some decaboxylases remain active even after pasteurization. This implies that the amount of amine once formed will not be reduced during processing and might even increase during storage.

2.3.1 Mechanism of Histamine Production

Histamine is produced in foods through decarboxylation of histidine. This reaction is catalyzed by the enzyme histidine decarboxylase. Some early studies indicated that histamine was generated from autolysis(Geiger, Coutney and Schnakenberg, 1944). However, substantial evidence points out now that the decarboxylation results largely from the action of bacteria which possess the enzyme(Amold and Brown, 1978; Ababouch and Afilal, 1988). Thus, the presence of histamine in fish has been considered as an indicator of earlier microbial decomposition. Some histamine can also be produced by the endogenous decarboxylase present in the food. Scombroid fish and some of the non-scombroid species such as mahi-mahi (Hibiki and Simudu, 1959) and sardines (Ababouch and Afilal, 1988) possess large amounts of free histidine in the muscle tissue. This free histidine serves as a substrate for bacterial histidine decarboxylase. Proteolysis, either autolytic or bacterial, may play a role in the release of free histidine from tissue proteins (Ababouch and Afilal, 1988).

Histamine is thought to be one of the main toxicants in scombroid poisoing.

Scombroid poisoning is so named because the fish usually implicated in cases of poisoning, tuna, mackerel etc. belong to the suborder scombroidei. These fish have a higher concentration of basic amino acids and imidazole derivatives than that found in the normal musculature of slaughter animals and other fish. (Ferencik, 1970). This may be an important factor in the formation of higher levels of histamine in their flesh. Since histidine decarboxylase is an inducible enzyme, the higher levels of histidine will favour its induction. Also, increased levels of free histidine will favour rapid formation of histamine.(Ferencik, 1970; Edmunds and Eitenmiller, 1975). A limiting factor in the formation of histamine in fish muscle is the release of histidine from muscle proteins. Ferencik, stated that autolytic proteases are much more important in this respect than the proteolytic enzymes of the contaminant microflora.



Histidine Histamine

Fig. 1.1 shows the formation of histamine

Histidine decaboxylase is not widely distributed among bacteria. Bacteria in a variety of genera, including *Proteus, Hafnia, Enterobacter, Klebsiella,*

Lactobacillus, Streptococus, Clostridium, Citrobacter, Vibrio Escherichia, Providertcia, Salmonella and Pseudomonads, possess histidine decaboxylase. Enteric bacteria appears to be the most important histamine-producing bacteria in fish.

Proteus morganii, *Klebsiella pneumoniae* and *Enterobacter aerogenes* belong to the category of prolific histamine producers while *Hafnia alvei Citrobacter freundii* and *Escherichia coli* are slow producers of histamine (Behling and Taylor,1982).

2.4 HANDLING PROCESSING AND STORAGE CONDITIONS AFFECTING THE PRODUCTION OF HISTAMINE IN FOOD

Various processing methods, handling and storage conditions and environments could affect production of histamine in foods.

The histamine content of delayed iced skipjack tuna was significantly higher than those immediately iced on board the fishing vessel. After 8 days of storage, the histamine contents were 4.14mgN percent and 8. lmgN percent for immediately iced and delayed iced skipjack tuna respectively. (Putro and Saleh, 1985). Orejana (1983), showed that 12-hour delay in the icing of frigate mackerel causes much higher subsequent levels of histamine production at 0°C than would be expected.

Skipjack used for local consumption are mainly obtained from artisanal fishermen who have no proper on-board handling facilities, whereas those for export are derived from modem pole and line fishing equipped with on-board freezers. Consequently, skipjack caught by artisanal fishermen have inferior quality compared to those from pole and line fishing vessels. This is due to the fact that the fish are held without chilling (icing) for several hours (up to 8

hours) under direct sunlight at relatively high temperatures and relative humidity. Apparently, this is one of the main reasons that skipjack tuna consumption in Indonesia has been implicated with histamine poisoning. (Putro and Saleh, 1985).

Icing, therefore, plays an important role in minimising the production of histamine in skipjack tuna. Proper handling of skipjack is expected to reduce the possible hazard of histamine poisoning, especially in those tuna caught by artisanal fishermen without proper handling facilities on board the fishing vessel.

Histamine is not produced in frozen storage. However, Pan *et al.,* (1981) observed that prefreezing shelf life and thawing methods affect histamine formation in fish. For mackerel chilled up to 2 days before freezing, thawing in running water or in still air caused little difference in histamine content. However, histamine increased during prolonged pre-freezing cold storage. Thawing in running water did not lead to further increase, while thawing in still air caused a further 33 percent increase in histamine formation. Therefore thawing in running water and reduction in pre-freezing handling is recommended. (Baranowski and Pan, 1985). Behling and Taylor (1982) pointed out that fish exposed to 20°C for a short period(l day) will yield high levels of histamine following subsequent storage at refrigeration temperatures.

Smith *et al.,* (1980) also found a more rapid production of histamine when mackerel were iced after 24 hours at ambient temperature. Negligible histamine, production occurred at fish storage temperatures of 0°C or below (Baldrati *et al,* 1980). However, histamine production has still been observed below 10°C (Baldrati *et al.,* 1980, Cattaneo and Cantoni, 1978; Dabrowski, Kolokowski

and Markiewicz, 1968; Park *etal.,* 1980; Pan *et al,* 1981; Sakabe, 1973) except at a lower rate.

With storage of tuna, herring and mackerel at 4°C for 3 -4 days, histamine content exceeded 100mg/kg(ppm). On the 5th day, histamine content reached 596ppm in mackerel, 978ppm in herring and 3720ppm in tuna (Rubach, Offizorz andBreyer, 1981).

A comparison of the quality of fish stored for at most 21 days at ice to fish ratio of 1:4 and 1:2 revealed that the latter ice to fish ratio yielded substantially lower histamine levels than the former at all sampling periods (Orejana *et al.,* 1983). This further underscores the importance of ice in minimising histamine production in fish.

Histamine content in the ungutted mackerel was ten (10) times more than in the gutted fish after storage at ambient temperature for 140h (Hardy and Smith, 1976). In mackerel, although the liver has a lower content of free histidine than the muscle, it produced histamine faster than the muscle during storage. (Femandez-Salguero and Makie, 1979). This evidence seemed to show a relationship between gutting and histamine formation.

Seasonal variation in histamine formation has been reported. Free histidine content in herring varied with seasons from 260 to l,600mg/kg being highest in summer (Hughes, 1959). Less histamine was found in sardine and bonito during winter than in other seasons under the same handling conditions (Simidu and Hibiki, 1954). Confirmed histamine poisoning outbreaks caused by smoked mackerel occurred during late summer when both fish and water have the highest temperature and the mackerel is at its fattest (Skovgaard and F.llp.mann 1978). There seems to be a definite connection between season, temperature,

histidine content and histamine formation in fish products.

It was determined by Joosten and Stadhouders (1987) that biogenic amines were not produced in Gouda and Maasdam cheese made from pasteurized milk with sufficient hygienic care.

Pottasium sorbate at 0.5 percent effectively inhibited growth and histamine production by *Proteus morgcmii* and *Klebsiella pneumoniae* and the sodium salts of hexameta phosphate and polyphosphate at 2 percent slowed down the histamine production rate (Taylor and Speckhard, 1984). It could, therefore, be possible to use antimicrobial agents together with ice or refrigerated sea water to inhibit microbial growth and histamine production.

2.5 TOXICOLOGY OF BIOGENIC AMINES

Although biogenic amines are needed for many critical functions in man and animals, consumption of food containing high amounts of these amines can have toxicological effects. Biogenic amines may exert vasoactive effects (tyramine), psychoactive effects or both (histamine). Psychoactive amines act on the neural transmitters in the central nervous system, while voasoactive amines act, either directly or indirectly on the vascular system. (Lovenberg, 1973) Pressor amines are vasoactive amines that cause a rise in blood pressure.

Amines such as histamine, tyramine, putrescine, cadaverine, spermine and spermidine possess vasoactive properties, making their presence in food a potential public health concern. These vasoactive compounds increase capillary permeability thereby producing a fall in blood pressure in the systemic circulation. Large doses of these amines notably histamine, produce signs of shock and allergic reactions. Symptoms that can occur after excessive oral intake of biogenic

amines are nausea, respiratory distress, hot flush, sweating, heart palpitations, headache, bright red rash, oral burning, and hyper or hypotension. (Rice *et al.,* 1976).

A typical phenomenon is the 'cheese reaction' usually caused by high levels of tyramine in cheese. (Brink *et al.,* 1990). However, the most notorious food-borne intoxications caused by biogenic amines are related to histamine. Biogenic amines such as tyramine and beta phenylethylamine have been proposed as the initiators of hypertensive crisis and dietary induced migraine in certain patients (Stratton, Hutkins and Taylor, 1991).

Although the toxicity of individual biogenic amines in general is beyond all doubt, it is very difficult to determine the exact toxicity threshold of these compounds. For instance, the toxic dose is strongly dependent on the efficiency of detoxification, which may vary considerably between different individuals. Furthermore, the effects of histamine and tyramine may be potentiated by other consumed compounds (Taylor, 1983), including other biogenic amines (Hayashi, 1954); Bjeldanes *et al.,* 1978). Putrescine and Cadaverine, which both seem to have a much lower pharmacological activity than histamine and tyramine, also interact with the amine oxidases and thereby hamper the detoxification of histamine and/or tyramine. The use of certain drugs [Mono amine oxidase (MAO) inhibitors] decrease the efficiency of the detoxification system. In addition, consumption of alcoholic beverages result in an increased sensitivity towards biogenic amines(Brink *et al.,* 1990). Even though all humans are succeptible to scombroid poisioning, the symptoms can be severe for the elderly and those taken medications such as isoniazid.

Notwithstanding all uncertainties stated, histamine levels above 500-

lOOOmg/kg food are considered potentially dangerous to human health. This level is based on the concentrations found in food products involved in histamine poisoning (Taylor, 1983). A legal upper limit of 1 OOmg histamine/kg food and 2mg/l alcoholic beverage has been suggested (Brink *et al.,* 1990).

In evaluating the toxicological status of amine containing foods, one should not focus exclusively on the concentration of one particular biogenic amine. The effect of potentiating agents, including other amines in the food, the amine content of other dietary components, alcohol and certain drugs are very important and should therefore not be neglected. It is quite conceivable that simultaneous consumption of a number of food products (such as wine, cheese, sauerkraut and fermented sausage) results in biogenic amine poisoning whereas consumption of each of these products alone does not present any problems. It should also be stated that secondary amines such as putrescine and cadaverine can react with nitrite to form carcinogenic nitrosamine (Askar and Treptow, 1986).

2.5.1 Potentiation of Histamine Toxicity

The toxicity of histamine appears to be enhanced by the presence of other biogenic amines found in foods that can inhibit histamine metabolizing enzymes in the small intestine. (Stratton, Hutkins and Taylor, 1991). Pure histamine has been administered in relatively high doses to humans with no apparent ill effect (Arnold and Brown, **1978)** leading to the suggestion that scombroid poisoning by spoiled fish is caused by histamine acting synergistically with other diamines, primarily putrescine and cadaverine, present in the fish (Bjeldanes *et al.,* **1978).**

Both putrescine and cadaverine may interfere with the normal histamine detoxification system of the intestine (by competing with histamine as substrates for diamine oxidase under certain conditions) implying that the combined ef-

fects of histamine and the diamines may be required for production of scombroid poisoning. Potentiation of histamine by inhibition of histamine-metabolising enzymes occurs only at cadaverine/histamine or putrescine/histamine ratios of 5:1 or greater (Hui and Taylor, 1985). Potentiators would act by inhibiting intestinal histamine catabolising enzymes, which would result in greater transport of histamine across cellular membranes and into the portal circulation (Taylor, 1983).

Spermine has been reported to liberate endogenous histamine in intestinal fluids of the guinea pig(Pamt, 1948). Also, histamine taken with a meal (bread, milk and butter) has been reported to be absorbed to a greater extent than histamine consumed by itself (Mitchell and Code, 1954). The presence of other amines therefore augments histamine toxicity in foods.

2.5.2 Detoxification of Histamine

Histamine in foods is not necessarily hazardous. A fairly efficient detoxification system exists in the intestinal tract to metabolize ingested histamine and histamine which may be formed by intestinal bacteria. The detoxification system is composed of two distinct enzymes: diamine oxidase which coverts histamine to imidazoleacetic acid and histamine N-methyltransferase which catalyzes a methylation reaction. The methylated product can then be metabolized by monoamine oxidase to methyl imidazoleacetic acid (Taylor, 1983). This detoxification system is adequate for handling normal dietary intakes of histamine. However, it apparently fails to detoxify large doses of histamine that can be ingested with spoiled fish.

2.5.3 Catabolism of Biogenic Amines

Very little information is available on the catabolism of biogenic amines by bacteria. Inman, tyramine may undergo one of several different catabolic reactions as shown in (Fig.2.1).

Oxidation by monoamine oxidase is one of the more important pathways,

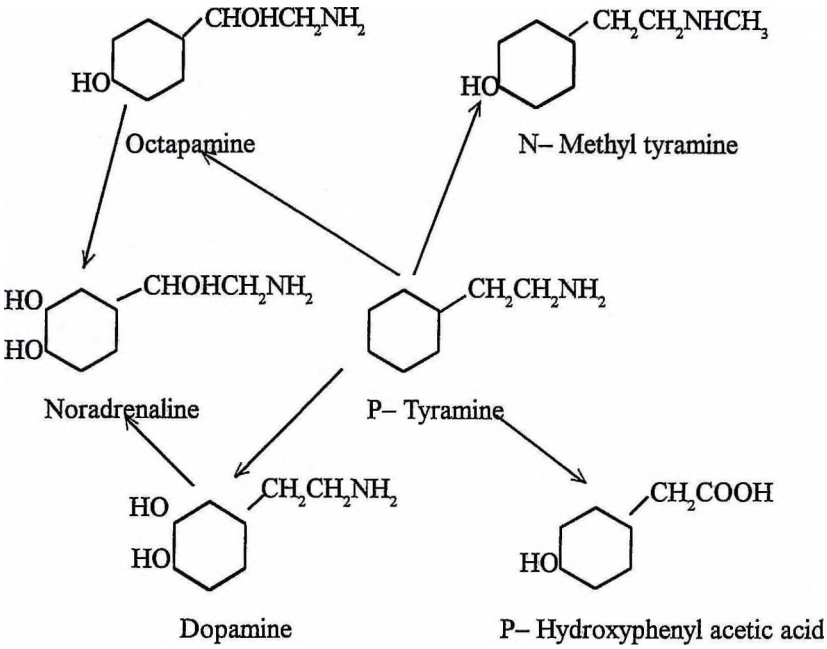


Fig 2.1: Catabolism of Tyramine

Histamine can be catabolized by several routes (Fig 2.2). It can be oxidatively deaminated by diamine oxidase, methylated to form 1-methyl histamine, or its side chain can be methylated or acetylated. (Franzen and Eyesell, 1969).

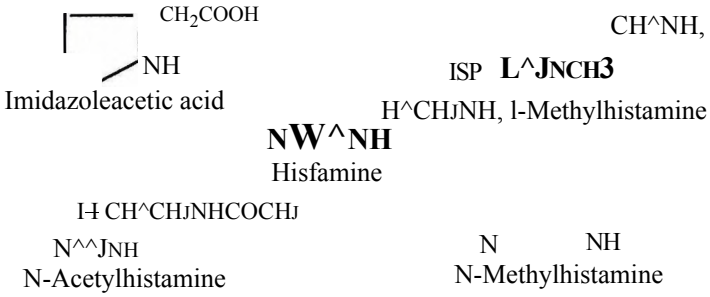


Fig. 2.2: Catabolism of histamine

2.6 CONTROL OF BIOGENIC AMINES IN FOODS

It was shown by Watts and Brown (1982) that carbon dioxide (CO^ modified atmosphere could reduce the formation of biogenic amines in pacific mackerel *{Scomber japonicus)*.

Controlled meat fermentations via pure cultures can also reduce the risk of toxic histamine and tyramine formation during prolonged aging processes where food products are exposed to "wild" microbial degradation (Smith Palumbo, 1983). Pure cultures, lacking the enzymes required for the conversion reactions (leading to formation of biogenic amines), control the fermentation process and inhibit potentially dangerous, naturally-occurring micro-organisms. The addition of lactic acid bacteria cultures has been suggested to prevent histamine and tyramine accumulation by control of natural fermentation (Eitenmiller *et al.,* 1978; Taylor *et al.,* 1978).

Caurie *et al.,* 1974, reported anti-oxidant, microbistatic and microbicidal properties in wood ash. Wood ash also possesses the capability to reduce the moisture content and increase pH to levels unfavourable for most bacteria and thereby preventing the formation of biogenic amines.

In sausages manufactured with starter culture, the levels of tyramine and cadaverine as well as their precursors tyrosine and lysine were lower than in sausages manufactured without starter culture. Levels of histamine were also lower, although there were no differences in levels of histidine. These results therefore support the use of starter culture to decrease formation of biogenic amines. (Maijala*etal.,* 1996).

Histamine accumulates at a very slow rate at storage temperature of 0°C or below. Consequently, the use of proper icing (at least 1:2 ratio of ice to fish), refrigerated sea water and chilled sea water should prevent histamine poisoning. Sprinkling fish with salt which is used in some developing countries to preserve pelagics has been shown to be inefficient (Ababouch, Aloui and Bust, 1986; Ababouch and Afilal, 1988). Storage of fish at refrigerated temperatures ranging from 4 to 10°C may also prove to be insufficient, as several storage trials at these temperatures have shown substantial histamine formation over long periods.

Addition of glucose or ribose reduces the histamine content of fish meal. This is due to the maillard reaction between the amino group of histamine or histidine and reducing sugars. Ribose is more favourable to the reaction than glucose. (Toyama *et al.,* 1982).

In the production of lactic acid fermented foods, starter cultures should be used which are amino-acid decarboxylase negative and which actively repress non-starter organisms, preferably also non-starter lactic acid bacteria.

High levels of biogenic amines can be prevented if proper hygienic care during processing is practised, contamination and microbial activity often due to mishandling and temperature abuse during storage is controlled and low initial contamination of the food is taken care of.

2.7 REGULATORY LIMITS FOR HISTAMINE IN FISH

Due to the uncertainty regarding the threshold toxic dose for histamine, most countries do not have firm regulatory limits on the allowable/ acceptable levels of histamine in foods including fish.

However, several countries have adopted or are in the process of evaluating allowable levels of histamine in fish.

In studies conducted by Simidu and Hibiki (1955), it was estimated that the threshold toxic dose (TTD) for histamine in fish is approximately 60mg/ lOOg. Due to the difficulty in determining the TTD, several countries have adopted maximum allowable levels of histamine in fish. The US Food and Drug Administration (FDA), for instance, established a hazard action level (HAL) of 50g/100g in tuna products based on the investigation of previous histamine poisoning outbreaks and the defect action level (DAL) of 20mg/100g.

The European Economic Community (EEC) regulations on the other hand stipulates a Defect action level to be lOmg/lOOg. and a maximum allowable leveltobe20mg/100g. (FAO, 1994).

The hazard action level(HAL) is an amount constituting a known human health hazard and a defect action level (DAL) represents some mishandling of the fish.

2.8 ANALYTICAL METHODS FOR HISTAMINE DETERMINATION

Several methods are available for the analysis of histamine in foods. The quantitative analytical techniques involve three steps.

Extraction of histamine from fish using methanol or trichloroacetic acid (TCA);

ii) purification of the fish extract by removing interfering materials, using ion exchange chromatography or butanol;

iii) quantification by spectrophotometry at 495nm after derivatizing histamine in the purified eluate with a diazonium reagent. This colorimetric method relies on the reaction of histamine with a diazotized aromatic compound

to form a coloured complex. This coloured complex is allowed to stand at 0°C for 1 Ominutes prior to the measurement of absorbance at 495nm using distilled water as a reference. A standard curve is prepared by using 1ml aliquots of a standard histamines solution (0-80mg histamine/0.2 NH_4C1). Or quantification by spectrofluorometry (at 3 50mn for excitation and 450nm for emission) after derivatizing histamine with orthophthaldehyde. This is the fluorimetric method and is based on the reaction of histamine wtih orthopthaldehyde to form a product which fluorscence.

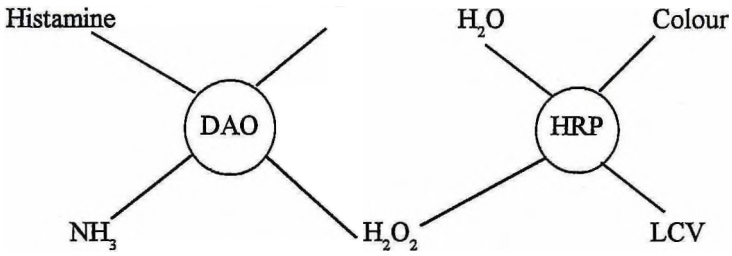
These methods are reproducible and sensitive. However, they require skills and expensive instruments which may not always be available. The semi-quantitative chemical methods are much simpler but not as sensitive and accurate as the quantitative methods. They involve some type of chromatographic step, usually paper or thin layer chromatography (TLC) to separate histamine from the interfering substances. In TLC, silica gel is an adequate stationary phase and methanol: ammonia(2:1) or chloroform:methanol:ammonia(2:2:l) are good solvent systems (Lieber and Taylor, 1978; Ababouch.Alaoui and Busta, 1986); Ababouch and Afilal, 1988). These semi-quantitative methods can be performed in many situations because they do not require elaborate equipment and a technician can assay numerous samples with a minimum of time and effort.

An enzymatic method for the semi-quantitative analysis of histamine has been developed (Lerke *et al.,* 1983) which can be used routinely.

The basis for this technique is that the enzyme diamine oxidase catalyses the conversion of histamine to imidazole acetaldehyde with the concurrent conversion of oxygen to hydrogen peroxide. Hydrogen peroxide is converted back to oxygen and water by the action of horseradish peroxidase. Concurrent to this reaction is the oxidation of leuco-crystal violet (a colourless compound) to crystal

violet, an intensely purple compound. The colour formed should be directly proportional to the amount of histamine present. Due to the very complex nature of the reaction a somewhat less than ideal results are found.

The technique is illustrated below:



(Lerke *et al,* 1983)

DAO=diamine oxidase, HRP=horse-radish peroxidase; LCV = Leuco-crystal violet.

There are newer techniques that have been developed for the analysis of histamine in foods. These techniques include high performance liquid chromatography(HPLC), gas chromatography(GC) and radio immuno assay(RIA). These techniques are more accurate and offer more flexibility but are available to only the most sophicated analytical laboratories. They offer, however, a major advantage over the other methods: they allow the simultaneous analysis of histamine and other putrefactive amines in foods.

Many researchers have turned to the use of high performance liquid chromatography(HPLC) for the determination of biogenic amines in foods. Many of these methods depend on derivatization with either orthopthaldehyde (Subden, Brown and Noble, 1978; Davis *et al.,* 1979; Saria, Lembeck and Stofitsch, 1981;

Tsurata, Kohashi and Okhura, 1981; Robert *etal.,* 1983 or dansyl chloride (Mietz and Karmas, 1977). Dansyl derivatives, which have a napthalene structure, are excellent derivatives for primary amines. They are easily formed and detected by a uv detector. There is, however, one procedure (Mett and Sturgeon, 1982) in which histamine is anlaysed directly without derivatisation.

A mono clonal antibody-based ELISA (enzyme-linked immuno sorbent assay) for the determination of histamine in foods has been developed.

Monoclonal antibodies (Mabs) to histamine were prepared by immunising mice with histamine-protein conjugate. Four mabs were obtained which both exhibited high affinity for histamine after chemical derivatisation and showed no cross-reaction with five other biogenic amines (Cadaverine, putrescine, spermine, spermidine, tyramine) or with structurally related compounds such as histidine or serotonin.

Using these antibodies, a competitive inhibition ELISA (CIELISA) was developed, available in the range lO-lOOng/ml. The assay has been used to quantify the histamine content of fish extracts previously treated for histamine derivatisation using 1,4-benzoquinone (Serrar *et al,* 1995).

There have been attempts by several authors to use indirect methods to estimate histamine levels in fish loads.

Frank (1985) developed a nomograph to predict histamine production in skipjack tuna knowing the time-temperature fluctuation history of the fish after catch. Olley and Mcmeekin (1985) have reported the use of temperature-function integrators. This method monitors temperature fluctuations and integrates these to provide an equivalent number of days(shelf-life) at a reference temperature. The application of these indirect methods in commercial handling and

processing fish is very limited.

The choice of the particular method used for histamine analysis depends on the need for accuracy, time considerations, technical skills, expense and availability of reagents and equipment needed.

2.9 IMPORTANCE OF WATER ACTIVITY IN CONTROLLING FOOD SPOILAGE

Water activity is an important parameter for controlling food spoilage and enhancing the shelf life of foods. Most bacteria do not grow below $a_w = 0.91$ and most moulds cease to grow below $a_w = 0.8$. Table 2.3 shows the a_w of some food and their susceptibility to spoilage by microorganisms. Water activity (a_w), rather than water content, determines the lower limit of available water for microbial growth. The water activity value determines the food's microbiological stability, stability to oxygen, enzymatic deterioration, colour changes and other parameters (Luck, 1977; Robinson, 1976; Heidelbaugh and Karel 1975).

2.10 TRADITIONAL METHODS OF FISH FERMENTATION

Fermented fish sauces have been consumed since ancient times and the earliest reported is "Garum" (finest quality made from viscera and blood) which was highly prized in the Roman era (Badham, 1854). Fermented fish is any fishery product which has undergone degradative changes through the enzymatic or microbiological activity either in the presence or absence of salt (FAO, 1993). Fermented fishes are characterised by a strong odour attributable to enzymatic and microbiological activity in the fish muscle.

Various processing methods in fish fermentation are used in different localities. The particular method used depends largely on availability of salt and

Tabic 2.3

Water Activity of Some Foods and Susceptibility to Spoilage by Microorganisms'"

Range a_w Micro organism generally inhibited by lowest aw in this range	Examples of food generally within this range of a..
1.00 - 0.95 *Pseudomonas, Eschrichia, Proteus, Shigella, Klesiella, Bacillus, Clostridium perfringens,* some yeasts	Highly perishable foods (fresh and canned fruits, vegetables, meat, fish) and milk; cooked sausages and breads; foods containing up toca 40 percent (w/w) sucrose or 7 percent NaCl
0.95 - 0.91 *Salmonella, Vibrio parahaemolyticus C. Botulinum, Serratia, Lactobacillus, Pediococcus, some molds,Rhodotorula, Pichia*	Some cheeses (Cheddar, Swiss, Muenster, Pro - volone), cured meat, (ham), some fruit juice concentrates; food containing 55 percent (w/w) sucrose or 12 percent NaCl
0.91 - 0.87 Many yeasts (*Candida, Torulopsis, Hamsenula), Micrococcus*	Fermented sausage (salami), sponge cakes, dry cheeses, margarine; foods containing 65 percent (w/w) sucrose (saturated) or 15 percent NaCl
0.87 - 0.80 Most molds (mycotoxigenic peinieilla), *Staphylococcus aureus,* most densed milk chocolate syrup, maple and fruit *Saccharomyces (baillii)* spp., syrups, flour, rice pulses containing 15-17 *Debaryomyces* percent moisture, fruit cake country style ham, fondants, Ijigh-augar cakes	Most fruit juice concentrates, sweetened con-
0.80 - 0.75 Most halophpilic bacteria, mycotoxigenic aspergilli	Jam, marmalade, marzipan, glace fruits some marshmallows
0.75 - 0.65 Xerophilic molds (*Aspergillus chevalieri, A. candidus, Wallemia sebi),rained Saccharomyces bisporus* molasses, raw can sugar, some dried fruits, nuts	Rolled oats containing ca. 10 percent moisture, nougats, fudge, marshmallows, jelly
0.65 - 0.60 Osmophilic yeasts *{Saccharomyces rouxii)* few molds (*Aspergillus echinulatus, Monascus bisporus*)	Dried fruits containing 15-20 percent moisture some toffees and caramels, honey
0.50} } } } }	Noodles, spaghetti, etc. containing ca. 12 percent moisture; spices containing ca. 10 percent mois- ture
0,40} } } No microbial proliferation	Whole egg powder containing ca. 5 percent mois- ture
0.30} } } } } }	Cookies, crackers, bread crusts, etc. containing 3-5 percent moisture
0.20}	Whole milk powder containing 2-3 percent mois ture; dried vegetables containing ca. 5 percent moisture; com flakes containing ca. 5 percent moisture; dehydrated soups; some cookies, crackers

* Adapted from Beuchat (1981)

the food habits of the local people. Three main techniques have clearly emerged as methods commonly practised in many African countries. These are:

i) fermentation with salting and drying;

ii) fermentation and drying without salting and

iii) fermentation with salting but without drying (FAO, 1993).

Some of the major fermented fishes consumed in Ghana are *momone, kako, koobi,* and *ewurefua.*

Momone is a soft product with a very pungent and sometimes offensive smell. It is susceptible to larvae infestation (maggots), mould growth and bacterial spoilage especially if the salt level is low. Various species of fish such as catfish, barracuda, seabream, thread fin, croaker, grouper, bonito macekrel, herrings, squid, octopus, bumper, snapper, ribbon fish. (FAO, 1993) can be used.

Kako, koobi, ewurefua are dried products with a mild to strong odour. These are susceptible to fragmentation, insect infestation and mould growth. For long-term storage (four to six months) they have to be redried periodically in order to maintain quality. Shark, skates, ray are used for making kako while trigger fish is used for making ewurefua. For the processing of koobi, tilapia is used.

2.10.1 **Processing of** *koobi*

Various species of tilapia can be used. The fish is scaled and gutted. The fish is then thoroughly washed. Dry salt is rubbed into the gills, the belly cavity and on the surface. The fish is then allowed to ferment for two to three days before being dried for two to four days. More salt may be sprinkled on the fish during drying (FAO, 1993).

2.10.2 Salting

The absorption of salt during fish curing results in the removal of water from the flesh to a level that impedes microbial growth and enzymatic activities. The concentration of sodium chloride in Ghanaian solar salt may be below the standard level of 96.6 percent (Sefa-Dedeh, 1973; Owusu, 1971). When high levels of salt are used in fish fermentation, the primary objective is to select the halophilic micro-organisms which will effect a controlled degradative process on the organic compounds in the fish muscle to bring about the desired flavours in the product.

In Ghana, where solar salt is readily available and inexpensive, fermented fish is heavily salted. Solar salt, however, is noted for its poor microbiological quality (Sefa-Dedeh and Youngs, 1976; Lupin, 1977;F.A.O. 1981).

The use of salt in fish processing may either be by dry salting (Kenching) or wet salting. Salting plays multiple roles in fish processing. It affects the state of the proteins, inhibits some enzymes systems while enhancing others (Haard,1991) and reduces the moisture content of the fish muscle. Salt also enhances the flavour of the prouducts. (Deng and Tomaszewski, 1980).

2.11 IMPORTANCE OF QUALITY ASSURANCE IN TRADITIONAL FISH PROCESSING

There are no formal quality assurance methods in traditional fish processing. However, the traditional fish processors have a means of determining the quality of fish (by looking at the gills etc). HACCP can make it possible to incorpo-

rate formal quality assurance into traditional fish processing. Hazard Analysis and Critical Control Point (HACCP) is a quality system which aims at the production of microbiologically safe products of defined quality specifications by controlling the quality of raw materials and processing operations. HACCP is a preventive method that is systematically applied during food handling, processing and storage by relying on the anticipation, identification and assessment of microbiological hazards as a preventive measure to ensure safe food.

HACCP does not rely on elaborate laboratory analysis of finished products, its principles can therefore be incorporated into various levels of food processing operations. In the artisanal Koobi processing where there are no facilities available for analysing food, the HACCP concept can be carried out by relying on visual observation of the Tilapia and other processing material as well as the unit operations and simple tests, such as time/temperature monitoring and determination of approximate pH with pH strip. The HACCP concept therefore offers the opportunity to integrate formal management of quality into traditional and non-traditional food processing in Ghana.

KEY CONCEPTS OF HACCP

Implementation of the HACCP quality system in food processing is based on certain key concepts and principles. In order that the HACCP system is understood and fully appreciated, the following basic terms are defined as follows: Hazards are any biological, chemical or physical property that may cause an unacceptable health risk or quality defect in food. Their elimination or reduction to acceptable levels is essential to the production of safe food of defined

quality.

A Critical Control Point (CCP) is a raw material, location or process at which control can be exercised to prevent or minimise a hazard.

Monitoring is a planned programme of observations and simple checks of CCPs to ensure that critical limits are not exceeded.

Corrective measures are actions that are taken immediately when a CCP is monitored and found not be under control.

Verifications are traditional laboratory analysis and audits carried out to ensure that the HACCP System is working effectively.

PRINCIPLES OF HACCP

Implementation of HACCP is based on seven principles and for incorporation into *Koobi* processing these are defined as follows:

1. Hazards

   Hazards are any biological, chemical or physical property that may cause an unacceptable health risk or quality defects such as histamine associated with *Koobi* processing. Their elimination or reduction to acceptable levels is essential to the production of safe food of defined quality.

2. Critical Control Points

   Operational steps which can be controlled to eliminate or minimise the occurrence of the identified hazards.

3. Critical Limits Target levels and tolerances, which should be met to ensure that each identified CCP is under control. They are stated as chemical, physical, biological or sensory parameters which are easy and rapid to

monitor such as apperance, odour and pH.

4. Monitoring

A control plan is established to monitor each CCP to ensure that it is under control that is the critical limits are not exceeded.

5. Corrective Actions

Measures to be taken when the monitoring results show that particular CCP is not under control.

6. Documentation and Record Keeping System.

The commitment of artisanal fishermen and *koobi* processors and the systems procedures and working instructions that are described and assembled in a manual.

7. Verification

Verification is carried out to ensure that the HACCP system is working correctly through a review or audit of the system as well as chemical, physical and microbiological analysis of raw materials, intermediary and the finished product.

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Fresh Tilapia were obtained from the Weija lake, near Accra. These fishes were stocked in an ice-chest filled with ice and transported to the laboratory within thirty minutes of purchase in a vehicle. Solar Salt, used for traditional fish curing was obtained from Panbros Salts Ltd. Accra.

3.2 METHODS

Field surveys were conducted on processors of *koobi* and also artisanal fishermen A semi-structured questionnaire was used. (Appendix 1).

3.2.1 Survey of Fishermen

Questionnaires were used for the survey (Appendix 1) as well as observation of fishermen at work. Twenty (20) artisanal fishermen were interviewed.

3.2.2 Survey of *Koobi* Processors

The survey was in the form of interviews guided by a questionnaire (Appendix 1) as well as observation of the processors at work. Twenty (20) *Koobi* processors were interviewed.

3.3 LABORATORY WORK

3.3.1 Processing of Koobi

The fresh Tilapia were scaled. The fish were then gutted through a slit at the anal opening and washed clean with water. After removing the entrails and

washing the opened fish, coarse salt was rubbed onto the flesh, the gills and the belly cavity.

Some of the fishes were immersed in sodium benzoate before salting and drying. The fishes were then arranged in layers alternating with salt. .Salting took one day after which the Tilapia were dried for seven days at various temperatures.

3.3.2 Salting Levels

Various amounts of Salt were used for the processing of *Koobi.*

(a) *High Salt level (50 percent)*

A salt quantity corresponding to 50 percent of the body weight of the Tilapia was used as high salt level according to Abbey *et al.,* (1995).

(b) Intermediate Salt level corresponded to 25 percent of the body weight of the Tilapia Abbey *et al.,* (1995).

(c) *No Salt*

No salt at all was added to the Tilapia.

(d) *Artisanal Salting*

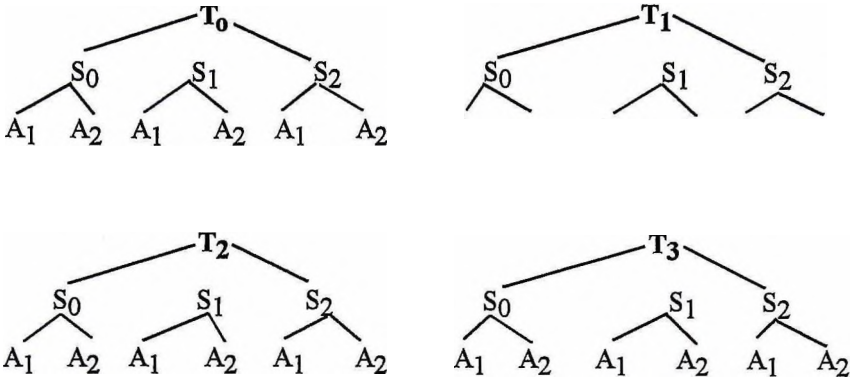
Adequate amounts of salt was added to the Tilapia, typical of the way, salting is done by the artisanal *Koobi* processors.

*Plates 1-4 show stages in *koobi processing.*

3.3.3 The use of sodium benzoate

Some Tilapia samples were immersed in 0.4 percent sodium benzoate for either one hour or two hours before been given varying salt treatment and dried at different temperatures as illustrated in the experimental design below.

The experimental design



where T_0 j_s ambient temperature, ^1 is solar dryer temperature, ^2 is refrigeration temperature and T3 j_s freezing temperature.

S_0 is No salt, S1 is intermediate salt and S2 is high salt.

A] is one hour immersion of fish in 0.4 percent sodium benzoate, A2 is two hour immersion of fish in 0.4 percent sodium benzoate.

3.4 CHEMICAL ANALYSIS

3.4.1 **Histamine Analysis**

The colorimetric method for histamine determination was used. Principle: This method relies on the reaction of histamine with a reagent to produce a coloured complex. The method usually involves a coupling reaction with diazotized aromatic compound followed by a clean-up to exclude interfering compounds. (Hardy and Smith 1976).

REAGENTS

DIAZOTISED COMPOUND

(a) Prepared by dissolving 0.0052 moles (0.894g) of P-bromoaniline in 9ml concentrated HC1 and diluted to 100ml with water.

(b) After chilling, 2ml of solution was added to 2ml chilled 5 percent Na $N0_2$ in a 100ml volumetric flask chilled in ice-bath.

(c) After 5 minutes, 5ml of 5 percent Na $N0_2$, was added slowly and the volume diluted after standing for another 5 minutes.

(d) The solution was allowed to stand for 15 minutes but not more than 12 hours prior to use (Grudemeier and Andrews 1965).

***Tri Chloroacetic Acid (TCA)** — 2.5 percent TCA was prepared for use.

*** Acetate Buffer** — ,2N: 4.025g of sodium acetate was added to 2.3ml Acetic acid and made up to 200ml (Normally 1 litre is prepared). The pH of the solution was standardised to 4.63.

Sodium Carbonate Anhydrous — Na_2C0_3 - 5 percent was prepared.

***Clean up Column** — 12mm internal diameter

***Amberlite CG-50 Resin** (100-200 RESIN)— lg of resin in 10ml 0.2N acetate buffer pH 4.63 was prepared. The slurry was poured into the column which has previously been filled halfway with tap opened. The column was washed with

100ml of the acetate buffer and prepared for use.

Sample Preparation

lOg of the minced flesh of tilapia was blended with 100ml 2.5 percent T.C.A solution and then filtered.

Clean up of Sample Solution

The clean-up of this solution was accomplished by ion-exchange chromatography.

(i) The TCA sample solution was neutralized to PH 7 with IN KOH. Seventy-five(75)ml of this neutralized solution was applied to the prepared column.

(ii) The column was washed with 100ml acetate buffer to remove interfering substances.

* Histamine was eluted with exactly 25ml of 0.2N HC1.

*A blank determination using similar volume of2.5 percent TCA was performed and the process outlined above followed.

*lml of HC1 eluate was added to 15ml 5 percent Na_2C0_3 in a stoppered test tube previously chilled in ice water bath,

*2ml of chilled diazotised reagent was added to the mixture and allowed to stand at 0°C for 10 minutes prior to absorbance measurement.

*Absorbance was determined at 495mm using 0.5ml distilled water as a reference.

♦Standards were prepared using 1ml aliquots of histamine solution (0-50ug histamine/ml 0,2 NH_4C1) for a standard curve.

Glass Ware

100ml volumetric flask

Test tube with corks

Beaker

Waring blender

Calculation

Concentration in Ug/g = 3.3 x F/E x W concentration (ug/ml) from graph,

where F = volume after neutralisation

E = volume of extract after Alteration

3.4.2 Sodium Chloride Analysis

The salt levels was estimated by titrating against silver nitrate (AOAC, 1990).

Reagent

(a) Silver nitrate standard solution — O.IN and standardised against O.IN NaCl containing 5.844g of pure dry NaCl/1.

(b) Ammonium thiocyanate standard solution. O. IN and standardised against O.IN Ag $N0_3$

(c) Ferric Indicator — standard solution of $Fe\_NH_4(S0\ J.,.\ 12H,,0$.

Determination

A known volume of O. IN Ag $N0_3$ solution (more than enough to precipitate all chlorine as AgCl) was added to the sample. Then 20ml $HN0_3$ was also added. The mixture was then boiled on a hot plate until all solids except AgCl dissolves

(usually 15 minutes). The solution was titrated with 0. IN ammonium thiocyanate solution until all the solution becomes permanent light brown.

Calculation

Percent salt in lOOg of sample is given by

$$\frac{(35\text{-}T)\text{x } 0.005844\text{x}100}{\text{weight of fish}}$$

where $35cm^3$ is the volume of $AgN0_3$ added and T is the titre.

3.4.3 Moisture Content

This involves a measurement of free water content on evaporation. The dish and lid were washed clean with vim. These were rinsed thoroughly with distilled water and dried in an oven for 1 hour to *lVi* hours. The can and lid were then cooled in a desiccator for 45 minutes and weighed to a constant weight.

Five grammes (5g) of the sample is then weighed into the can. After this the dish with the lid and the sample is dried in a fan-driven oven at 104°C for 4 hours and then cooled to a constant weight.

CALCULATION

(can + sample) - sample = lot of sample

(can + sample) - can + sample on drying

= Loss in lot = moisture content

$$\frac{\text{Loss in lotx } 100}{\text{wt. of sample}} = \text{percent moisture}$$

(AO AC 1990)

3.4.4 Water Activity

Water activity (a_w) was measured with an **HI** 8564 Thermo-Hygrometer. This method consists of sealing the fish in a container which is held at a constant temperature until the water vapour in the air reaches equilibrium with the moisture in the product. The humidity above the product is then determined by a probe. The meter measure both temperature and equilibrium relative humidity. The a_w was determined from the relationship

$$a_w = \frac{ERH}{100}$$

where a_w = water activity and

ERH = equilibrium relative humidity percent)

3.4.5 **Total Viable Count**

Ten grammes (10g) of the fish was weighed into a sterile stomacher bag. A prepared 90ml sterile peptone water was added and then blended in a stomacher blender for 60seconds.

One ml of the blended fish sample was pipetted into a sterile 9ml peptone water and shaken for 60seconds. This is known as the 10_{-1} dilution. Another 1ml was pipetted from the 1 O'[1] into another sterile 9ml peptone water. This was also known as 10^{-2} dilution. Serial dilutions are continued in this fashion to 1 O'[6]dilution. One ml of these dilutions were pipetted aspetically into sterile petri-dishes and about 15-20ml of the required agar medium cooled to about 40-45°C was poured aseptically and gently into the petri-dish containing the fish mixture. This was swirled gently to get a homogenous mixture and then allowed to settle.

This was finally incubated at 37°C for 24hours after which viable microbial colonies were counted.

3.5 HISTAMINE AS INDEX OF SPOILAGE

3.5.1 **Post-catch deterioration Index**

Calculated as:

$$\frac{\text{Histamine levels after catch - Histamine levels at fresh catch}}{\text{Histamine level at fresh catch}} \text{ xlOO}$$

3.5.2 **Processing Depression Index**

Calculated as:

$$\frac{\text{Histamine level after processing - Histamine levels at fresh catch}}{\text{Histamine level at fresh catch}} \text{ xlOO}$$

3.5.3 **Storage Deterioration Index**

Calculated as:

$$\frac{\text{Histamine levels at storage - Histamine levels at fresh catch}}{\text{Histamine level at fresh catch}} \text{ xlOO}$$

3.6 DEVELOPMENT OF HACCP SYSTEM FOR SALTING AND FERMENTATION OF TILAPIA

Field observations of artisanal fishermen, *koobi* processors and laboratory analysis of *koobi* were used to develop the HACCP quality system for *koobi* processing.

3.7 ANALYSIS OF RESULTS

The levels of histamine, sodium chloride, moisture, water activity and bacterial counts were analyzed by one-way analysis of variance and paired difference analysis of Scheffe's test and Fischers protected LSD. The correlations with the formation of histamine as explained by sodium chloride levels moisture content, water activity and bacterial counts were analysed by correlation coefficients (Pearson). Calculations were performed using SPSS 6.0.

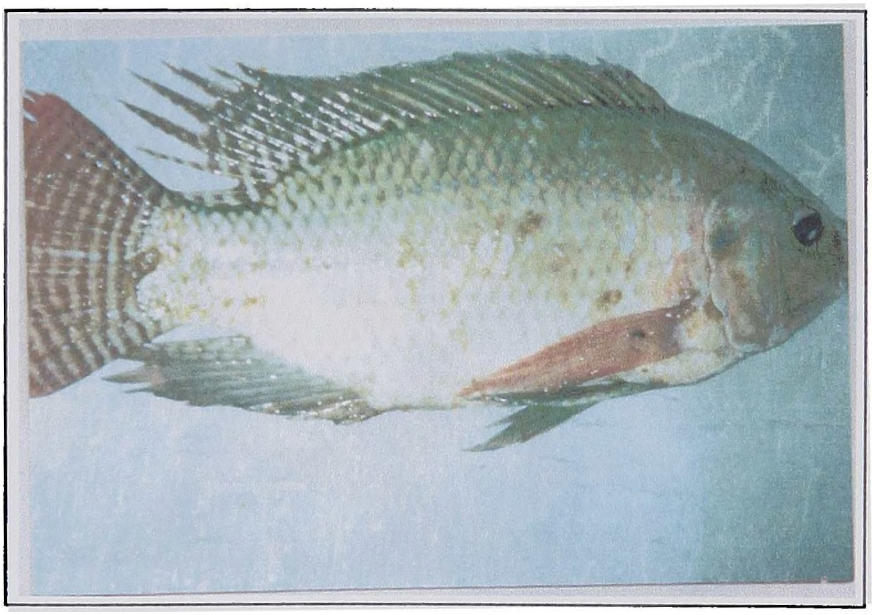


Plate 1: Fresh Tilapia

cite 2 f; Koobi being processed by artisarnal fish processc

Plate 3: Koobi displayed at a retail outlet

Plate 4: Koobi being dried on baskets

4.0 RESULTS AND DISCUSSION

FIELD WORK

4.1. SURVEY OF ARTISANAL FISHERMEN

4.1.1 **Social Profile**

This survey was conducted in order to understand the social profile of the artisanal fishermen and how this might affect their handling practises.

All the twenty(20) respondents were males aged between 19 to 60 years. Most of them (70 percent) had primary education or some form of it while few (20 percent) had no primary education at all. A very small number of the respondents (10 percent) had secondary education (O'level) or some level of it. Due to the low educational background of these artisanal fishermen, they tend to rely almost entirely on fishing for their livelihood.

4.1.2 **Post-Catch Handling Practices**

Few of the artisanal fishermen interviewed (10 percent) said they iced the Tilapia soon after catch. Only 15 percent of the respondents indicated having ice in their boats. Delayed icing may therefore be the major contributory factor to histamine production in such fishes.

On the other hand most of the fishermen (70 percent) interviewed said they try to avoid inflicting mechanical injury on the Tilapia. This implied that they use the appropriate fishing net sizes and thus adopted good catching practices.

It was quite evident that the fishermen recognised the need for good catch-

ing as well as post-catch handling practices. The delay in icing the fresh Tilapia was probably out of ignorance for the critical role prompt icing plays in controlling microbial and chemical deterioration of Tilapia.

4.2 SURVEY OF *KOOBI* PROCESSING IN GHANA

4.2.1 **Social profile of *Koobi* processors**

The aim of this survey was to get acquainted with the social profile of the *Koobi* processors and probably have insights into certain practises by the *Koobi* processors.

Twenty (20) traditional fish processors with ages ranging between 18 and 64 years were interviewed. These *Koobi* processors were all women. These processors have a low educational background. Indeed, ninety percent of them had no formal education with the remaining 10 percent having primary education or some level of it. As a result of their very low educational background most of these fish processors may not be privy to fishery information available in books, journals, magazines and the media. Frequent communication with these artisanal fishennem in the local dialects by extension officers would therefore be very helpful.

4.2.2 **Handling, Processing and storage by *Koobi* processors**

Eighty-five percent of the respondents indicated that they ice the fish soon after purchase but the source of the ice and the quantity of ice used varied appreciably. Indeed Seventy percent of them said that they ice the fish immediately after purchase. The impression, therefore, was that most of the processors acknowledge the importance of icing. That was quite encouraging.

Some respondents said that they obtained the ice from friends who have

deep freezers, others also said that they prepared ice from deep freezers they own at home while another group indicated that they purchased the ice from the market.

The quantity of fish in a batch ranged from 50 to 70. This may be in excess of the amount of ice procured (£1,000-02,000 per batch). The ice to fish ratio may therefore not be enough to control chemical and microbial deterioration under conditions when processing of the Tilapia is delayed. Prompt Processing of Tilapia should therefore be advocated.

Other improper handling conditions such as abusive handling temperatures, use of dirty water for washing the fish, improper packaging and transport of Tilapia and the concomitant bruising of the Tilapia, cited by other fishery researchers as possible causes of fish deterioration were also investigated. Ninety-five (95) percent of the respondents, agreed that exposing the Tilapia fish to the sun is not a good practice. Their explanation was that the Tilapia gets spoilt faster when it is exposed to the sun. The impact of abusive handling temperatures on histamine production seems therefore to be very low. All the respondents agreed that washing the Tilapia fish with dirty water was not good. They explained that this practice can cause illness when the fish is consumed.

Dirty water contaminated with faecal matter could harbour coliforms such as *E. coli* which are prolific histamine producers in foods. It is therefore important that clean, uncontaminated water is used to wash the fish.

Fifty percent of the respondents used vehicles, 20 percent used head load, 20 percent used push cart while 10 percent used baskets to convey their Tilapia. None of the respondents claimed any training in the handling of fish.

They said that their knowledge in fish handling has been acquired through

observing fellow processors as well as many years experience in the *Koobi* processing business. There was certainly the danger of bruising the Tilapia and thereby facilitating microbial entry into the fish when they are packed into baskets.

There was no variation or very slight variation in the way *Koobi* was processed among the respondents.

All the respondents stated that they washed and scaled the fish after which they, washed and salted the fish before finally drying the fish for about a week on mats. Most of the artisanal fish processors used coarse salt. Fine Salt causes faster penetration and thus control further deterioration of the fish but causes flaking of the fish.

All the respondents indicated that a greater proportion of the Tilapia they used for processing koobi had been bruised.

On the mode of storage, most of the respondents, (80 percent) stated that they store the *Koobi* in baskets while the remaining 20 percent stored their koobi in pans or trays.

Koobi stored in baskets may be relatively less exposed to microbial and insect infestation and therefore more likely to be free from post-processing or storage contaminations.

It became quite clear during the interviews that the fish processors were aware of most of the bad handling processing and storage practices.

The major problem observed with these *koobi* processors is the general unhygienic conditions of the processing environment as well as the equipment used for processing.

This was found to be consistent with findings by (Dibbs 1961; Kagan 1970; Amu 1973; Bonsu 1976), that fresh fish is not well-handled and stored in Ghana.

It will, therefore, be necessary to undertake some form of sensitization drive to ensure that the hygienic conditions of the equipment as well as the general environments of the koobi processors are improved to minimize the presence of food toxicants as well as pathogens in our foods in general and *Koobi* specifically.

4.3 CONDITION OF RETAIL SAMPLES

Samples of Koobi were purchased from ten (10) outlets and analysed for their Histamine content, sodium chloride levels, moisture content, water activity (aw) and unicubial load. Histamine levies are shown in Figure 4.1 and other parameters shown in Table 4.1 respectively. ANOVA was used to determine a significant or non-significant differences in the mean values obtained and the Scheffe's mean separation was used where there were significant difference in the means.

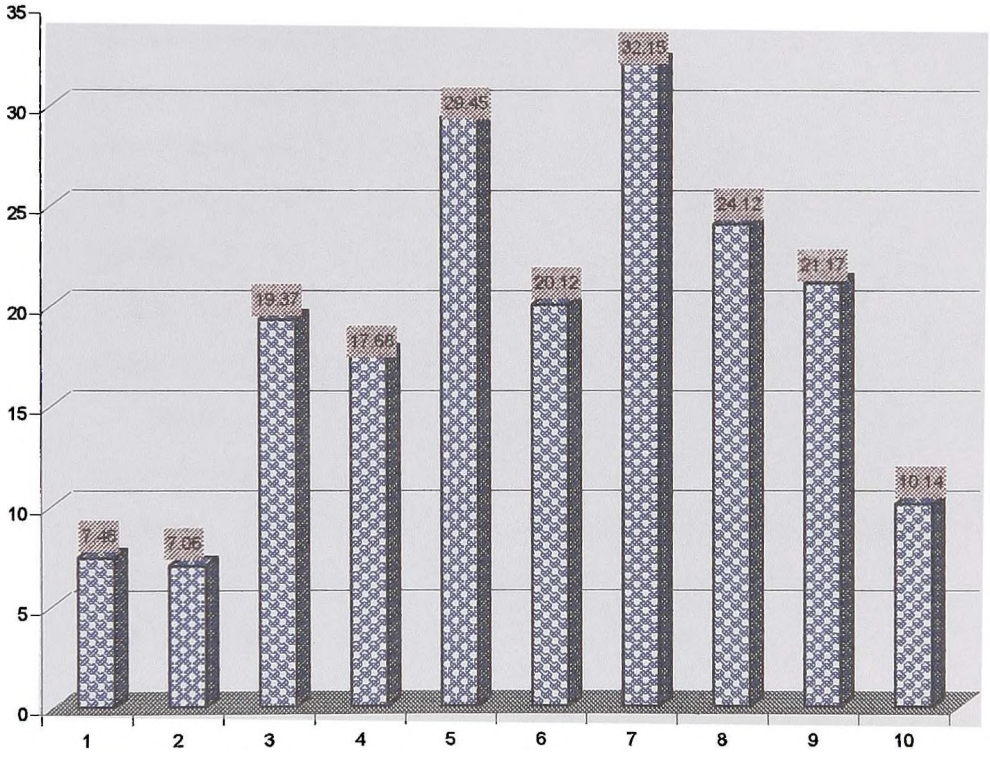
Table 4.1

Sodium chloride, a_w, and Microbial counts in *Koobi* from Different Retail Outlets

Retail outlets sample	Sodium chloride(%)	Moisture content(%)	Water activity (a_w)	Total viable counts
1	15.89	30.18	0.55	$5.93x10^5$
2	17.82	35.17	0.58	$2.42x10^s$
3	19.68	41.44	0.62	$1.53xl0^5$
4	13.39	41.25	0.60	$1.58xl0^4$
5	16.25	40.18	0.60	$9.33x10^3$
6	16.14	30.00	0.51	$9.63x10^3$
7	16.10	40.15	0.61	$1.25x10^s$
8	17.34	39.96	0.62	$9.80xl0^4$
9	15.45	32.60	0.52	$2.54x10^s$
10	16.20	33.18	0.57	$1.28x10^s$

Fig. 4.1 Histamine Condition (ug/g) in *koobi* from Different Retail outlets.

Retail outlet number	Name of market/location	Date of procuments
1.	31st Dec. Market, Accra Central	4/8/98
2.	Kaneshie market, Kaneshie	4/8/98
3.	Mallam Atta, New Town	5/8/98
4.	Tuesday Market, Korle-Gonno	5/8/98
5.	Salaga Market, Accra Central	11/8/98
6.	Kwasiagyaso, Adabraka	12/8/98
7.	La market, Labadi	18/8/98
8.	Control Market, Dansoman	18/8/98
9.	Abeka Market, Abeka	19/8/98
10.	Dome Market, Dome	19/8/98



The Histamine levels in salted and dried Tilapia, *Koobi* from retail outlets were generally very low. The Histamine levels ranged from 7.06mg/g to 32.15mg/g. (Figure. 4.1). Pan *etal.* (1982), however, established that high levels of histamine can be produced in Japanese canned tuna sampled from retail markets.

Histamine production in food is by the decarboxylation of histidine through a reaction catalysed by the enzyme histidine decarboxylase. Geirger, Courtney and Schanakenberg (1944) reported that histamine was generated from autolysis. Research, however, indicates that the decarboxylation reaction results largely from bacterial action (Arnold and Brown 1978; Ababouch *et al.* 1991.

The conditions in the ten (10) retail outlets were quite similar. These outlets were marked by untidy surroundings. Differences in histamine observed among the retail samples should therefore not be attributed to differences in the conditions in the retail outlets.

The histamine status of retail *koobi* sample may be a reflection of the history of the fish. It was established by Yoshinage and Frank (1982) that the composition and type of histamine-forming bacteria in fish can be influenced by factors such as feeding habits, geographical location, fishing gear, nets, season, water temperature, water salinity, the water quality at harvest, the way the product is handled after harvest and market environs. Samples which were handled well and processed more promptly may produce relatively lower histamine levels. On the other hand, samples which were improper handled and delayed for processing may produce higher histamine levels.

Early processing and proper handling of the Tilapia may tend to reduce or even stop the autolytic as well as bacterial action on the fish and may lead to

lower histamine production.

Some retail Koobi samples had histamine levels (around 7.00 ug/g) similar to that observed in fresh Tilapia. In the fresh Tilapia, (microbial decarboxylation of histidine to histamine may be at its lowest) endogenous decarboxylation of histidine to histamine may therefore be the prime source of histamine production. It is thus quite tenable that in those retail samples with histamine content similar to that observed in the fresh Tilapia, endogenous histamine production was the main source of histamine rather than bacterial histamine production. Autolytic enzymes were thus very important in this direction.

The relatively low levels of histamine observed in such retail *Koobi* samples could be attributed to very good post-catch handling, processing and storage practises. It may be a pointer to the fact that if proper post-catch handling, processing and storage practises are adopted, the histamine level in Koobi could indeed be kept at levels comparable to that in the fresh Tilapia (that is pre-processing levels).

It could therefore be stated that in samples with histamine levels comparable to that of the fresh Tilapia, endogenous decarboxylation was the major source of histamine production whereas samples with histamine levels far in excess of that of fresh Tilapia, microbial decarboxylation may be the predominant source of histamine production.

The differences in histamine levels observed among the retail samples may be attributed to varying post-catch handling, processing and storage by the fish processors.

Differences in catching, post-catch handling and storage by fishermen before the fish is purchased by the fish processors could also be an important

factor accounting for the observed differences in histamine levels in the retail samples. The state of the Tilapia before it is processed is therefore very vital. Differences in histamine levels could also be due to differences in the promptness of processing of Tilapia by the artisanal fish processors.

The sodium chloride levels in the retail samples varied from 13.3 9 percent to 19.68 percent (Table 4.1). This range of sodium chloride concentration was too high for microbial histidine decarboxylase activity Microbial proliferation and enzymatic activity may thus be inhibited by the high salt content of the *Koobi* samples.

In laboratory cultures, histamine production by bacteria occurred at 8 percent sodium chloride (Ababouch *etal.,* 1991) and up to 12 percent sodium chloride (Gunaratne and Samarajeewa, 1995).

It is however doubtful that histamine production could go on at sodium chloride levels beyond 12 percent. At such salt levels, only halophilic organisms are favoured and since most of the histamine producing bacteria are not halophilic, histamine production would be checked by the salt levels in *Koobi.* The significant differences in salt content among the retail samples (P = .0001, Appendix II) could be the result of the salt type used. While most artisanal fish processors use coarse salt for curing the Tilapia, a small number of these processors use fine salt for curing the Tilapia. The use of fine salt or coarse salt would result in different levels of salt penetration and absorption.

Salting may result in the removal of water from the fish muscle and thereby reducing the water activity.

Micro organisms require an aqueous environment in which to carry out the solute exchanges accompanying growth and reproduction. A micro organ-

ism which is not in contact with an external aqueous system of reasonable extent is effectively isolated from its environment even though some gases from endogenous processes may continue to be given off for a time and liquid or solid wastes or product of autolysis may accumulate around the cell.

The moisture content of a food is therefore an important factor affecting microbial and enzymatic activity. The moisture content of *Koobi* samples from retail outlets ranged from 30.00 percent to 41.44 percent (Table 4,1). This is in the range of intermediate moisture foods. However, moisture content seems to be an inexact indicator of the susceptibility of a product to microbial activity. A factor which appears to be more closely related to conditions leading to the onset of microbial growth and activity is the water activity of food. The availability of water (a_w), attained at equilibrium is a major factor determining the chemical, enzymatic and microbial stability of foods (Trailer and Christian 1978; Rockland and Stawart, 1981).

The a_w values of *Koobi* from retail outlets ranged from 0.62 to 0.51 (Table 4.1). This range of water activity is too low to support microbial proliferation and enzymatic activity. It is quite apparent that the range of aw (0.62 to 0.51) observed in *Koobi* samples from retail outlets may not be conducive for bacterial decarboxylation of histidine to histamine. The levels of histamine observed in the retail outlet samples may therefore not increase appreciably over prolonged storage of a few weeks or months. The major histamine producing bacteria *{Proteus, Pseudomanas, Escherichia, Shigeila* etc.) are active within a_w range of 1.00 to 0.91 and as such histamine production through microbial action is likely to be greatly inhibited at a_w ranges observed in the *koobi* samples.

There were significant differences in a_w among the koobi samples from retail outlets (P = .0001,Appendix II). These differences could be the result of varying drying time, temperature and storage methods as well as differences in salt concentration in the *Koobi,* The microbial counts were quite low ($2.54x10^3$-$1.28x10^s$). This may be due to the high salt levels which tended to remove water from the fish resulting in low moisture content and water activity. This created conditions which may not be favourable for bacterial growth. Since most of the bacteria capable of histamine production are not part of the normal flora but represent post-harvest contamination during handling, processing and marketing. Cooling, short storage and good handling practices can avoid histamine formation (Ababouch, 1986).

4.4 CONDITION OF FRESH TILAPIA, SKIP JACK TUNA AND YELLOW FIN TUNA

Fresh tilapia obtained from the Weija lake, fresh salted skipjack tuna and yellow fin tuna procured from Pioneer Food Cannery, Tema were anlyzed for their histamine levels, sodium chloride content, moisture content, water activity and microbial counts. The results are shown in Fig. 4.2 and Table 4.2. This was done to establish conditions in the fresh state before processing and storage. It also served to compare the histamine levels in the fresh state of different types of fishes.

Fig. 4.2Histamine conc(ug/'g) in fresh skip jack tuna, yellow fin tuna & tilapi,

Type of fish

1. Fresh skip jack tuna
2. Fresh yellow fin tuna
3. Fresh Tilapia

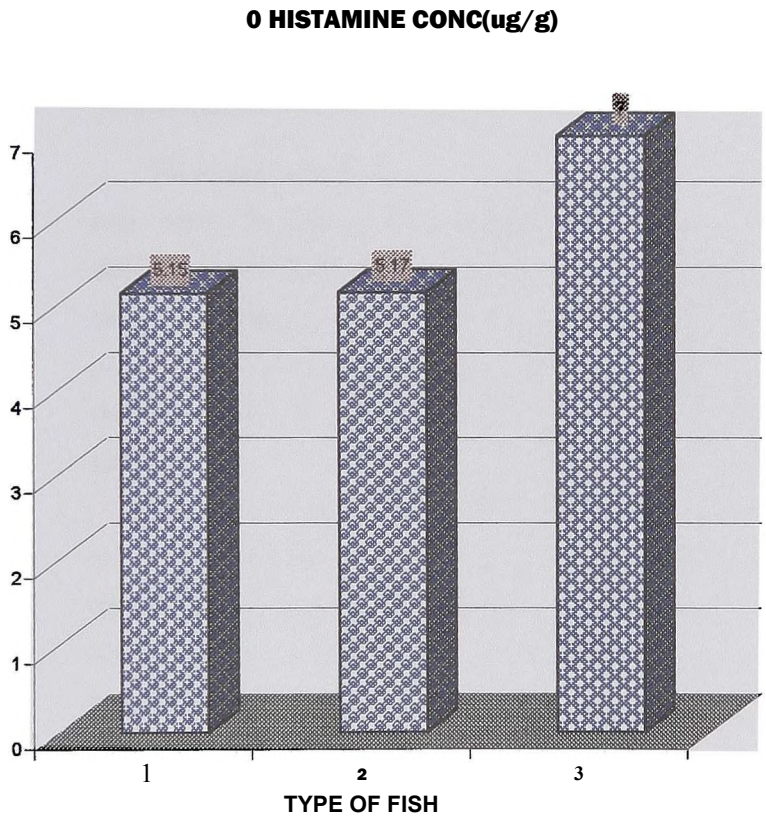


Table 4.2

Sodium chloride, moisture, water activity and microbial counts in fresh Tilapia, fresh salted skip jack tuna and fresh salted yellow fin tuna.

Fish sample	Sodium Chloride (%)	Moisture	Water content(%)	Total Viable Count Activity
Fresh Tilapia	0.8	80.20	0.75	6.80x10'
Yellow fin. tuna	2.41	70.04	0.68	l.OOxlO^3
Skipjack tuna	2.30	69.42	0.68	2.66xl0^3

The histamine levels were 5.15 mg/g, 5.17 mg/g and 7.00 mg/g in skipjack tuna, Yellow fin tuna and Fresh Tilapia respectively. (Fig. 4.2). These histamine, levels were very low and unlikely to pose any toxicological problems in man. This finding is consistent with that by Frank *et al.,* (1981); Nagyayam *et al,* (1985); Femandez-Salguero and Mackil (1987) that the content of histamine in fresh fish is very low and their appearance is therefore indicative of spoilage.

The histamine levels in fresh skip jack tuna and yellow fin tuna were found to be lower than that of fresh tilapia, even though spoiled tuna may contain histamine levels above the toxic limits stipulated by the U.S. Food and Drug Administration. The Tilapia was procured alive from the artisanal fishermen and handled in the best possible manner (like immediate icing and no bruises to the fish). It is thus difficult to explain why the histamine levels in fresh Tilapia was higher than that observed in fresh skip jack and yellow fin tuna which are scombroid fishes (which characteristically possess high levels of free histidine) and therefore expected to contain higher histamine levels even in the fresh state.

The fact that relatively lower levels of histamine are observed in fresh fishes (irrespective of type) may be an indication that evolution of high amounts of histamine occur during the latter stages of handling, processing and storage of fish

In fresh Tilapia, skipjack tuna and yellow fin tuna where histamine levels were relatively low endogenous histidine decarboxylase present in the fish muscle may be responsible for histamine production rather than bacterial histidine decarboxylase. Geiger et al., (1944) noted that histamine formation is through endogenous histidine decarboxylation.

Endogenous histidine decarboxylase may only play a significant role in histamine formation during the fresh state where bacterial population is minimal and therefore bacterial histidine decarboxylase activity is expected to be at its lowest.

The extremely low microbial counts recorded for fresh Tilapia, skipjack tuna and yellow fin tuna (Table 4.3) may be a reflection of the low level of microbial histidine decarboxylase activity in fresh Tilapia and tuna.

The low levels of histamine in fresh skipjack tuna and yellow fin tuna therefore tends to support the assertion by Arnold and Brown (1978); Ababouch and Afilal (1988), that the decarboxylation of histidine to form histamine results largely from bacterial histidine decarboxylase activity. The absence of bacteria with positive histidine decarboxylase activity is therefore vital to ensure minimal production of histamine.

The sodium chloride levels ranged from 0.8 percent to 2.41 percent (Table 4.3). These salt levels are not likely to inhibit microbial proliferation and enzymatic activity. Very low levels of bacterial counts were however recorded. This could be due greatly to the application of ice to the fish as soon as it was caught. Icing of Tilapia, skipjack tuna and yellow fin tuna as well as good handling practices might have prevented bacterial proliferation. It could, therefore,

be inferred that icing and good handling practise played a very significant role in minimising bacterial growth and activity and hence histamine production.

There was a positive correlation between the moisture content (r = 0.88); water activity (r = 0.75); and histamine production (see Appendix XI).

The high positive correlation between water activity and histamine production suggests the importance of water for enzyme activity. Water may influence chemical reactivity in different ways. It may act as a reactant, solvent, change the mobility of the reactants by affecting the viscosity of the food systems. Water may also form hydrogen bonds or complexes with the reacting species. Chemical reaction rates generally accelerate with increasing water activity due to increased reactant mobility.

Most of the histamine producing bacteria require an a_w in the range 1.0 to 0.91. (Table 2.3). The a_w values were 0.75 in fresh Tilapia and 0.68 in both skipjack tuna and yellow fin tuna. These values may not be enough for the growth and activity of histamine forming bacteria.

A high negative correlation (r = -0.84) was observed between the water activity and salt content. (Appendix XI). This suggests a lowering of the water activity through increased salting .

The bacterial flora on newly-caught fish depends on the enivronment in which it is caught rather than on the fish species (Shewan, 1977). Generally, fish caught in very cold, clean waters carry lower numbers whereas fish caught in warm water have slightly higher counts. The slight difference in microbial count observed between Tilapia and the two species of tuna may be a reflection of the different environments of the Tilapia and Tuna species.

The low microbial counts and histamine levels in fresh Tilapia, skipjack

tuna and yellow fin tuna places heavy reliance on proper handling, processing and storage methods to ensure that higher microbial counts are not developed during these stages which may lead to production of elevated levels of histamine,

4.5 EFFECT OF POST-CATCH HANDLING CONDITION ON HISTAMINE PRODUCTION AND OTHER FACTORS IN TILAPIA

Handling of Tilapia constitutes an important post-catch condition. Abusive handling of fish has been cited as one of the major causes of fish deterioration.

Tilapia, under various handling conditions were analyzed for their histamine levels, sodium chloride content, moisture content, water activity and microbial counts. The histamine levels are indicated in Fig. 4.3 while the other parameters are shown in Table 4.3

The histamine and sodium chloride levels werre recorded on a fresh weight basis.

Fig. 4.3 The effect of various post-catch handling conditions on histamine production in *koobi*

Handling Conditions

1. Bruised Tilapia, iced immediately after purchase from fishermen.
2. Unbruised Tilapia but delayed icing for one hour after purchase from fishermen.
3. Unbruised Tilapia but delayed icing for 24 hours after purchase from fishermen.
4. Bruised Tilapia and delayed icing for one hour after purchase from fishermen.
5. Bruised Tilapia and delayed icing for 24 hours.
6. Unbraised Tilapia iced immediately after purchase from fisherman (control).

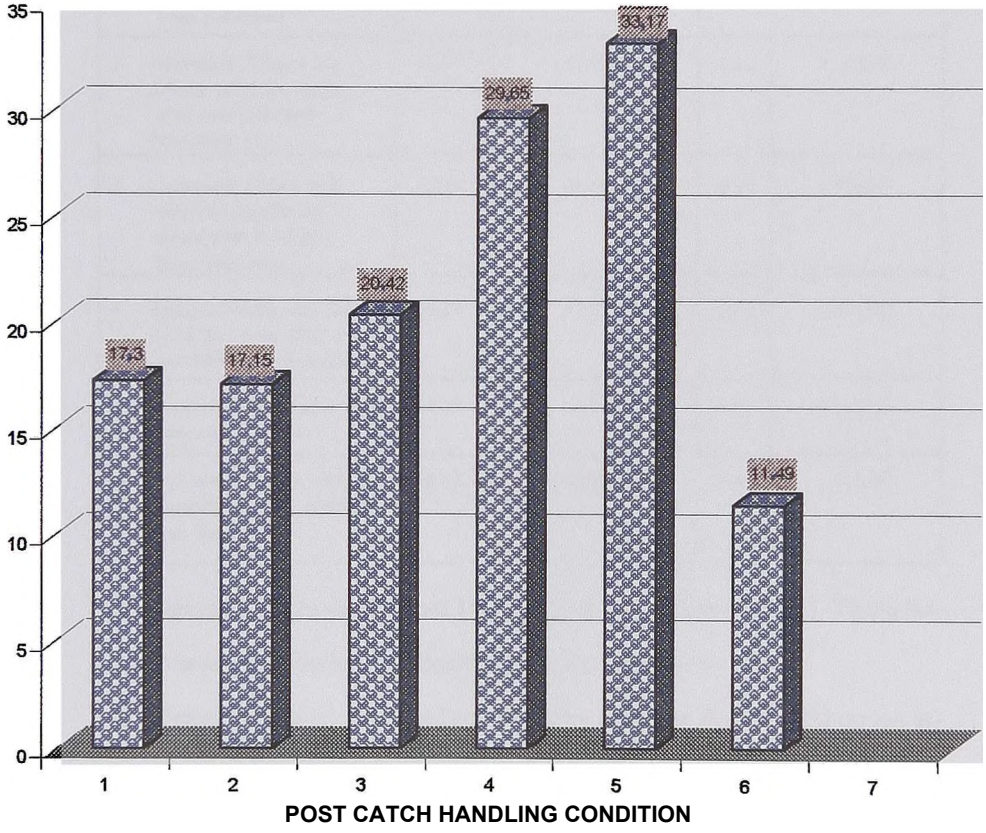


Table 4.3

NaCl, moisture, aw and Microbial Counts in Tilapia under various Post-catch handling conditions before salting and drying for one week

Post-catch handling condition	Nacl (%)	Moisture content (%)	a_w	Total viable count
1. Bruised Tilapia, iced immediately after purchase from fishermen	14.99	47.10	0.63	$3.04x10^4$
2. Unbruised Tillapia but delayed icing for lhour after purchase from fishermen	15.07	47.15	0.62	$2.76x10^4$
3. Unbruised Tilapia and delayed icing for 24 hours after purchase from fishermen	15.23	46.52	0.61	$1.52x10^5$
4. Bruised Tilapia and delayed icing for lhour after purchase from fishermen	15.27	47.01	0.63	$2.33xl0^3$
5. Bruised Tilapia and delayed icing for 24 hours	17.19	44.13	0.64	$9.76xl0^2$
6 Unbruised Tilapia iced immediately after purchase from fishermen	16.82	48.76	0.65	$3.16xl0^1$

The histamine levels varied from 11.49ug/g to 33.17jig/g (Fig.4.3). These histamine levels are too low to constitute any health hazards.

Unbruised Tilapia iced immediately after purchase from the fishermen attained a temperature range of 10-13°C and had the lowest histamine level (11.49mg/g). This may be due to the fact that icing minimized microbial and enzymatic activity and thereby prevented the production of elevated levels of histamine. Enymic activity is generally decreased by low temperatures and increased by a rise in temperature up to a point (optimum temperature). Icing may thus be very important in reducing the activity of autolytic enzymes in the

fish as well as the microbial enzymes and thereby lowering histamine production considerably. The histamine levels in samples numbers 1, 2 and 3 did not differ significantly from each other (Appendix IV).

The highest histamine levels were recorded for Tilapia samples that were bruised and also icing delayed.

There was no significant difference in histamine content between bruised samples in which icing was delayed for one hour and those in which icing was delayed for 24 hours (Appendix IV). However, unbruised Tilapia, iced immediately after catch differed significantly from Tilapia samples which were braised and icing also delayed in their histamine levels. The flesh of healthy live or newly-caught fish is sterile as the immune system of the fish prevents the bacteria from growing in the flesh. When the fish dies, the immune system collapses and bacteria are able to proliferate freely. Micro organisms enter the muscle tissue of the fish via the skin from the water in which the fish were held. The source of these micro organisms could have been the gill cavity, the gut or the environment.

Bruising of Tilapia (as a result of improper fishing practices such as the use of wrong size of fishing nets) can therefore cause a greater number of bacteria to enter the fish leading to enhanced microbial and enzymatic activity. This may in turn cause the production of higher amounts of histamine.

It was pointed out by Putro and Saleh (1985) that the histamine content of skipjack tuna in which icing was delayed was significantly higher than those immediately iced on board the fishing vessel.

It may be possible to have a low microbial population comprising a high proportion of histamine forming bacteria.

This may explain why bruised Tilapia in which icing was delayed for 24hours had a relatively lower microbial load (9.76x102) but yet registered the highest histamine levels (Figure 4.3).

It is quite obvious that good handling practices may be required to curb histamine production. The type of handling practise adopted (that is whether the fish is bruised, unbruised, iced immediately or delayed icing) did not significantly affect the Nacl levels, moisture content and water activity as indicated by P values of 0.2229, 0.5826 and 0.2058 for Nacl, moisture content and water activity respectively. Even though, the bacteria counts of the different samples were also not significantly different from each other (P = 0.3098) the histamine levels showed significantly differences (P = .0001). This may be an indication of the cummulative effect of these factors (Nacl, moisture, water activity and microbial counts) on histamine production.

Unbruised Tilapia in which icing was delayed for 24 hours had the highest bacterial count (1.52×10^5, Table 4.3). This is an indication of the inhibitory effect of icing to bacteria proliferation and highlights the importance of prompt icing. On the other hand bruised Tilapia, iced immediately after purchase from fishermen recorded quite a high bacteria load (3.04×10^4, Table 4.3). Brusing of Tilapia may leave the fish more valuerable to bacteria invasion and proliferation.

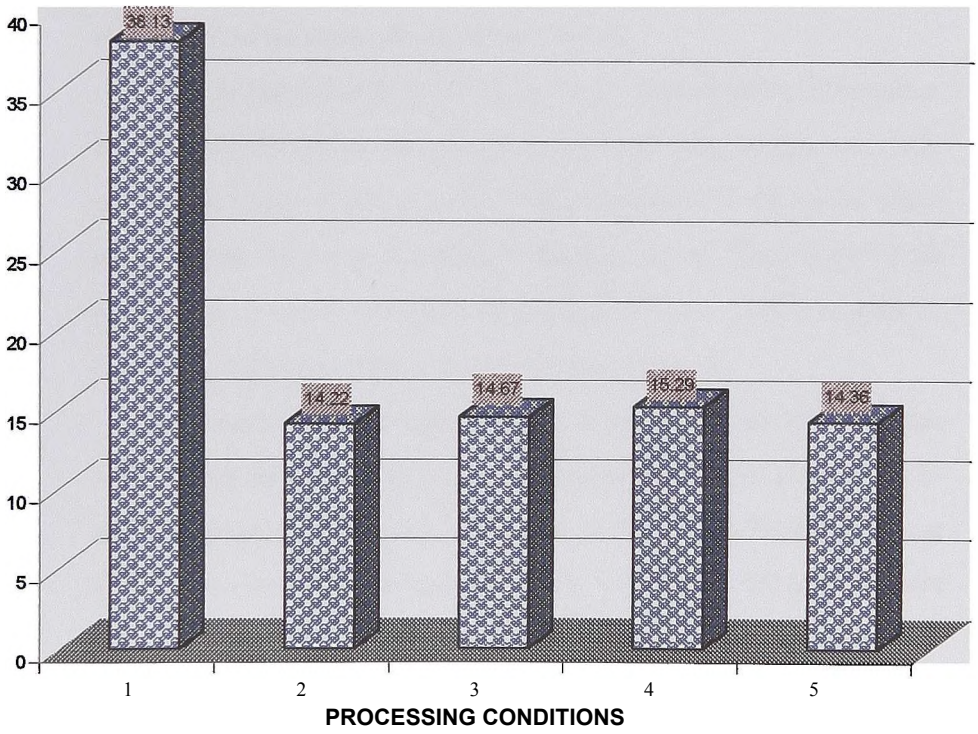
4.6 EFFECT OF VARIOUS PROCESSING CONDITIONS ON HISTAMINE PRODUCTION AND OTHER FACTORS IN TILAPHA

Samples of Tilapia subjected to various processing conditions (gutting, scaling, salting and steaming) were analysed for their levels of histamine, Nacl, moisture,

Fig. 4.4 The effect of various processing conditions on histamine production in *koobi*

Processing Condition

1. Gutted, Scaled and Unsalted *Koobi*
2. Gutted unsealed and normally salted *koobi*
3. Gutted, scaled and normally salted *koobi*
4. Ungutted, scaled and normally salted *koobi*
5. Gutted, scaled and heavily-salted *koobi*



water activity and microbial counts. The results are indicated in Fig. 4.4 and Tables 4.4 and 4.5.

4.6.1 Effect of gutting, scaling and salting on histamine production and other factors in Tilapia

The histamine contents of gutted and ungutted *Koobi* were 14.36mg/g andl5.ug/g (Fig. 4.4) respectively and these did not differ significantly from each other.

This observation seems to be consistent with findings by Orejana *et al.* (1983) that the ungutted frigate mackerel showed slightly higher histamine levels than that of the gutted sample on the 12th day.

Work by Hardy and Smith (1976), however, contradicted this observation. It was observed by Hardy and Smith that histamine was higher in gutted mackerel than in ungutted mackerel. This was probably due to the spread of gut mesophiles or visceral proteinases over the flesh of the mackerel during the gutting process which could have led to the production of elevated levels of histamine (The viscera contain active proteolytic eraymes).

This assertion is supported by the observation that mackerel muscle treated with partially purified typsin-like visceral proteinases showed pronounced increase in histamine production (Pan and Kuo 1983); Smith, Hardy and Young (1980), however, found that mackerel caught in March (9-16°C ambient) there was no difference in the rate of histamine production between gutted and ungutted fish and there was only a marginal difference in May, when the temperature rose to 18.30 at certain times. However, in July, when the temperature range was 12°C-23°C, the ungutted fish produced 37mg histamine/1 OOg flesh in four hours and the gutted fish 40mg in ten hours.

The benefit of gutting may be the removal of microganisms as well as enzymes which could contribute to histamine production. Scaled *Koobi* and Unsealed *Koobi* did not differ significantly in their histamine contents. However, scaled *Koobi* produced a slightly higher histamine level than the unsealed *Koobi.* This may be attributed to the scales of the Tilapia serving as an additional barrier for microbial entry into the muscle of the fish.

If cells are bathed in a hypertonic solution, the cells will shrivel because of a net transfer of water out of the cells. This phenomenon known as crenation and results in a lowering of water activity of the cell. This can be employed in food preservation by salting. The surface of the Tilapia is treated with salt and thereby forms a solution hypertonic to bacteria cells. Bacteria on the Tilapia then tend to shrivel and die.

Salting, may also inhibit some enzyme systems and also affect the state of the fish proteins and thereby curbing the action of both autolytic proteases and microbial enzymes and thus lower the histamine production in *koobi* considerably.

Unsalted Tilapia, showed the highest level of histamine. This tends to indicate the inhibiting role of salt to histamine production. The sodium chloride levels were similar in both the gutted and ungutted *Koobi.*

In scaled *Koobi* and the unsealed *Koobi,* there was a marked difference in the salt levels. Scaled *Koobi* had the higher sodium chloride level (14.98 percent) while the unsealed *Koobi* had sodium chloride levels of 10.23 percent. The lower salt levels observed in unsealed *Koobi* could be the result of the scales preventing easy penetration and absorption of salt. Scaling is thus an important operation on which permits a direct contact between the fish and the curing agent (that is the salt).

There were no significant differences between the gutted and ungutted *Koobi* in their moisture and water activities.

There was however a significant difference between the gutted and ungutted *Koobi* in their microbial load. The microbial count in the ungutted *Koobi* was markedly higher than in the gutted *Koobi.*

Scaled and unsealed *Koobi,* showed similar levels of moisture content as well as water activity (Table 4.4). Unsalted Tilapia had a relatively higher microbial count. High salt levels checks microbial poliferation and therefore its absence tends to favour microbial growth.

Table 4.4
Sodium chloride, moisture water activity and microbial counts in "Koobi" given various treatments and sun-dried for one week

Treatment	NaCl(%)	Moisture(%)	a_w	Total viable count
Gutted + Normal Salting	15.03	49.80	0.65	3.32 x 10‘
Ungutted + Normal Salting	15.77	48.16	0.65	1.56 x1O 4
Scaled + Normal Salting	14.98	49.52	0.65	2.79 X 10^2
Unsealed + Normal Salting	10.25	50.37	0.63	1.81 x1O 2
Gutted Scaled and Unsalted	0.70	48.81	0.66	3.49 x 10^s

4.6.2 The effect of thermal processing on histamine production and other factors in Tilapia.

Three separate batches of Tilapia were procured from the Weija lake on separate days in the fresh state and iced immediately after purchase. Each bach con-

sisted often (10) tilapia. These tilapia samples were sent to the laboratory, steamed and then analysed for the level of histamine, sodium chloride, moisture water activity

The histamine levels in steamed Tilapia ranged from 8.19mg/g tol3.47mg/g (Table 4.5).

Table 4.5

Histamine, moisture, aw and microbial counts in steamed Tilapia.

Sample	Histamine Concentration (ng/g)	NaCl (%)	Moisture (%)	a_W	Total viable count
batch 1	8.19	0.7	72.16	0.70	0.00
batch 2	13.47	0.8	70.17	0.65	0.00
batch 3	13.25	0.8	70.24	0.69	0.00

The level of histamine in fresh Tilapia is around 7.00(j,g/g. The fact that the histamine levels in the steamed Tilapia was more than that of fresh Tilapia may be an indication that heating does not reduce the histamine levels already formed. It is quite conceivable that histamine levels might even increase marginally during heating.

However, the low levels of histamine in the three (3) batches could be attributed in part to the heating conditions which might have inhibited microbial and enzymatic activity as implied by the zero microbial count (Table 4.5).

The significant difference observed between batches 2 and 3 on one hand and batch 1 on the other hand in terms of histamine content may be a measure of pre-steaming histamine levels.

4.7 EFFECTS OF VARIOUS STORAGE CONDITIONS ON HISTAMINE PRODUCTION AND OTHER FACTORS

Freshly caught Tilapia was stored under different condiitions of temperature namely frozen storage and storage at ambient temperatures.

These samples were then analysed for their histamine levels and other factors such as Nacl levels, moisture content water activity and total viable count. The result for the histamine levels in Tilapia under frozen storage for 12 weeks and that at ambient storage for 8 weeks are displayed in Figure 4.5 and Figure 4.7 respectively.

The effect of frozen storage time on water activity in *Koobi* was also investigated and the results shown in Figure 4.6. While the Nacl levels, moisture content and total viable count for the two storage conditions are indicated in Tables 4.6 and 4.7.

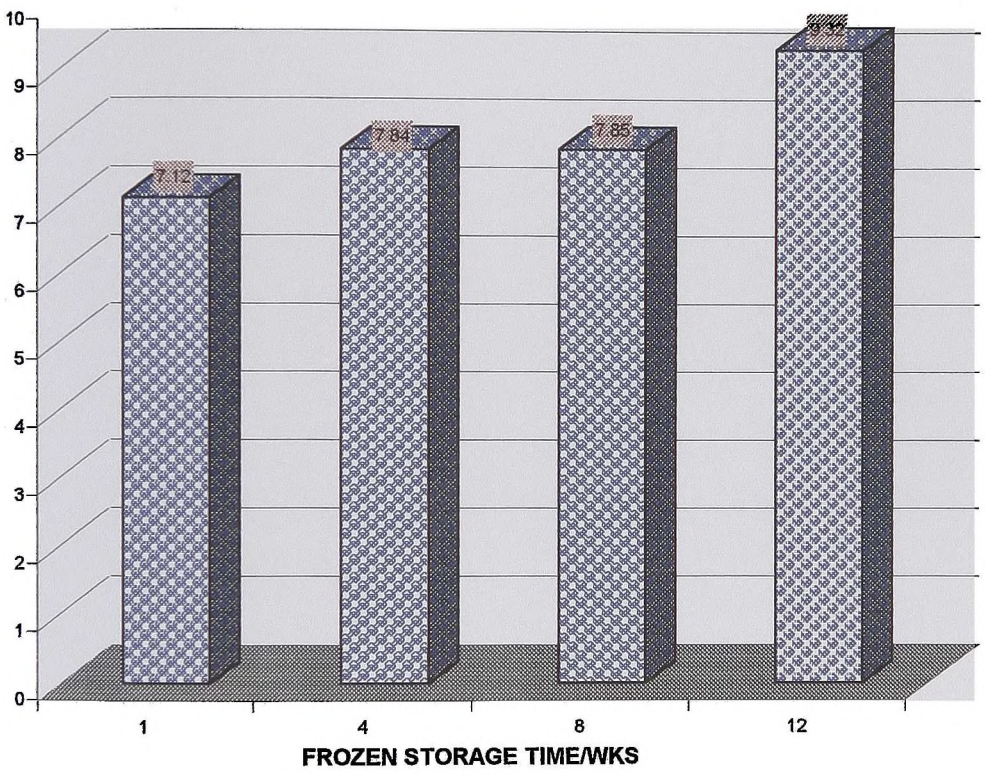
Table 4.6
Sodium chloride, Moisture content and microbial counts in Tilapia kept under frozen storage

Time/weeks	NaCl(%)	Moisture(%)	Total viable count
1	16.48	69.18	$2.15xl0^2$
4	15.01	68.24	0.00
8	15.13	62.17	0.00
12	16.20	60.15	$1.67xl0^3$

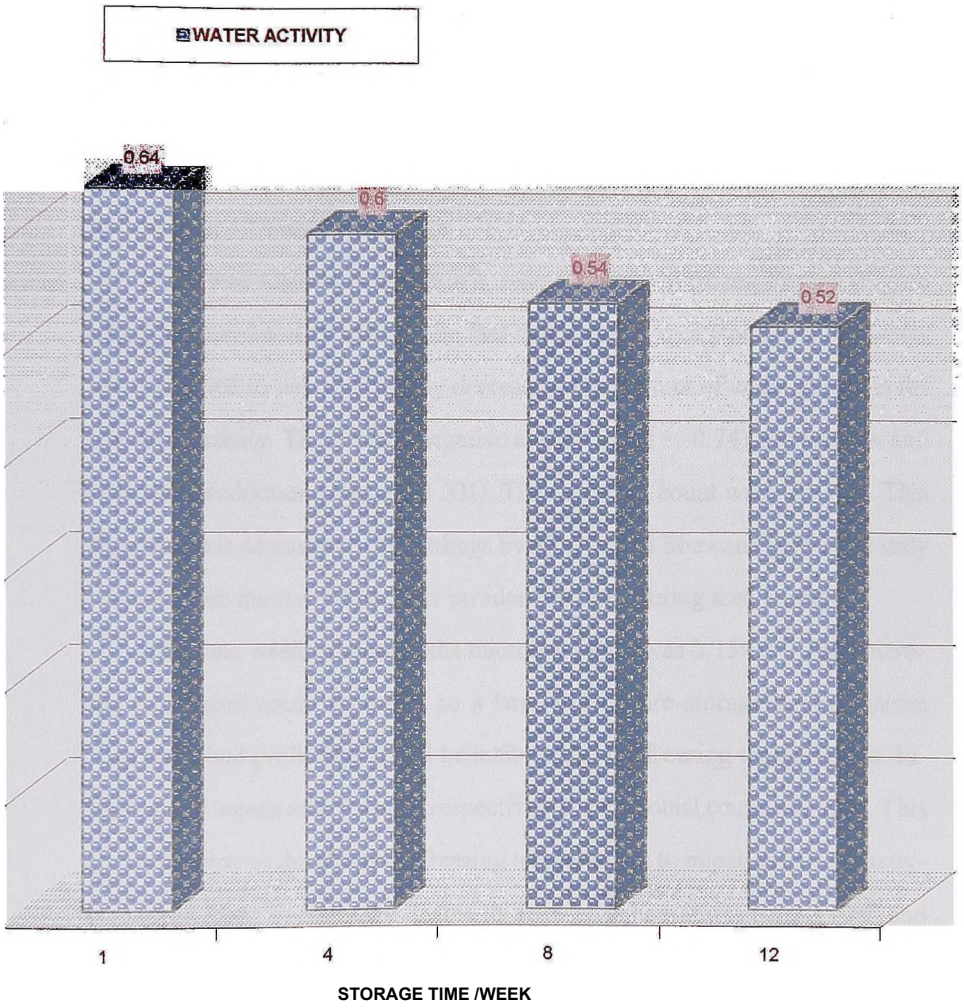
4.7.1 Histamine levels and other factors in Tilapia under frozen storage.

The histamine levels increased from, 7.12mg/g to 9.32mg/g (Fig.4.5) over the twelve weeks of frozen storage of Tilapia. This marginal increase in the evolution of histamine indicates the importance of freezing temperatures in minimizing histamine production. This observation conforms to findings by Ababouch

Fig. 4.5 The effect of frozen storage on histamine production in *koobi*



Fig, 4.6: Effect of frozen storage time on water activity(a_w)in *koobi.*



(1982) that histamine accumulates at a very slow rate at storage temperature of 0°C or below. It is, therefore, possible to keep Tilapia under storage for several months without evolution of marked histamine levels.

Work by Femandez-Salguero and Mackie (1979) on mackerel also established that the histamine levels in sterile muscle and mince remain steady or show only a slight increase even after 25 days at 0°C.

The moisture content of the Tilapia decreased from 69.18 percent to 60.15 percent after 12 weeks of storage.

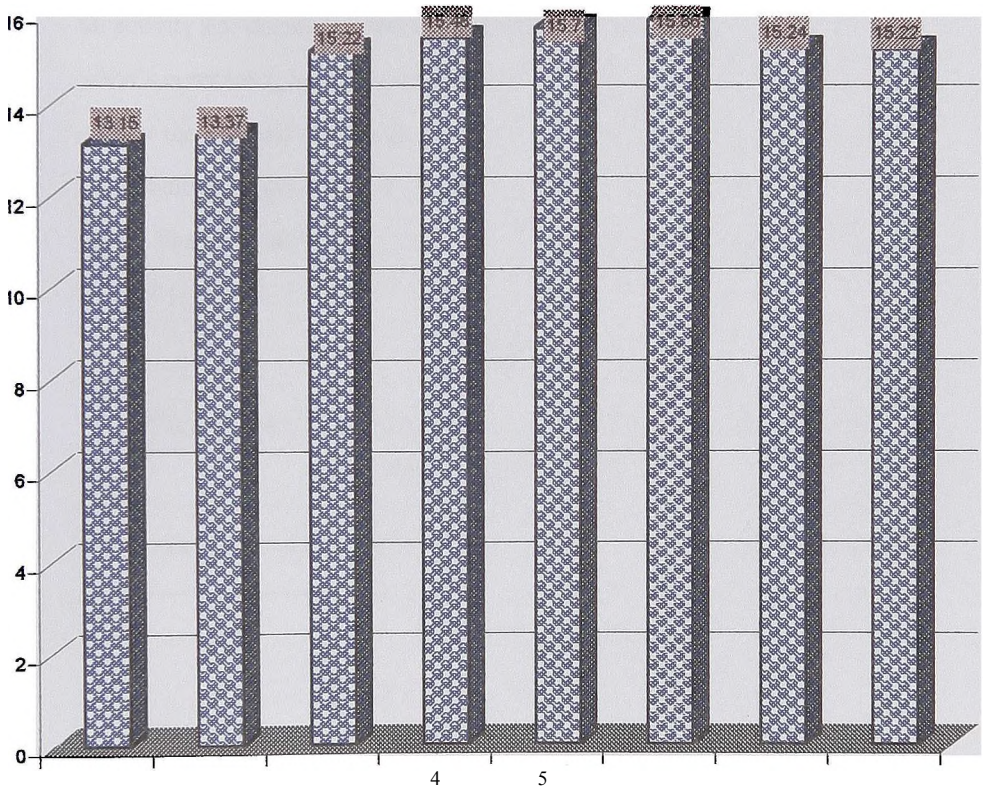
There was a steady decrease in a_w over 12 weeks of frozen storage. (Figure 4.6). This may be attributed to the fact that under frozen storage, more water was converted to ice, and thereby decreasing the amount of water available for microbial activity. There was a negative correlation (r = -0.74) between aw and histamine production (Appendix XII). The microbial count was very low. This observation is consistent with findings by Murray and Shewan (1979) that only a very limited number of bacteria invaded the flesh during iced storage.

After one week of storage the microbial count was $2.15x10^2$. This microbial population could represent to a large extent, pre-storage contamination since microbial proliferation will be inhibited by the freezing temperatures. Indeed after 4 weeks and 8 weeks respectively the microbial count was zero. This is an indication of the ability of freezing temperatures to impair microbial activity. Indeed many bacteria are unable to grow at temperatures below 10°C and even psychrotrophic organisms grow very slowly, and sometimes with extended lag phases when temperature approach 0°C. However, after 12 weeks of storage, the microbial count rose to 1.67×10^3 (Table 4.6).

This rise in microbial count may be more attributable to contamination during thawing rather than microbial growth/proliferation during storage.

Fig 4.7 Effect of storage (ambient) on histamine production in *koobi,*

@ HISTAMINE CONC (Ug/g)



STORAGE TIME/WKS

4.7.2 Histamine levels and other factors in Tilapia under ambient storage

The histamine levels ranged from 13.15M-g/g to 15.22jig/g (Fig,4.7). The histamine levels increased from the first week of storage till the 7th and 8th weeks when it started reducing. It is, however, not very clear the factors which might have caused a decline in histamine production.

The moisture content decreased steadily over the storage period. The water activity also decreased over the storage period till the 6th, 7th and 8th weeks, when a constant a_w was observed throughout (Table 4.7). The reduction in the a_w over the first half of storage could be the direct result of moisture loss to the surroundings till gradually an equilibrium was established and the a_w remained stable. The microbial load increased from $2.2x10^2$ in the first week of storage to $1.3x10^s$ in the 8th week.

Table 4.7

NaCI, moisture, a_w and microbial counts in "Koobi" sun-dried and stored for 1-8 weeks

Storage Time/weeks	NaCl(%)	Moisture%)	a_W	Total viable Count
1	17.16	40.18	0.60	2.2xl0^2
2	17.42	35.76	0.60	9.6x10^2
3	17.13	33.29	0.58	4.1xl0^4
4	17.77	32.88	0.55	6.2x10"
5	16.55	32.02	0.55	8.3xl0^5
6	17,32	31.67	0.54	8.7x10^s
7	17.12	30.14	0.54	6.0x10^s
8	16.44	30.09	0.54	1,3x10^s

4.8 THE INFLUENCE OF SODIUM BENZOATE, VARYING SALTING LEVELS AND DIFFERENT DRYING/STORAGE TEMPERATURES ON HISTAMINE PRODUCTION IN *KOOBI*

The Histamine levels, sodium chloride content, water activity, moisture content and microbial counts were determined for Tilapia under varying treatment with salt and sodium benzoate and different drying/storage temperatures. Sodium benzoate was applied to the Tilapia for either 1 hour or 2 hours before salting, (high salting, intermediate salting or no salting). The tilapia samples were then dried at different temperatures (ambient, solar dryer, refrigerator and fresh)

The results are displayed in Figures 4.8-4.11 and Tables 4,8—4.11.

Table 4.8

Sodium chloride, water activity, (a_w), moisture and microbial count in salted Tilapia dried at ambient temperatures for one week

Sample treatment*	Sodium chloride (%)	Moisture content (%)	Water activity (a_w)	Total viable count
A	18.32	46.21	0.62	7.90x10*
B	18.15	46.21	0.62	2.18x10*
C	15.61	49.54	0.63	$2.62xl0^2$
D	15.68	49.54	0.62	$2.34x10^3$
E	0.70	60.49	0.67	$1.25x10^s$
F	0.80	62.13	0.67	$8.30x10^s$
G	0.70	37.00	0.56	$1.42xl0^7$

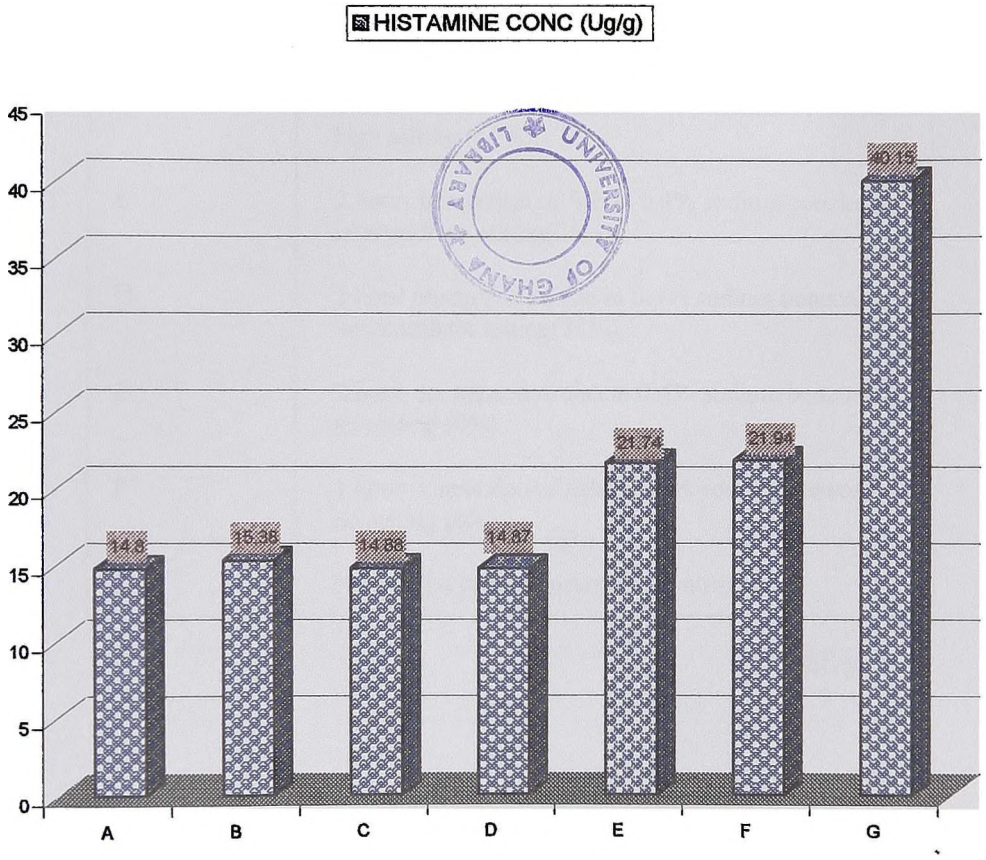
♦Treatment identification indicated in Box 4.1

4.8.1 *Koobi* sample treated with sodium benzoate (for 1 or 2 hours) before given varying salt levels (high salting, intermediate salting and no salt) and dried at ambient temperatures

Koobi sample which had been immersed in sodium benzoate for two hours and then given high salt treatment before drying at ambient temperature had a histamine content of 14.80 mg/g while those samples immersed for one hour and also given high salt treatment contained 15.38 mg/g of histamine. (Fig. 4.8).

Fig. 4.8 Effect of ambient temperature on histamine production in *koobi* under treatment with sodium chloride and sodium benzoate

Treatment identification	Treatment
A	2 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
B	1 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
C	2 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
D	1 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
E	2 hour immersion of fish in 0.4% sodium benzoate + no salting (0%)
F	1 hour immersion of fish in 0,4% sodium benzoate + no salting (0%)
G	No salt, no sodium benzoate (control).



Box 4.1

Treatment identification	Treatment
A	2 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
B	1 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
C	2 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
D	1 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
E	2 hour immersion of fish in 0.4% sodium benzoate + no salting (0%)
F	1 hour immersion of fish in 0,4% sodium benzoate + no salting (0%)
G	No salt, no sodium benzoate (control).

Also, histamine levels in *Koobi* samples which had been immersed in sodium benzoate for two hours but given intermediate salt treatment was 14.88 ug/g while those koobi samples given the same intermediate salt treatment but immersed in sodium benzoate for one hour had a histamine content of 14.87(j.g/g. All the above treatments did yield histamine levels which were not statistically significant (Appendix VII).

Koobi prepared using Tilapia which had previously been immersed in sodium benzoate for two hours but without any salting had a histamine content (21.74(j.g/g) which did not differ significantly from *Koobi* prepared using Tilapia which had previously been immersed in sodium benzoate for one hour also without any salting (21.94|ag/g)

There was, however, a significant difference in the histamine levels between the latter treatments (sodium benzoate but no salt) and the former treatments (sodium benzoate and salt). Those samples which were salted (whether high or intermediate) had a lower histamine content than those samples which were not salted. This underscores the importance of salting in reducing histamine production. There was a marked elevation in histamine evolution when neither sodium benzoate nor salt was used. (40.15(ig/g).

High salting levels creates conditions which are not favourable for microbial activity, growth and proliferation. Ions of neutral salts increase the solubility of proteins. These ions react with charges on proteins, decreasing the electrostatic attraction between opposite charges of neighbouring protein molecules. The solvation layer which accompanies ions of neutral salts also tends to increase solvation of the proteins and to enhance their solubility. Increasing the solubility of proteins by adding neutral salts is called “salting in”. If the concen-

tration of neutral salts is increased to a high level, in many instances the protein precipitates. This phenomenon apparently results because the excess ions (not bound to the proteins) compete with proteins for the solvent. The decrease in solvation and neutralization of the repulsive forces allows the proteins to aggregate and precipitate. This effect is called "salting out" and leads to denaturation of the fish proteins. One major consequence of denaturation is that en2yme activity, if originally present is decreased or lost. Decarboxylation of histidine to histamine may be greatly hampered. The result is that high levels of salt will impeede microbial decarboxylation of histidine to histamine. Under high salt condition, only halophiles are favoured and since most histamine producing bacterial are not halophilic, histamine production would be impaired. At salt concentrations of 18.32 percent observed in some *koobi* samples (Table 4.8) almost all histidine decarboxylating bacteria may be rendered incapable of histamine production. This conforms with work by Yunizal *et al.* (1985) which suggested that salt may play an important role in inhibiting histamine formation. Work by Taylor and Speckhard (1984), established that sodium chloride did not inhibit bacterial growth or histamine production by bacteria at concentrations of 0.5, 1.0 and 2.0 percent at 32°C.

The level of sodium chloride (around 2 percent) in sea water may, therefore, not be inhibitory to bacterial growth and histamine production. In order for salting to achieve an inhibitory effect on microbial histidine decarboxylase activity, intermediate or high levels of salting should be used during fish processing. Hanson *et al.* (1985), however, noted that histamine formation could still take place during the process of salting and drying.

Hafnia alvei, a halophilic histamine producing bacteria may be responsible

for the histamine production under such conditions (high salinity). It was also pointed out by Negandra *et al.* (1988) that only marginal increases in histamine concentrations occur after salting of mackerel. This further lends credence to salting as an effective tool for checking histamine production in *Koobi.* Sodium benzoate seemed to help in checking histamine production in *Koobi.* Sodium benzoate probably killed many histamine forming bacteria and thereby lowering the potential for histamine formation considerably. This reflects the relatively lower microbial counts observed when sodium benzoate was applied to the fish

Koobi samples which were immersed in sodium benzoate (for 1 hour and 2hours) had a microbial count of $8.30x10^5$-$1.25x10^5$. While those sample with neither salting nor immersion in sodium benzoate had a microbial count of $1.42x10^7$. This difference may be the manifestation of the role of sodium benzoate as an antimicrobial agent.

This observation seems to agree with work by Taylor and Speckhard (1984) where potassium sorbate at a concentration of 0.5 percent inhibited growth and histamine production by selected strains of *Proteus morganii* and *Klebsiella preumoniae* in a trypticase-soy broth fortified with histidine (TS BH medium). The use of antimicrobial agent may provide an additional impediment to bacterial histamine production particularly during periods of prolonged storage on ice or in refrigerated sea water.

Sodium benzoate is an antimicrobial agent with a broad spectrum of effectiveness and therefore a good promise as an effective inhibitor of growth and histamine production by bacterial in *Koobi.* It is quite conceivable that sodium chloride and sodium benzoate played a complementary role in checking microbial proliferation.

In situations where the *Koobi* samples were treated with both sodium benzoate and sodium chloride the microbial counts were relatively lower. (Table 4.8) salting also reduces the availability of water (a_w).

In samples with no salting but treated with sodium benzoate, the a_w was 0.67 whiles those treated with both sodium benzoate and salt had a a_w of 0.62-0.63. The lowering of the a_w might be accomplished through the dissolution of the salt by water in the fish and thereby reducing the quantity of water left. There was a strong negative correlation (r = -0.94) between sodium chloride and histamine (Appendix XI).

This could probably be due to the creation of high salinity and reduction in water activity, both of which are not conducive for microbial growth and enzymatic activity. Consequently, high salt concentration in the fish tended to inhibit histamine production.

This assertion is greatly supported by the high negative correlation (r = -0.90) between sodium chloride and aw and the strong positive correlation (r = 0.83) between aw and histamine . (Appendix XI).

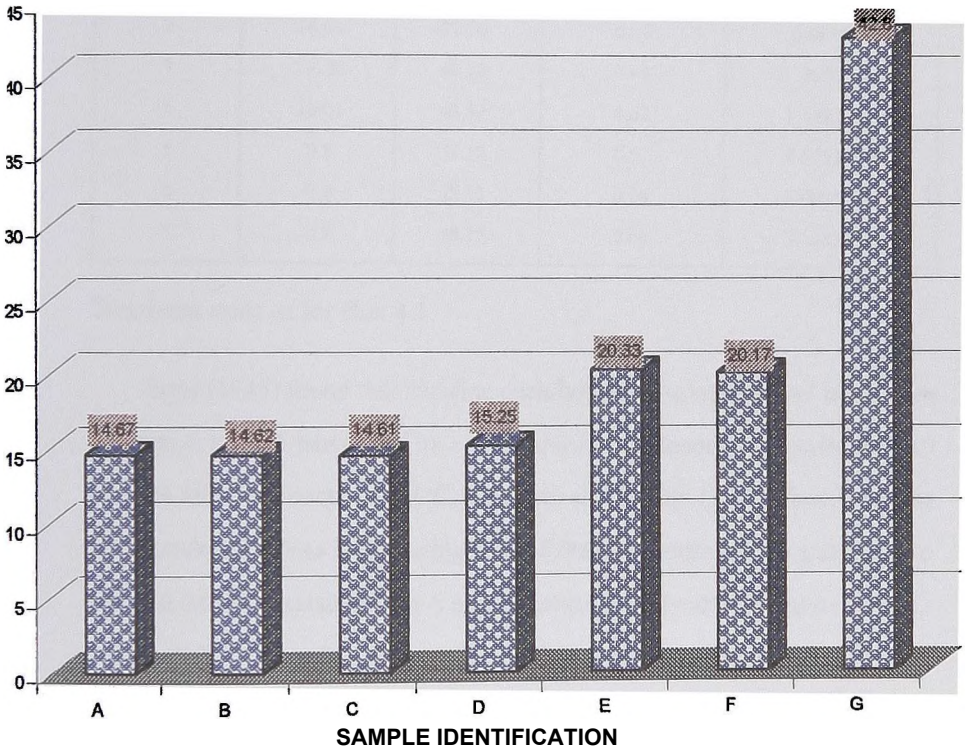
The *Koobi* samples were dried at temperatures around 28°C. This temperature is favourable for the proliferation of mesophiles. Most of the histamine producing bacteria are mesophilic and therefore their growth and activity is favoured by ambient temperatures.

4.8.2 *Koobi* sample treated with sodium benzoate (for 1 or 2 hours) before given varying salt levels (high salting, intermediate salting and no salting) and dried in a solar dryer.

The histamine levels ranged from 14.61mg/g to 42.80ug/g (Fig.4.9). This histamine range was not markedly different from that observed in *Koobi* samples dried at ambient temperatures. As explained by Epps(1945) the bacterial

Fig. 4.9: Effect of solar dryer temperatures on histamine production in *koobi* treated with sodium chloride and sodium benzoate.

Treatment identification	Treatment
A	2 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
B	1 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
C	2 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
D	1 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
E	2 hour immersion of fish in 0.4% sodium benzoate + no salting (0%)
F	1 hour immersion of fish in 0.4% sodium benzoate + no salting (0%)
G	No salt, no sodium benzoate (control).



histidine decarboxylase seems fairly sensitive to temperatures higher than ambient and therefore one would not expect significant increases in histamine levels above ambient temperatures.

Table 4.9
Sodium chloride, moisture, aw, and microbial count in Tilapia given various treatments before drying in a solar dryer for one week

*Sample treatment	Sodium chloride(%)	Moisture content (%)	Water activity(%)	Total viable count
1	17.36	35.25	0.58	0.00
2	16.61	37.16	0.60	6.0x10*
3	15..32	40.70	0.63	0.00
4	15.01	40.32	0.62	1.48xl0^2
5	0.8	55.12	0.67	6.57xl0^5
6	0.8	55.13	0.66	7.00x10^J
7	0.8	48.19	0.66	3.53x10^8

Treatment same as for Box 4.1

Epps (1945) found that histidine decarboxylase isolated from *Clostridium perfringes* lost 25 percent of its activity during the second 5 minutes of a 10 minutes assay conducted at 38°C. Kwabata and Suzuki (1959) found that the histidine decarbxylase from resting cells of *Proteus morgcmii* was stable for 96 hours at 24°C but retained only 5 percent of the activity after 24 hours at 37°C. For *E. Coli,* roughly 50 percent of the activity was lost after one hour at 35°C (Gale 1940), similar results for *Klebsiellapreumoniae* were found by Baranowski, Brust and Frank (1984). These data, therefore, tend to indicate that inactivation of histidine decarboxylase occurs rapidly at slightly elevated temperatures.

The moisture levels ranged from 35.25 to 55.12 percent. The moisture content of *Koobi* samples dried in the solar drier was lower than that of their

counterparts dried at the higher temperatures. This may be attributed to the higher temperatures experienced in the solar dryer (about 42°C) which resulted in the evaporation of more water from the flesh of the fish and thereby reducing the moisture content of the fish.

The a_w values obtained for the *Koobi* samples may not be high enough for microbial and enzymatic activity (Table 4.9) samples which were not salted had a higher a_w reflecting the ability of salt to reduce a_w.

The total viable count of bacteria was very low (except samples which were not salted). This may be due to the combined effect of the sodium chloride and sodium benzoate in checking microbial growth. The high salt levels also reduced the aw of the *Koobi* which might have created unfavourable conditions for microbial and enzymatic activity.

Koobi samples which were neither treated with salt nor sodium benzoate had a relatively higher microbial load ($3.53x10^s$). Such samples also showed higher histamine levels (42.80mg/g). This may be an indication of a positive correlation between microbial counts and histamine production.

There was a strong negative correlation between sodium chloride and a (-0.82) as well as sodium chloride and histamine (r = -0.95) (Appendix XI). This clearly underscore the importance of salting in reducing histamine production in *koobi*.

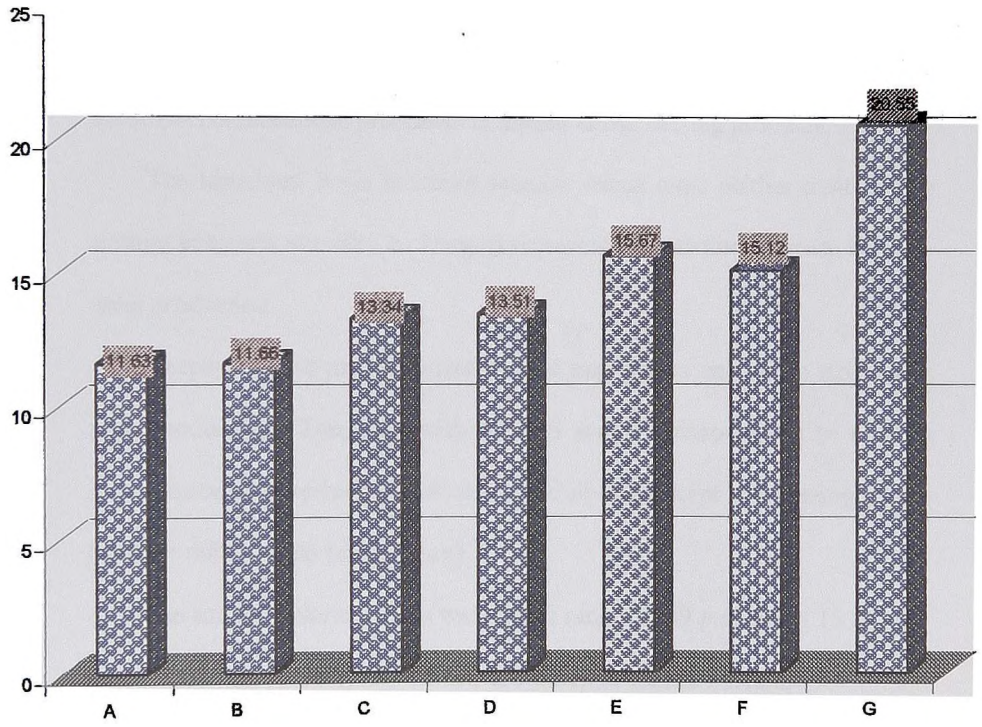
4.8.3 *Koobi* samples treated with sodium benzoate (for 1 or 2 hours) before given varying salt levels (high salting, intermediate salting and low salting) and kept in a refigerator.

The histamine levels varied from 11.63mg/g to 20.55mg/g (Fig.4.10). These were again very low and highly unlikely to pose any toxicological problems.

Low temperature enzyme activity by mesophilic organisms may play an

Fig. 4.10 Effect of refrigeration temperature on histamine production in *koobi* treated with sodium chloride and sodium benzoate

Treatment identification	Treatment
A	2 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
B	1 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
C	2 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
D	1 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
E	2 hour immersion of fish in 0.4% sodium benzoate + no salting (0%)
F	1 hour immersion of fish in 0.4% sodium benzoate + no salting (0%)
G	No salt, no sodium benzoate (control).



important role in histamine formation if a sufficient number of these organisms have been produced prior to cold storage.

Although Klebsiella pneumoniae did not grow well at 2°C, it remained viable and produced histamine in small quantities and its resting cells produced histamine at 4°C (Baranowski, Brust and Frank, 1984).

Psychrophiles may also be important agents of histamine production in *Koobi* kept in the refrigerator.

As observed by Van Spree Kens, (1986), some psychrophilic photobacteria are important histamine producers in mackerel and herring products.

The histamine levels in *Koobi* samples which were neither treated with sodium benzoate nor salt (20.55|ig/g) represented a marked increase in histamine production.

Keeping Tilapia in a refrigerator alone may not be enough to stop histamine production. Treatment with salt and sodium benzoate can be used to reduce histamine production in *Koobi* especially when kept at low temperature (such as refrigeration temperature).

The sodium chloride levels were in the range 17.69 percent to 15.80 percent. These levels as stated earlier, are likely to inhibit bacteria growth and enzymatic activity. Histamine production under such high salt conditions will, therefore, be minimized.

Table 4.10

Sodium chloride, moisture, aw and microbial counts in Tilapia given various treatments before being kept in the refrigerator for one week

*Sample treatment	Sodium chloride (%)	Moisture content (%)	Water activity (a_w)	Total viable count
A	17.69	65.02	0.65	0.00
B	17.55	66.40	0.62	0.00
C	15.74	66,14	0.64	0.00
D	15.80	67.00	0.64	0.00
E	0.8	68.15	0.68	$3.50x10^{s}$
F	0.7	68.00	0.67	$1.94x10^{s}$
G	0.8	70.01	0.65	$3.53x10^{s}$

*Sample Treatment same as for Box 4.1

The sodium chloride level in unsalted Tilapia was from 0,8 to 0,7. Microbial and enzymatic activities are likely to proceed under such salt levels. This may explain the higher evolution of histamine under conditions where salting was not done (Fig.4 10).

Even though the moisture content was relatively high, the a_w was not correspondingly high (Table 4.9). This could be the result of some water being frozen and therefore unavailable for microbial and enzymatic activity.

Koobi samples prepared by immersing the Tilapia in sodium benzoate and sodium chloride recorded zero viable counts. While samples which were not salted produced significant levels of microbial counts ($3.53xl0^{5}$ -$3.50x10^{s}$). This clearly demonstrates the ability of salting to inhibit microbial activity.

This assertion is further supported by the negative correlation (r = -0.79) between sodium chloride and histamine (Appendix XII).

4.8.4 *Koobi* samples treated with sodium benzoate (for 1 or 2 hours) before given varying salt levels (high salting, intermediate salting and low salting) and kept in a freezer

The histamine levels ranged from 11.02ug/g to 13.1 lug/g (Fig.4.11). The freezing temperatures (-2°C) was highly unfavourable for en2ymatic and microbial activity and histamine production was not likely to occur at such low temperatures.

Even for Tilapia neither immersed in sodium benzoate not salted, the histamine level was relatively low (compared to other storage drying temperatures).

This seems to agree with findings (Baldrati *et al.* 1980; Behling and Taylor 1982; Cattaneo and Cantoni 1978; Hardy and Smith 1976; Pan *et al.* 1982). That negligible histamine production occurred at fish storage temperatures of 0°C or below.

Table 4.11

Sodium chloride, moisture, aw and microbial counts in Tilapia given various treatments and kept in the freezer for one week

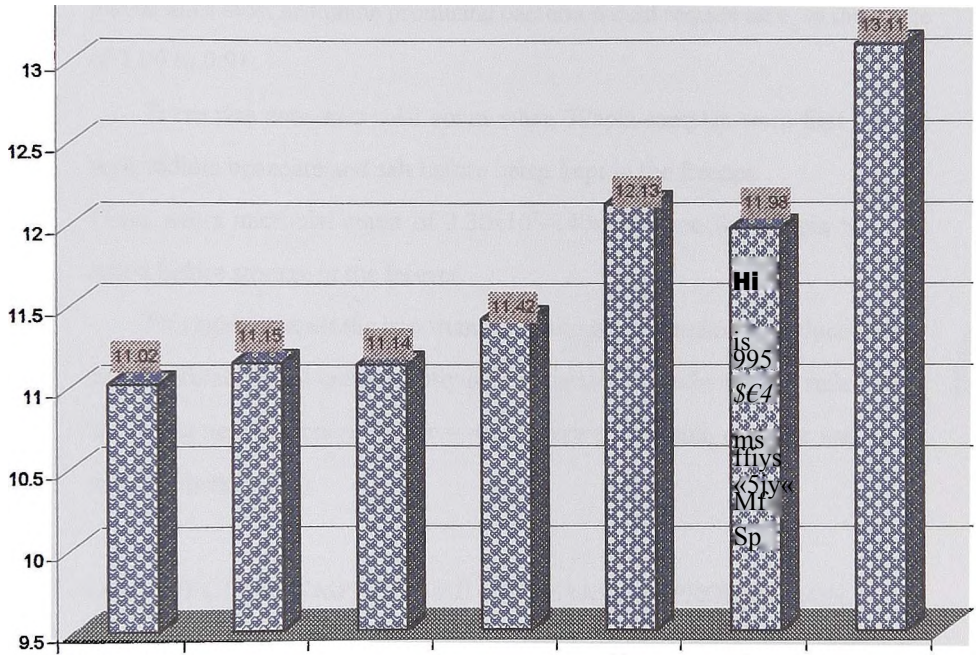
♦Sample treatment	Nael (%)	Moisture content (%)	^{a}w	Total viable count
A	16.72	65.33	0.65	0.00
B	16.60	67.70	0.64	0.00
C	14.88	67.40	0.65	0.00
D	14.85	67.62	0.65	0.00
E	0.8	68.88	0.67	$3.53xl0^2$
F	0.8	69.11	p.66	$1.40xl0^3$
G	0.8	66.84	0.61	$3.30x10^2$

* Treatment same as Box 4.1.

Fig. 4.11 Effect of freezing temperature on histamine production in *koobi* treated with sodium chloride and sodium benzoate.

Treatment identification	Treatment
A	2 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
B	1 hour immersion of fish in 0.4% sodium benzoate + high salting (50%)
C	2 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
D	1 hour immersion of fish in 0.4% sodium benzoate + intermediate salting(25%)
E	2 hour immersion of fish in 0.4% sodium benzoate + no salting (0%)
F	1 hour immersion of fish in 0.4% sodium benzoate + no salting (0%)
G	No salt, no sodium benzoate (control).

0 HISTAMINE CONC (Ug/g)



SAMPLE IDENTIFICATION

There was no significant, difference among the various samples of *Koobi* in their histamine levels.

The sodium chloride levels ranged from 16.72 percent to 14.85 percent. Under such high salinity, it is doubtful that significant microbial activity can take place

The sodium chloride levels in the fresh unsalted Tilapia was 0.8 percent. The a_w values obtained (0.61-0.67) seemed to suggest that microbial and enzymatic action by histamine producing bacteria would be significantly impaired since most histamine producing bacteria would require an a_w in the **range** of 1.00 to 0.91.

There was zero microbial count when Tilapia samples were first treated with sodium benzoate and salt before being kept in the freezer.

There was a microbial count of $3.30xl0^2$-$140xl0^3$ when the Tilapia was not salted before storage in the freezer.

This again stresses the importance of salting as a measure to reduce microbial proliferation and consequently inhibit histamine production as reflected in the strong negative correlation(r = -0.75) between sodium chloride and histamine (Appendix XII).

4.9 EFFECT OF TEMPERATURE ON HISTAMINE PRODUCTION, SALT CONTENT, MOISTURE CONTENT, WATER ACTIVITY AND MICROBIAL COUNTS IN KOOBI

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4.9.1. Effect of Temperature on Histamine production

Temperature is an important factor affecting microbial and enzymatic activity. For enzyme systems, temperature plays two roles that may have opposite effects on the velocity of the enzyme reactions. Although an increase in tempera-

ture generally increases the rate of reaction it also causes denaturation of the enzyme which decreases the reaction rate. Optimum temperature is a function of both of these factors. This concept of a temperature optimum is an operational parameter rather than a true characteristic of enzymes (Whitaker 1972).

Although most enzymes are denatured at subfreezing temperature, some remain quite active after freezing and thawing and can even exhibit significant activity in partially frozeen systems.

Histamine production did not differ significantly in *Koobi* samples which were kept in either the freezer or the refrigerator(Appendix XtV)

Koobi samples which were dried in either the solar drier or in the laboratory room differed significantly from those kept in the refrigerator or the freezer in terms of histamine content. Shewan and Murray(1979) showed that above 8°C bacteria penetrated into the flesh of fish along the collagen fibres. At lower temperatures however, bacterial growth is a surface phenomenon.

Higher levels of histamine were produced when the koobi samples were dried at either the ambient or solar dryer temperatures than when the samples were in refrigerator or the freezer. This could be attributed in part to elevated enzymatic and microbial activity at the ambient or solar dryer temperatures. Temperature seems to have an influence on histamine production, therefore to minimize histamine production, low storage temperatures may be employed.

4.9.2 **Effect** of **temperature** on sodium chloridt **levels**

Temperature did not seem to play any significant role in the penetration and absorption of salt by the Tilapia.

Keeping the Tilapia samples in the freezer (-4 to -2°C), refrigerator (4-

7°C), solar dryer (38 to 42°C) or in the laboratory room (28 to 30°C) did not affect the sodium chloride content of the fish (Appendix XIV).

This could be attributed to the thermo stable chemical nature of sodium chloride. Sodium chloride has a high melting point (801°C) and would therefore not undergo any drastic chemical change at the solar dryer temperature (42°C) and ambient temperatures (28°C). Even though, the salt content of steamed *Koobi* was not determined, it is not expected to change for the same reason.

It is also known that sodium chloride depresses the freezing point of ice. The high salt content of *Koobi* would therefore increase the temperature at which the *Koobi* wil freeze during frozen storage.

4.9.3 Effect of Temperature on the Moisture Content

Temperature can affect the rate of moisture loss or gain in food. The moisture content of food could therefore be affected by temperature.

Koobi samples dried in the solar drier differed significantly from those kept in the laboratory room in their moisture content (Appendix XIV).

This may be due to the fact that at refrigeration temperatures (4°C) and below the rate of moisture loss/gain from the Tilapia was very low. (evaporation varies directly with temperature).

On the other hand, there was a pronounced difference in the moisture contents of samples dried in the solar drier (42°C) and those at ambient temperatures (28°C) because at such elevated temperatures the rate of moisture loss was relatively higher. This caused a marked difference in the rates of moisture loss in the samples dried in the solar drier and that dried at room temperature.

4.9.4 Effect of Temperature on the a_w

Koobi samples kept in the refrigerator, solar drier, or at room temperatures were not significantly different from each other in their aw levels (Appendix XIV).

However, *Koobi* samples which were either kept in the solar drier or the freezer differed from each other in their a_w levels.

Temperature did not therefore seem to have a profound influence on aw. This could probably be explained in part by the fact that sodium chloride levels did not differ significantly with temperature variation. Since aw is affected by salt levels, it is conceivable that the nearly similar levels of salt tended to ensure a constant aw irrespective of temperature variation.

4.9.5 Effect of temperature on microbial count

It is well known that both enzymatic and microbiological activity are greatly influenced by temperature. However, in the temperature range from 0 to 25°C, microbiological activity is relatively more important, and temperature changes have greater impact on microbiological growth than on enzymatic activity.

The histamine producing bacteria are certain enterobacteriaceae, some vibrio sp. a few colostridium and lactobacillus sp. The most potent histamine producers are *Proteus marganii, Klebsiella pneumoniae,* and *Hafnia alvei* (Stratton and Taylor, 1991). These bacteria can be found on most fish, probably as a result of post-harvest contamination. They grow Well at 10°C but at 5°C growth is, greatly retarded and no histamine was produced by *Proteus morganii* when temperatures were less that 5°C at all times (Klausen and Huss 1987). However, large amounts of histamine were formed by *Proteus morganii* at low tempera-

ture (0-5°C) following storage for up to 24th ours at high temperature (10-25°C) even though bacterial growth did not take place at 5°C and below. Many studies agree that histamine producing bacteria are mesophilic. However, Ababouch *et al* (1991) found considerable histamine production in sardines at temperatures less than 5°C and Van spree Ken (1987) has reported on histamine production by photobacterium sp. which are also able to to grow at temperature less than 5°C.

Temperature variation did not cause any significant difference among the various samples of *Koobi* in their microbial load (Appendix XTV).

This could partly be attributed to the good handling and processing practices which ensured minimal contamination of the Tilapia samples with bacteria. It could also be that, both sodium chloride and sodium benzoate acted as antimicrobial agents and thereby checking microbial proliferation irrespective of favourable conditions of temperature or otherwise.

4.10 HISTAMINE AS AN INDEX OF DETERIORATION

Histamine is mainly found in food which was subject to microbiological and biochemical deterioration due to handling processing, ripening or storage. Higher concentrations of biogenic amines may be found in food containing protein produced in microbiotic processes as well as in microbiotically deteriorated proteins like fish. The presence of histamine is, therefore, a criterion when controlling the quality of food like fish. The use of histamin^ as a basis for deterioration serves to highlight the toxicological importance of histamine in food spoilage.

By the use of the term 'deterioration index', there is a greater emphasis on food spoilage which in turn draws attention not only to histamine content but

also the possible microbiological hazards associated with the food. Processing of food is done to retard deterioration of the food. The "processing depression index" is thus a measure of the extent to which food spoilage has been controlled or retarded.

The "Deterioration index" was determined for Tilapia under various handling and storage conditions while the "processing depression index" was also determined for Tilapia under various processing conditions. The "Post Catch Deterioration index" was obtained by subtracting the "histamine level at fresh catch from the histamine level after catch" and then dividing the result by tfie "histamine level at fresh catch".

The "Storage Deterioration index" was obtained in a similar fashion by substracting the "histamine levels at fresh catch" from the "histamine level at storage" and dividing the result by histamine level at fresh catch".

The determination of the "processing depression index" was done by substracting the "histamine levels at fresh catch from the "histamine levels after processing" and dividing the result by "histamine level at fresh catch" The results are displayed in Tables 4.12 4.13 and 4.14, 4.15 and 4.16.

Bruised Tilapia which was iced 24 hours after purchase had the highest deterioration index. This implies that it has the highest degree of spoilage. Even though the level of histamine observed in Bruised Tilapia in which icing was delayed for 24 hours was far below the toxic limits'stipulated by the U.S. Food and Drug Administration, the rather pronounced excess over that of fresh Tilapia (see Table 4.2 and 4.3) which manifested as high Deterioration index, points to the need to exercise good handling and processing methods to check produc-

tion of elevated histamine levels. Unbruised Tilapia, iced immediately after catch had the lowest deterioration. The low deterioration index associated with unbruised Tilapia iced immediately after purchase show the importance of ice in reducing **histamine** levels by impeding microbial action.

Table 4.12 Post-Catch Deterioration Index

	Post-Catch handling condition	Deterioration Index (%)
1.	Bruised Tilapia, iced immediately after purchase from fishermen	144.71
2.	Unbruised Tilapia not iced immediately after purchased (delayed icing for 1 hour)	145.00
3.	Tilapia not iced immediately after purchase (delayed icing for 24 hours)	191.71
4.	Bruised Tilapia not iced immediately after purchase (delayed icing for 1 hour)	323.57
5.	Bruised Tilapia not iced immediately after purchase (delayed icing for 24 hour	373.86
6.	Unbruised Tilapia, iced immediately after purchase	64.14

Bruised Tilapia could be a good point of entry^y bacteria and, therefore, higher levels of histamine could be expected. Improper handling resulted in a faster spoilage rate. This was due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria.

The unsalted Tilapia had the highest processing Depression Index (Table 4.13). This may be the result of the importance of salt in retarding histamine production. Salt, as noted earlier could affect the biological activity of proteins by causing denaturation of the proteins. In the unsalted Tilapia, therefore, a much higher biological activity is expected and consequently a higher decarboxylation of amino acids (histidine) to histamine may occur.

Also, the salt creates conditions which are unfavourable for microbial proliferation and, therefore, microbial decarboxylase activity is drastically hampered. Thus, in the unsalted state, microbial decarboxylation of histidine to histamine is likely to proceed at a higher level.

The gut is a potent source of enzymes whose activity is critical for the development of the texture and aroma of cured fish (Haard 1991; Norda *et al.* 1982).

Gutting, therefore, rids the Tilapia of these enzymes. The result is that enzymatic activity is greatly reduced. This may cause a reduction in histamine production.

The gut is also known to be harbour micro organisms. These microorganisms could also be an important source of histamine production. These reasons may account for the relatively higher processing depression index in the ungutted Tilapia. Scaling did not seem to depress histamine<production very appreciably (Table 4.13).

Table 4.13

Processing Depression of Tilapia under varying Processing Conditions

Treatment	Processing Depression Index
Gutted + normal salting	105.00
Ungutted + normal salting	118.43
Scaled + normal salting	109.57
Unsealed + normal salting	103.14
Unsalted, gutted and scaled	444.71

Three separate batches (each batch made up of 10 Tilapia samples) of Tilapia were procured from the Weija lake on separate days in the fresh state and iced immediately after purchase. These iced Tilapia samples were sent to the laboratory, analysed for their histamine levels at the fresh state. The tilapia samples were steamed and then analysed for their histamine levels.

The processing depression index was calculated as:

Histamine levels of steamed Tilapia - Histamine levels at the fresh state

Histamine levels at the fresh state.

Table 4.14

Processing Depression index of different batches

Steamed Tilapia

Batch	Processing Depressi6n Index (%)
Batch 1	17.00
Batch 2	92.43
Batch 3	89.29

The drastic difference between batch 1 and the other two batches of Tilapia may be an indication of the varying degree of spoilage of the Tilapia before steaming. It also confirms the point that histamine is stable to thermal processing.

Storage Deterioration Index

During frozen storage (below 0°C) the growth of spoilage and pathogenic micro-organisms is reduced, thus reducing the spoilage rate and reducing or eliminating some risks.

Temperature reduction also reduces the rate of enzymatic reactions, in particular those linked to early Post mortem changes extending, if properly applied, the rigor mortis period.

Fish temperature reduction is by far the most important effect of ice utilization. Therefore, the quicker the ice chills the better. Although cold-shock reactions have been reported in a few tropical species when iced, leading to a loss of yield of fillets (Curran *et al,* 1986), the advantage of quick chilling usually outweights other considerations.

Tilapia in frozen storage had very low storage deterioration index (Table 4.15). There was however a steady increase in deterioration over the storage period.

Table 4.15
Storage Deterioration Index of Tilapia under Frozen Storage

Time/weeks	Storage Deterioration Index
1	^{f}1.71
4	12.00
8	12.14
12	33.14

The storage deterioration index was relatively higher in *Koobi* samples kept in baskets. This could be due to more favourable temperatures for both microbial and enzymatic activity at the ambient storage temperatures.

The storage deterioration index increased steadily over the storage period but started declining at the 7th and 8th weeks.

Table 4.16

Storage Deterioration Index of Tilapia under Ambient storage(in baskets)

Time/weeks	Storage Deterioration Index (%)
1	87.86
2	91.00
3	117.43
4	121,14
5	124.29
6	126.57
7	117.71
8	117.43

4.11 HAZARD ANALYSIS AND CRITICAL CONTROL POINTS (HACCP) IN *KOOBI*

Elaboration of the Process Flow Diagram

Fresh Tilapia — CCP

Scaling of Tilapia

gutting of Tilapia — CCP

Washing of Tilapia

Salting of Tilapia — CCP

Fermentation/ Drying of Tilapia

IDENUFCATION OF HAZARDS

Raw Material

Tilapia (the raw material) constitutes a very important CCP in *koobi* production since it is a point where colifonn bacteria and other pathogenic bacteria may be introduced into the food through improper handling of the Tilapia. This CCP can be controlled by adopting good handling practices such as prompt icing of the Tilapia and avoiding mechanical damage to the fish.

Gutting of Tilapia

Gutting may help rid the Tilapia of proteolytic enzymes (which may speed up the autolytic process in the Tilapia) and also bacteria (which may also increase the quantity of food toxicants produced).

Ungutted Tilapia could for instance produce higher proportion of histamine. This CCP can be controlled by gutting Tilapia used for *koobi* production.

Salting of Tilapia

Use of salt with poor microbiological quality may result in the introduction of certain bacteria into the food. This CCP can be controlled by using salting with good microbiological quality.

Cleaning and hygiene

Good housekeeping, good personal hygiene of *koobi* processors, cleaning of equipment and processing environments constitute a general CCP in *koobi* production. Non compliance with this c ode of conduct and practices will lead to contamination of materials during processing thus posing a hazard.

Potable water, clean equipment and containers etc. should be used at all times.

Table 4.17

Hazards and Critical Control Points in the production of *koobi*

Product flow	Hazard	Preventive Measure	Degree of Control
Live Tilapia	Contamination with pathogenic bacteria	Monitoring of environ-ment	CCP-2
Catch and atch. handling	Growth of bacteria	(Temperature and Time Control	CCP-1
Chilling	Growth of bacteria	(Temperature and Time Control	CCP-1
Landing	Excessive contamina-tion and/or growth of bacteria	Hygienic handling Temperature and Time Control	CCP-1
Transport of Tilapia to processing Site	Mechanical Inqury Growth of bacteria	Proper packing of fish in an appropriate container	
Scaling Gutting Washing	Contaminated water (faecal and chemical contamination)	use water which meets WHO drinking water standards and alsouse clean salt	CCP-1
Fermentation/ Drying		Dry on clean surfaces andenvironments	

Critical limits for the production of *koobi*

The critical limits established for each **CCP** will ensure that the *koobi* produced has the desired microbiological, toxicological and sensory quality.

The critical limits are that Tilapia to be pro9essed should not be badly damaged and also have a putrid smell.

The water used for washing the Tilapia should be clean colorless, odorless and neutral in taste.

The Tilapia should also not harbour coliforms (such as E.coli salmonella, shigella etc.). It is important that the Tilapia is gutted so that certain microorganisms as well as proteolytic enzymes in the gut which might hasten the process of autolysis are eliminated.

The salt used should not contain microorganisms. Salt of good microbiological quality should therefore be used.

Salting of the Tilapia should be done soon after gutting and washing of Tilapia. Delay in salting the tilapia may give rise to faster autolysis of the tilapia as well as a higher microbial infestation of the fish.

Efficient housekeeping which will involve keeping the processing area and also the equipment used in processing the *koobi* clean should be strictly adhered to.

• Establishment of a monitoring system for each CCP

All critical control points (CCP) should be monitored regularly and the findings recorded.

• Corrective measures

Corrective measures should be embarked upon when monitoring results show that a CCP is not conforming to the ciritical limits.

For instance, rotten tilapia with maggots on it should not be used for *koobi* processing.

• Verification and Audits

Verification

The microbiological safety and organoleptic quality of the *koobi* produced should be verified periodically with external assistance obtained from the Food Research Institute, Ghana Standards Board or an established laboratory.

Verification will involve microbiological, chemical and sensory analysis of the *koobi.*

External Audits

Once a year all activities at the *koobi* processing site should be audited by an external auditor appointed by the Food Research Institute to assess the effectiveness of the HACCP System.

• Establishement of a record keeping system

Activities carried out in the operation of the HACCP system should be recorded.

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 On the social profile of the artisanal fishermen, it can be concluded that:

(a) The artisanal fishermen were males with very low educational backgrounds.]

(b) As a result of their low educational background they depend almost exclusively on fishing for their livelihood.

5.2 On the post-catch handling practices adopted by the arti sanal fishermen it can be concluded that:

(a) Most of the artisanal fishermen did not ice the tilapia soon after catch. Delayed icing may therefore be a major contributory factor to histamine production.

(b) Most of the artisanal fishermen avoided inflicting mechanical injury on the tilapia.

(c) Generally, the artisanal fishermen recognised the need for good handling practices and indeed to a large extent handled the fish well.

5.3 On the social profile of the *koobi* processors it can be concluded that:

(a) The *koobi* processors were females with very low educational backgrounds. This implied that these *koobi* processor's cannot avail themselves to the numerous information on fish processing available in magazines and journals and thereby improve on their processing methods.

5.4 On the handling, processing and storage practices adopted by the *koobi* processors it can concluded that:

(a) Most of the *koobi* processors iced the Tilapia soon after purchase.

(b) Most of the *koobi* processors also avoided exposing the Tilapia to the direct impact of the sun. The impact of abusive handling temperatures on

histamine production seems therefore to be low.

(c) There was no marked variation in the way *koobi* was processed.

(d) Most of the *koobi* processors stored the *koobi* in baskets.

5.5 On the histamine content of fresh Tilapia, skip jack tuna and yellow fin tuna it can be concluded that:

(a) Histamine levels are generally low in fresh fish.

(b) The histamine contents of fresh Tilapia, skip jack tuna and yellow fin tuna were quite similar.

(c) This could be a clear indication that most fishes irrespective of the type contain similar levels of histamine once they are in the fresh state.

5.6 On the histamine content of *koobi* from retail outlets it can be concluded that:

(a) Histamine poisoning is not likely to occur in *koobi* since the levels of histamine observed in the retail samples were far below the toxic limits stipulated by the U.S. Food and Drug Administration.

(b) *Koobi* samples from different retail outlets or even the same outlet could differ in their histamine contents.

5.7 On the effect of post-catch handling prior to processing on production of histamine it can be concluded that:

(a) Bruising of the Tilapia used for *koobi* processing could cause the production of elevated levels of histamine.

(b) Prompt icing of Tilapia was very important ^minimizing histamine production in *koobi* since microbial and enzymatic activity are checked at low temperatures.

(c) Post-catch handling condition can indeed influence the level of histamine

production. Stringent/Proper post-catch handling practices can help minimize histamine production in *koobi.*

5.8 On the effect of processing conditions on histamine production in *koobi* it can be concluded that:

(a) Gutting of Tilapia was important in reducing histamine production in *koobi.*

(b) Salting was a very crucial unit operation in *koobi* processing which tended to create conditions unfavourable for both microbial and enzymatic activity and thereby inhibited the production of high histamine levels in *koobi.*

(c) Scaling of Tilapia did not influence histamine production in *koobi* significantly.

(d) Steaming Tilapia did not reduce the level of histamine already formed. Histamine can thus be labelled as thermostable.

(e) The processing conditions can influence histamine production in *koobi.*

5.9 On the effect of storage conditions on histamine production in *koobi* in can be concluded that:

(a) Increased post-processing storage time caused marginal increases in histamine production up to a point beyond which the amount of histamine produced also declined marginally.

(b) Storage temperature can influence histamine production. Low storage temperatures were indeed very important in controlling histamine production.

(c) Storage condition can therefore affect histamine production in stored *koobi.*

5.10 On the influence of sodium benzoate, varying salt levels and different drying/storage temperatures on histamine production in *koobi* it can be concluded that:

(a) Both high and intermediate salting were effective in minimising histamine

production.

(b) Temperature had an important effect on histamine production.

(c) Sodium benzoate also helped immensely in arresting bacterial proliferation and consequently histamine production.

5.11 On the cumulative effect of handling, processing and storage on histamine production in *koobi* it can be concluded that:

(a) The handling, processing and storage methods adopted can influence the amount of histamine formed.

5.12 On the effect of HACCP quality system on histamine production in *koobi* it can be concluded that:

(a) The level of histamine and perhaps associated amines can be controlled by adopting the HACCP qualify management system.

It is therefore, recommended that extension officers impart the concepts of HACCP to both the artisanal fishermen and the fish processors to curb the production of histamine and other food toxicants.

Artisanal fish processors should also be encouraged to transport the Tilapia alive to the processing site since in this state, bacterial deterioration of the fish is virtually non existent.

Finally, even though histamine is the most important indicator of amine poisoning, the presence of other amines potentiates scombroid poisoning. It is therefore necessary to determine the amine profile of *Koobi.*

REFERENCES

Ababouch, L; and Afilal M. E. 1988. Histamine forming bacteria isolated from spoiling sardines. Food Microbiology. Vol. 2 page 385 - 389

Ababoucl, L. Afilal, M. E. Rhafiri, S. and F. F. Busta. 1991. Identification on of histamine producing bacteria isolated from Sardine (sardina pilchardus) stored in ice and at ambient temperature (25C) Food Microbiology 8:127 - 36

Ababouch, L; Alaoui, M.M; and Busta F.F. (1986) Survey of histamine levels in commercially processed fish in Morocco. J. Food. Protection 49:904-908

Abbey L D, Hodari - Okae M, and Osei - Yaw 1995. Studies on Traditional and Improved methods of storage of fermented fish (Momone) Ghana/Netherlands Artisanal Fish processing Applied Research Project.

Amu, B. 1973; In Fermented fish in Africa. A study on Processing, marketing and consumption. GAO Fish. Tech. Paper 329.

Anon. 1980 Scrombrotoxin in Moroccan Sardines. Communicable Disease Report 80 No. 27

Arnold S.H and Brown W.D. 1978. Histamine (?) toxicity from fish products. Adv. Food. Research 24 : 113-54

Askar and Treptow. Jn Occurrence and formation of biologically active amine in foods. Int. Journal of food microbiology 1990 P.78

Badham 1854. Microbiology of fermented food Vo.2 (Ed). Brian J.B.3.

Baldrati G; Fornari, M.B.; Spotti, E; and Incerti I, 1980. Effect of temperature on histamine formation in fish rich in free histidine Ind. Conserve. 55:114-122

Baranowski John and Pan, B. S. (1985). Effects of freezing and subsequent thaw abuse. In Histamine in marine products: Production of bacteria, measurement and prediction of formation. FAO fish. Tech. paper (252):P.36

Baranowski, J. D. Brust P. A.; Frank H. A; 1984. Growth and resting cell histidine decarbooxylase characteristics of Klebsiella pneumonia UH-II. J. Food Biochem. 43:217-219

Bauer Von F; T Schabrum Rr. and Sick K 1989. In formation of Biogenic Amines during ripening of Dry sausages as affected by starter culture and Thawing Time of Raw Materials. J-food sci. 60 (6) pp 11877-1190.

Behling A. R. And Taylor S. L. 1982.Bacterial histamine production as a function of temperature and time of incubation. J. Food. Sci. 47:1311-1317

Beuchat L. K. 1981. In water activity: Theory and application in food (eds) Rockland L. B. and L.K. Beuehat, L. K. N.Y. Marcel Dekker.

Bjeldanes L.F, Schutz D.E and Morris, M.W; 1978. On the aetiology of Scombroid poisoning: Cadaverine potentiation of histamine toxicity in guinea pig. Food Cosmetology and Toxicity. 167. 157-159.

Bonsu, B 1976 In Fermented fish in Africa. A study on Processing, marketing and consumption. FAO Fisheries Tech. Paper 329

Brink B.T., Damink, C; Joosten H. M. J.; and Huis in't veld J. H. J. 1990 Occurrence & formation of biologically active amines in foods. Int. J. of food microbiology, 11:73-84.

Cattaneo, P; and Cantoni C.; 1978 Identification and rapid determination of histamine in fish samples. Ind. Aliment 17:303-307

Caurine, M; Tung-Ching, L; Solomon, M; and chichester C.O. 1974. Hot smoke fish curing. Journal of National Science council of Sri Lanka 2 (1):77-86

Curran, C. A. Poulter, R. G., Brueton A, and Jones N R (1986). Effect of handling treatment on fillet yields and quality of tropical fish. J food Technology 21:301

Dabrowski, T. Kolokowski, E; and Markiewicz K. 1968. Histamine as an indicator of freshness of fish. 1 change of imidazole compounds in flesh of Baltic herring stored at 0 -2°C. Nahrung, 12:631-7

Dasgupta S. K. 1978 Biochemistry Macmillan company of India Ltd. P.5

Davis T.P.; 1979. High performance liquid chromatographic analysis of biogenic amines in biological materials as o-phthalaldenlyde dervatives. J. Chromatography 162:293-310.

Deng, J. C. And Tomaszewski, F.B 1980. The use of response surface methodology to determine the effects of salt, tripolyphosphate and sodium alginate on the quality of fish patties prepared from minced fish, croaker. In Advances in fish science and Technology, (ed) J.J. Connell P. 218-223.

Dibbs, 1961_In_Fermented fish in Africa. A study on Processing, marketing and consumption. FAO Fisheries Tech. Paper 329.

Dierick N; Vandekerckhove P; and Demeyer D; 1974. Changes in non protein nitrogen compounds during dry sausage ripening. J. Food Sci. 39.301-304.

Edmund W.J. and Eitenmiller R. R. 1975. Effect of Storage time and temperature on histamine content and histidine decarboxylase activity of aquatic species. J. Food Sci. 40:516-519

Edward, S. T, and Sandine, W. E. 1981. Public health significance of amines in cheese. In symposium: Microbial metabolites of importance in dairy products. J, Dairy Science 64:2431-2438.

Eitenmiller R.R. Koehler P.E. and Reagan P. E. 1978 Tyramine in fermented sausages: factors affecting formation of tyrosine and tyrosine decarboxylase J Food Sci. 43:689-693

Epps, H. M. R. 1945, studies on bacterial amino-acid decarboxylaes. 4 Histamine decarbooxylase from *Clostridium welchi* type A, Biochem. J. 39:42-46

FAO fish Tech. Paper. 334 1994. Assurance of sea food quality p. 32-35

FAO Fisheries Technical Paper 329. 1993 Fermented fish In Africa. A study on processing, marketing and consumption. 2- 34

FAO. 1981: The prevention of loses in cured fish. FAO fish. Tech. Paper 219-0 10-32

Ferencik. M. (1970)- Formation of histamine during bacterial decarboxylation of histidine in the flesh of some marine fishes. J. Hyg., eipdemiol, Microbiol, Immunology. 14:52-60

Fernandez-Salguero, J. And Mackie, I.M. Histidine metabolism in mackerel *(scomber scombrus)* 1979. Studies on histidine decarboxylase activity and histamine formation during storage of flesh and liver under sterile and non-sterile conditions. J. Food. Technology 14:131-139.

Frank, A. H. 1985. Use of nomographs to estimate histamine in marine priducts: Production by bacteria, measurement and Prediction of formation, (eds) Pan, B. S. and James, D. FAO Fish. Tech Paper. 252:18-20

Frank H. A, Yoshinaga, D. H; and Nip, W.K,; 1981. Histamine formation and honeycombing during decomposition of skipjack tuna, *Katsuwonus Pelamis* at elevated temperatures. Marine Fish Review 43 (10):9

Frauzen F, and Eyesell K. 1969. Biologically active amines found in man. Pergaman press New York.

Gale, E. F. 1940. The production of amines by bacteria. 1 The decarboxylation of amino-acids by strains of Bacterium coli. Biochem 392-413.

Geiger, E; Courtney G.; Schnakenberg G. 1944. The content and formation of histamine in fish muscle. Arch. Bio chem. Biophys. 3:311-319

**Grundemeier, E.W and Andrews A.C. 1965
Spectrophotometric determination of histidine and histamine in their mixtures Trans. Kansas Acad. Sci., 68 (1): 62-4**

Gunaratne, S. And Samarajeewa, U; 1995. Effect of processing on histamine in fish and dried-fish proceedings of the Third Asia Pacific Food Analysis Network, Manila Philippines

Haard N.F 1991 Protein Hydrolysis in sea food. (Private Communication)

Halasz A' Barath A, Simon-Sakardi, L and Holzapfel, W. 1994. Biogenic amines and their production by micro organisms in food. Trends food Sci. Technol. 5:42-49.

Hanson, S.W; Knowles M.J. and Al-Kasadi, A.S 1985 Histamine in southeast Asia cured fish and changes in histamine levels during salting and drying. FAO fisheries .Report 317 Suppl:3386-392

Hardy, R; and Smith J.G.M The storage of mackerel *{Scomber scoombus)* 1976 Development of histamine and rancidity J. Sci. Food Agric 27:595-9.

Hayashi, M. 1954 Investigations on food poisoning caused by ordinary putrefaction. Iv. Synergism between histamine and several biogenic amines. J. Pharm soc Japan. 74:1148-1151

Heidelbaugh, N. D and Karel M. 1975 In "Free Drying and Advanced Food Technology" (eds) Goldblith S. A., Rey L; and Rothnayer, W. W. Academic Press London P. 620

Hibiki S. And Simidu W. 1959 Studies of putrefaction of aquatic products. 26 Spoilage of fish in the presence of carbohydrates. Bull of Jap. Soc. of Sci. fish 24:913-915

Hughes, J; Horwitz M.A; Merson, M.H; Barker, W. H. Jr. And Gangarosa, E. J. 1977. Food borne disease outbreaks of chemical etiology in the United States. 1970-1974. American Journal of Epidemiology 105:233

Hughes, R.B. 1959. Chemical studies on the herring *(Clupea harengus)* 2. The free amino acids of herring flesh and their behaviour during post-Mortem spoilage. J. Sci. Food. Agric, 10:558-564

Hui, J.Y and Taylor S.L 1985. Inhibition of invivo histamine metabolism in rats by food borne and pharmacologic inhibitions of diamine oxidare, histamine N-methyltransferase and mono amine oxidase . Toxicol. Appl. Pharmacol 81:241

Hwang Deng-Fwu, Chang Sheng-Hsuing; Shiau Chyuan-yan; and Cheng Chang-Chia. 1995. Biogenic Amines in the flesh of Sail Fish *(Istio phorus platypterus)* Responsible for scombroid poisoning. J. Food sci. V. 60:926-928

Izquierdo-Pulido M; Font-Fabregas, J; and vidal-Carou, C 1995. Influence of saccharomyces cerevisiae var. Uvarum on histamine and tyramine formation during beer fermentation. Food chem.. 54(1) 51-54

Joosten HMLJ; and Stadhouders, J. 1987. Conditions allowing the formation of biogenic amines in cheese. Netherlands Milk and Dairy Journal 41:, 247-258

Kagan, E. 1970; In Fermented fish in Africa. A study on Processing, marketing and consumption. FAO Fisheries Technical Paper 329.

karmas E 1981. Biogenic amines as indicators of Seafood freshness. Food Sci. Technol. 17,20-23

Kim I-S and Bjeldanes Leonard E. 1979 Amine Content of toxic and wholesome canned tuna fish. J. food sci. 44. 922-923.

Klausen, N.K and H. H. Huss 1987 - Growth and histamine production by Morganella morganii under various temperature conditions. International J. food microbiol. 5 147 - 156

Kranner P; Bauer, F; and Hellwig, E, 1991. Investigations on the formation of histamine in raw sausages. P. 8899-891. In proceedings of 37th ICMST. 1-6 September, Kulmbach, Germany.

Kwabata, T. and Suzuki,s, 1959. studies on the food poisoning associated with putrefaction of marine products. 9. Factors affecting the formation of L (-)

histidine decarboxylase by *Proteus* morganii. Bull. Jap. Soc. Sci. fish. 25: 481-487

Lerke P.A; Porcuna, M.N; and Chin H.B. 1983 Screening test for histamine in fish J. Food sci. 48:155-157.

Lerke, P. A.; Werner, S. B; Taylor, S.L; and Guthertz L.S. 1978 Scombroid Poisoning. Report of an outbreak. West J. Med. 129:381

Lieber, E. R and S L. Taylor, 1978. Thin layer chromatographic Screening methods for histamine in tuna fish. J. Chromatographic 153:143 - 52

Lovenberg W. 1973. Some vaso active and psychoactive substance in food: amines, Stimulants, depressants and hallucinogens- In Toxicants occurring naturally in foods National Academy of Science, Washington 170

Luck, E 1977 classical intermediate moisture foods and the European food market in "Intermediate moisture foods" C . P. C. I. A. paris.

Lupin H.M, 1977 Principles of Salting and drying of Hake. In: Technical Consultation on the Latin American Hake Industry FAO Fish Tech. Report No 203, Supp: 1 40-42.

Mackie, I. M. and A. H. Ritchie, Free amino acids of fish flesh. Pro. Int. Congr. Food 1974 Science Technology, 4(1): 29-38

Maijala, Rita, Eerola, Susanna; Lievonen; Satu Hill, Pauli; and Hirvi, Timo-1996. Formation of Biogenic Amines during ripening of Dry sausages as affected by starter culture and Thawing time of Raw materials. J. Food Sci. 60(6\0:1187.

Merson. 1974. Scombroid fish poisoning. Outbreak traced to commercially canned tuna fish. J- Am. Med. Association 228:1268-1269

Mett, C.L; and Sturgeon, R. J; 1982 Cation exchange chromatography of histamine in the presence of ethyl ammonium chloride. J. Chromatography, 235: 536-538.

Middlebrooks, B.L; Toom Paul M; Douglas Willard L; Harrison Richard E and Mc-Dowell Sandra 1988. Effect of storage time and temperature on the microflora ^ and amine development in Spanish mackerel *(scomberomorus maculatusjff* ^ Journal of food science . 53(4), 1024-1029. 14

Mietz J. L; and Karmas E.;; 1977 chemical quality index of canned tuna as determined by high performance liquid chromatography. J. Food sci. 42:155-158.

Mitchell and Code In Amine Content f toxic and wholesome Canned Tuna fish. A research note. J. Food science 44:99 - 923

Murray, C. K.; Hobbs G. and R. G. Rilbert, RG1982. Scombrotoxin and Scombrotoxin-like poisoning from canned fish. J. Hyg. Cam. 88:215-1220.

Nagayama, T; Tamura, Y; Maki, T; Kan, K; Naoi, Y; and Nishima, T; 1985. Non-volatile amines formation and decomposition in abusively stored fishes and shell-fishes. Eise Kagaku 31(6): 362.

Negranda, E, and Cattaneo P. 1988. In Amine Centert of spoiled macherel. Arch. Biochem. Bio phys., 4: 245-246

Nencki - 1877. In Toxicants Occurring Naturally in foods. National Academy of Sciences 2nd ed. 170-178.

Noda, M; Van, T. V; Kusakabe, I; and Murakami, K. 1982. Substrate specificility and salt inhibition of proteinases isolated from the plyloric caeca and stomach of sardine. Agric. Bio. Chem. 46:5165-1569.

Norysiewicz, L; and Krikler D. 1981. Scombrotoxic atrial flutter. Br. Med. J. 282:1434-1438

Olley, J; and Me. Meekin, T.A; 1985-Use of temperature function integrators. In Histamine in marine products. Pan B.S. and James D. (Eds) FAO fisheries Technical Paper 252:24-29.

Orejana, F. M; Olley J; Sun Pan Bonnie; 1983 J. Food Sci. Technol. 7 (2):30-38.

Owusu, A. A.; 1971. Sodium chloride: Production, quality and possible uses of Common salt in Ghana. Industrial Research Institute 24-36

Pfanhauser, W and Pechaneku, U. 1984.]n formation of biogenic amines during ripening of dry sausages as affected by starter culture and thawing time of raw materials. J. Food Sci. 60 (6) 1187-1190.

Pan and James 1985. In Histamine in marine products: production of bacteria measurement and presiction of formation FAO fisheries technical paper (252): 30-32.

Pan B.S.; Yang B.H and Huang D.M. 1982. Histamine formation in canning of bonito mackerel. Food. Ind. Taiwan 14(2):84

Pan, B. S, and J. M Kuo, effect of Vise eral protease on histamine formation in mackerel. In proceedings of the sixth international congress of Food Science an technology. Dublin, Book Press 2:54

Pan, B. S; Yang B. H. And Huang D. M. 1981. Evaluation of tuna, bonito and mackerel processing by histamine formation. Food. Ind. Taiwan 13(11): 79

Park Y. H; Kim, S.S. Kim 1980, changes In histamine content of the muscle of dark-fleshed fishes during storage and processing. I Changes in histamine content in muscle of common. Mackerel, gizzard-shad and small sardine. Bull Korean. Fish. Soc. 13:15-22

Parrot J. L. 1948. In: Amine Content of toxic and wholesome canned Tuna fish. A research note J. Food Science 44. 922 - 923

Pechanek, U; P fannhauser, W; and Woidich, H; 1983. In formation of Biogenic Amines during Ripening of Dry Sausages as Affected by Starter culture and Thawing Time of Raw materials. J. Food. Sci. 60:1190-1192.

Plahar, W A. N. T Annan and F. Appoh.1996 Ghana Netherlands Artisanal fish processing and applied research project.

Putro S. and Saleh M 1985. Effect of Delayed icing. In: Histamine in Marine products: production of bacteria, measurement and prediction of formation. FAO fisheries Technical paper 252: 30-32

Rice S. L. and Koehler P.E. 1976. Tyrosine and histamine decarboxylase activities of *Pediococus, Cerevisiae and Lactobacilli.* Species and the production of tyrosine in Fermented Sausage J. Food Sci. 42:283-285.

Rice, S.; Eitenmiller R. S. and Koehler P. E. 1976. Biologically active amines in foods. J. Milk Food Technol. 39, 353-358.

Robert J. C.; 1983. Determination of histamine methyl histamines and histamine-o-phthalmethIdialdehyde complexes by two high performance liquid

chromatographic procedures. Application to biological samples J. Chromatography and Biomedical Application 272:275-278.

Robson, J N. 1976 Some introductory thoughts on intermediate moisture foods. In "intermediate moisture foods" (R. Davies, G. G. Birch and K. J. Parker eds) Applied Science Publishers. London. 32 -40

Rockland, L. B and Stewart G. F. 1981. Water Activity: Influences on Food Quality. Academic Press, New York.

Rubbish, K; OffiZorz, P; and Breyer, C; 1981. Effect of icing conditions. In Histamine in marine products: production of bacteria, measurement and prosecution of formation FAO Fishery Technical paper (252) 1985

Sakabe, Y; 1973. Studies on allergy-like food poisoning. Histamine production by *proteus morganii* J. Nara Med. Assoc. 24:248-256

Saria, A; Lembeck, F; Skofitsch, G; 1981. Determination of capsaicin in tissues and separation of capsaicin analogues by high performance liquid chromatography. J. Chromatography 208(1):41-6

Sayem -Ei-Daher N; Simard R.E; Fillion; and Roberge, A. G(1984) Extraction and determination of biogenic amines in ground beef and their relation to microbial quality. Food Sci. Technology.17,20-223

Schulze, V. K; Reusse U; Tellack J; 1979. In Histamine in fishery products: A review by L. Ababouch

Sefa-Dedeh. S and Youngs A.J (1976). The bacteriological quality of Ghanaian solar salt with reference to its use in fish preservation Ghana J. Sci. 16:7-13

Sefa-Dedeh-S. 1973. The chemical and microbiological quality of Ghanaian solar salt. B.Sc. Dissertation. University of Ghana.

Serrar D. Brebant R; Bruneau, S; Denoyel, G.A; 1995. The development of monoclonal antibody-based ELISA for the determination of histamine in Food: Application to fishery products and comparison with the HPLC essay. Food chem (54)85-91

Shewan J. M and Murray C.K. 1979. The microbial spoilage offish with special reference to the role of psychrophiles . In cold tolerant micro organisms in spoilage and the environment, edited by A.D Russell and R. Fuller. London, Academic Press, pp. 117-136.

Shewan J. M. (1977). The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In proceedings of the conference on Handling, Processing and Marketing of Tropical Fish, Tropical Products Institute, London 51 - 66.

Simidu, W and Hibiki S. 1955. Studies on Putrefactionn of aquatic products. Influences of certain substance upon histamine formation Bull. Jap soc. Sci. Fish (9) 808-810

Simidu, W. And Hibiki, S. 1954 Studies on putrefaction of aquatic products comparison on putrefaction of different kinds of fish. Bull. Jap. soc. sci. fish 20 40 :302-4

Skovgaard, N; and Elleman, G.; 1978. Histamine in marine products: Production of bacteria, measurement and predication of formation FAO fish. Tech. paper (252):41 - 42

Smith J.G.M, Hardy R and Young K.W, 1980. Seasonal changes: A seasonal study of the storage characteristics of mackerel stored at chili and ambient temperatures. In advances in fish science and technology, edited by J.J. Connell Farnham, England Fishing News Books Ltd. PP. 372-378

Smith Palumbo 1983. J. Int. Food Tech. 1994 Vol. 38 No 6 P 63

Stratton J.E. Hutkins R.W and Taylor S.L 1991. Biogenic amines in cheese and other fermented foods: A review. J. Food. Prot. 54(6) 60-470

Subden R.E, Brown. R.G.; and Noble A.C; 1978 Determination of histamine in wine and musts by reversed-phase high performance liquid chromatography. J. Chromatography 166:310-312

Taylor S. L and Speckhard M.W. 1984 Inhibition of bacterial histamine production by sorbate and other ant microbial agents. J. Food Prot. 47:508-511

Taylor S. L, Leatherwood M and Lieber E. R. 1978. A survey of histamine levels in sausages. J. Food. Prot. 41:634-637.

Taylor S.L 1983 Histamine poisoning associated with fish, cheese and other foods. FAO/WHO Monograph Cx/PH 83/11

Toyama R. In Fish smoking and drying. The effect of drying and smoking on the Nutritional properties offish (ed) by J. R. Burt. 1982. 42-45

Trailer, J. A. And Christian J. H. B. (eds)1978 Water activity and food. New York, Academic Press, 9.-11

Tsabrun, R, Sick, K, Bauer, F, and Kranner, In formation of Biogenic Amines during ripening of Dry Sausages as Affected by Starter culture and Thawing

Time o Raw materials. J. food Science 60:1187 - 1190

Tsurata. Y; Kohashi K; and Ohkura, Y; 1981 Simultaneous determination of histamine and N-methyl histamine in human urine and rat brain by using high performance liquid chromatography with fluorescence detection. J. Chromatography 224:105-110.

Udenfriend, S; Lovenberg W and Sjoerdsma A. 1959. Physiologically active amines in common fruits and vegetables. Arch. Biochem. Biophys. 85:487-490

Van Slyke L L. And Hart E.B. 1903. The relation of carbondioxide to proteolysis in the ripening of Cheddar cheese. Am. Chem. J. 30:1-24.

Van spreekens, K.J.A. 1986. Histamine production by the psychrophilic flora. In Seafood quality determination. Elsevier Amsterdam (eds) D.E. Krammer and J. Liston J. 309-318

Vanderkerckhove, P 1977. Amines in dry fermented sausage: a research note. J. Food Sci. 42, 283-285

Vidal-Carou, M.C; Izquierdo, M.L; Martin-Morro, M.C.; and Marine-Font A. 1990. Histamine and tyramine in meat products. Relationship with meat spoilage. Food Chem. 37: 239-249.

Waalkes, T.P.A; Sjoerdsma, C.R.; Creveling, H, Weissbach and Udenfriend, S. 1958. Serotonin, norepinephrine, and related compounds in bananas. Science 127: 648-650.

Watts, Daniel A; and Brown Duane W.; 1982. Histamine Formation in Abusively stored Pacific Mackerel Effects of $C0_2$ - Modified Atmosphere J. Food Sci. 47 1386-1388.

West, G.B. 1959. Tryptamine in tomatoes. J. Pharm. Pharmacol 11:319-320

Whitaker J. R. 1972. Principles of enzymology for the food sciences. New York, Marcel Dekker.

Yen, G.C; Chandra T; 1988 Biogenic amines in alcoholic beverages produced in Taiwan. J. SC. Food. Agric 44:3,2-80;15

Yoshinaga, D. H; and Frank, H. A; 1982. Histamine producing bacteria in decomposing skipjack tuna *(Katsuwonis Petamis).* Applied Environmental Microbiology 44:447

Yunizal, W.T.; Anggawati, W.T; and Putro, S; 1985 - Histamine formation in dried salted mackerel. FAO Fisheries Report (317) Suppl: 373-381.

APPENDIX I

QUESTIONNAIRE

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

Questionnaire No..... Date.....

1- Sex..... 2. Age.

3. ' Occupation..... 4. Level of Education
(i) No formal education
(ii) Primary school
(iii) Secondary school
(iv) Tertiary school
(v) Others (specify)

5. Do you carry ice in your fishing boat. Yes/No

6. Do you ice the Tilapia Immediately after catch.

7. Do you ice the Tilapia after purchase from fishermen. Yes/No. Why

8. How soon after purchase Do you ice the Tilapia

9. Where do you obtain your ice

10. How much ice do you buy? (cedis/batch)

11. Do you wash the Tilapia with clean water why?

12. How do you transport Tilapia to the processing site?

13., Do you consider exposing the fish to the sun a good practice?

14. How do you process the “koobi"?

15. Have you had any training in the handling, processing and storage of fish?.

16. How do you store the Koobi?

APPENDIX II

Type III Sums of Squares

SAMPLE	' 9"!	2010.452	223.384	254.969	0.0001
Residual	20	17.522	0.876		

Scheffe's S
Effect: SAMPLE
Dependent: HISTAMINE
Significance level: .05

	Count	Mean
Element 2	3	7.060
Element 1	3	7.460
Element 10	3	10.140
Element 4	3	17.680
Element 3	3	19.373
Element 6	3	20.120
Element 9	3	21.170
Element 8	3	24,117
Element 5	3	29.447
Element 7	3	32.150

Scheffe's S
Effect: SAMPLE
Dependent: Nacl
Significance level: .05

	Count	Mean	
Element 4	3	13.390	a
a Element 9	3	15.450	b
a Element 1	3	15.883	b c
b Element 7	3	16.100	b c
b Element 6	3	16.140	b c
b Element 10	3	16.200	b c
b c Element 5	3	16.250	b c
c Element 8	3	17.337	b c
d Element 2	3	17.820	c
d Element 3	3	19.680	

Type III Sums of Squares

Source	df	Sum of Squares	Mean Squ	F-Value	P-Value
SAMPLE	9	72.287	8,032	28,728	0.0001
Residual	20	5.592	0.280		

Dependent: Nacl

Type III Sums of Squares

Source	df	Sum of Squares	Mean Squ	F-Value	P-Value
SAMPLE	9	587.180	65.242	26.703	0.0001
Residual	20	48.865	2.443		

Dependent: MC

Scheffe's S
Effect: SAMPLE
Dependent: MC
Significance leved: .05

Scheffe's S
Effect: SAMPLE
Dependent: WATER
Significance level: .05

APPENDIX II

	Count	Mean
Element 8	3	30.000
Element 1	3	30.190
Element 9	3	32.600
Element 10	3	33.180
Element 2	3	35.170
Element 8	3	39.980
Element 7	3	40.150
Element 6	3	40.180
Element 4	3	41.247
Element 3	3	41.437

	Count	Mean	
a Element 6	3	0.510	a
a Element 9	3	0.620	a b
a Element 1	3	0.550	a b
a Element 10	3	0.570	a b
a b Element 2	3	0.580	a b
b c Element 4	3	0.600	a b
b c Element 5	3	0.603	a b
b c Element 7	3	0.610	a b
c Element 8	3	0.617	a b
c Element 3	3	0.620	b

type III Sums of Squares

Source	df	Sum of Squares	Mean Squ	F-Value	P-Value
SAMPLE	9	0.043	0.005	5.962	0.0001
Residual	20	0.016	0.001		

Dependent: WATER

Type III Sums of Squares

Source	df	Sum of Squares	Mean Squ	F-Value	P-Value
SAMPLE	9	7.331 E12	8.149 E11	1.570	0.1916
Residual	20	1.038 E13	5,191 E11		

Dependent: TOTAL VIABLE COUNT

Scheffe's S
Effect: SAMPLE
Dependent: TOTAL VIABLE COUNT
Significance level: .05

	Count	Mean	
Element 9	3	2540.000	a
Element 5	3	9333.333	a
Element 4	3	15766.667	a
Element 8	3	98000.000	a
Element 3	3	1.527 E 5	a
Element 2	3	2.423 E 5	a
Element 1	3	5.933 E 5	a
Element 6	3	9.633 E 5	a
Element 7	3	1250000	a
Element 10	3	1.277 E 6	a

None were significantly different at this level.

APPENDIX III

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	2	6.771	3.386]	4.7841	0.0572)
ResidU]	5	«S	0.733|		j

DependencHISTAMINE

Soheffe's S
Effect; SAMPLE
Dependent: HISTAMINE
Significance level;.G5

	Count	Mean	
Element 3	3	5.153	a
Element z	3	5.167	a
Element 1	3	7.000	a

None were significantly diffrent at this level.

Scheffe's S
Effect SAMPLE
Dependent: Nacl
Significance level:.05

	Count	Mean
Element 1	3	0.000
Element s	3	2.300
Element 2	3	2.410

Type II Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	2	11.110	5.555	13.620	0.0059
Residual	6	2.447	0.408		

DependentNacl

Type III sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	2	219.818	109.909	56.774	0.0001
Residual	6	11.615	1.936		

DependenfcMC

Soheffe's S
Effect SAMPLE
Dependent MC
Significance level:.05

	Count	Mean	
Element 3	3	69.420	a
Element 2	3	70.040	a
Element 1	3	80.200	b

Scheffe's S
Effect: SAMPLE
Dependent WATER
Significance level:.05

	Count	Mean
Element 3	3	0.680
Element 2	3	0.680
Element 1	3	0.750

Type III sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	2	0.010	0.005	12.250	0.0076
Residual	6	0.002	4.000 E-4		

DependentWATER

APPENDIX III

Type III sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
[SAMPLE 1	2	10363850.000j	5181925.000]	1.457]	0-3D50
!	6	&1?89822.1KP!	3556/"?7,Crid		

Dependent:TDTAL VIABLE CONTENT

Scheffe's S

Effect; SAMPLE

Dependent: TOTAL V1ABLEC0UNT

Significance level:.05

	Count	Mean
Element 1	3	68.333a
Element 2	3	1003.333a
Element 3	3	2663.333a

None were significantly dlffrent at this level.

ipppwmx iv

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
PCH	5	1025.99	205.198	90.519	0.0001'
Residual	12	27.203	2.267		

Depenendent:HISTAMINE

■Scheffe's S
Effect: PCH
Dependent: HISTAMINE
Significance level:.05

	Count	Mean	
Element 6	3	11.490	a
Element 1	3	17.130	b
Element 2	3	17.150	b
Element 3	3	20.420	b
Element 4	3	29.650	c
Element 5	3	33.170	c

Scheffe's S
iiffect: peh
Dependent Nacl
Sipificance leve!:.05

	Count	Mean	
Element 1	3	14.990	a
Element 2	3	15.070	a
Element 3	3	15.230	a
Element 4	3	15.270	a
Element 5	3	16.817	a
Element 6	3	17.190	a

None were smgnificantly level

Type ill Suras of Square

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
PCH	5	14.254	2.851	1.642	0.2229
Residual	12	20.835	1.736		

DependenCNacl

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
PCH	5	33,908	6.782	0.780	0.5826
Residual	12	104.272	8.689		

DependenfcMC
Scheffe's S
Effect: PCH
Dependent: MC
Significance level:.05

	Count	Mean
Element 5	3	44.130
Elam&nt 3	3	46.520
Element 4	3	47.010
Element 1	3	47.100
Element 2	3	47.150
Element 6	3	48.760

None were siglnlfantly different at this level

Scheffe's S
Effect: PCH
Dependent WATER ACTIVITY
Significance level:.05

	Count	Mean
Element 3	3	0.610
Element 2	3	0.620
Element 4	3	0.630
Element 1	3	0.630
Element 5	3	0.640
Element 6	3	0.650

None were sigislfantly different at this level

APPENDIX IV

Type III Sums of Square

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
PCH	5	0.003	0.001	1.714	0.2058
Residual	13	0.004	3.500 E-4		

Dependentwater

Type III Sums of Square

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
PCH	5	5.157 E 10	1.031 E 10	1.348	0.3098
Residual	12	91834339026	7.653 E 9		

DependentTOTAL VIABLE COUNT

Scheffe's S
Effect PCH
Dependent; TOTAL VIABLE COUNT
Significance level:.05

	Count	Mean
Element 6	3	316.000
Element 5	3	976.667
Element 4	3	2330.000
Element 2	3	27566,667
Element 1	3	30433.333
Element 3	3	152000

None were siginifantly diffrent at tMs level

APPENDIX V

■jpg III Sams of Squares

				F-Value	P-Value
BATCH	2	53.530	26.765	413.043	0.0001
Residual	G	0.389	0.065		

fipendent: HC

S'ilaKeVS

BlIM-BMCIl
Bapendenu HC
Significance Level: .05

S r ! * : - S
Effect: BATCH
Oependent: MC
Significance Level: .05

		Mean			
Element 1	3	8.190	Element 2	3	70.170
Element 3	3	13.250	Element 3	3	70.240
Element2	3	13.470	Elemenll	3	72.160

None were significantly different at this level

Type III Sums of Squares

Souro*	rt)	Sum of Squ ares '*>•/» Square	F-Value	?• Value
BATCH	**2**	7.651! 3.826	(1.786	0.4977
Residual	6	23.219! <1.870		

'Dependent MC

Type 111 Sums 01 Squares

BATCH	2	0.004	0.002	10.500	0.0110
iesidual	6	0.001	2.00 E4		

Dependent: WATER

Scheffe's S
Effect BATCH
Dependent: WATER
Significance level: .05

	Count	Mean
Elements	3	0.650
Element 3	3	0.690
Element I	3	0.700

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
BATCH	2	0	0	*1*	*1*
Residual	6	0	0		

Dependent: TVC

APPENDIX VI

Type III sums of squares

source		df	sum of squa	Mean Square	F-Value	
SAMPLE		3	7.682	2.561	10.597	0.0037
Residual		8	1.933	0.242		

Dependent: HISTAMINE

Fisher's Protected LSD

Dffect: SAMPLE

Dependent: HISTAMINE

Significance level: .05

	Count	Mean	
Element 1	3	7.120	a
Element 4	3	7.840	a
Element 8	3	7.850	a
iSeroant 12	S	9.320	h

Fisher's Protected LSD

Effect: SAMPLE

Dependent: MC

Significance level: .05

	Count	Mean	
Elementl2	3	60.150	a
Element 8	3	62.170	a
Element 4	3	68.240	b
Element 1	3	69.180	i»

Type III Sums Squares

Source		Sum of Squares		Mean Square	F-Value	P-Value
SIMPLE	3	178.4541		59.485	31.731	0.0001
Residual	8	14.997		1.875		

DependentMC

Type III Sums of Squares

Source	df	Sum of Squares		Mean Square	F-Value
SAMPLE	3	0.027	0.009	72.800	0.0001
Residual	8	0.001	1,250 E4		

DependentWATER

Fisher's Protected LSD

EffectSAMPLE

DependentWATER

Significance level:05

	Count	Mean	
Element 12	3	0.520	a
Element 8	3	0.540	a
Element 4	3	0.600	b
Element 1	3	0.640	c

Fisher's Protected LSD

EffectSAMPLE

DependentTOTAL VIABLE COUNT

Significance level:.05

	Count	Mean
Element 8	3	0.000
Element 4	3	0.000
Element 1	3	215.667
Element 12	3	1646.667

None were slgnlflcanity diffrenct at this level.

Type III Sums of Squares

Source		Sum of Squares		Mean Square	F-Value	P-Value
SAMPLE	3	567855.583		5672855.583	1.623	0.2594
Residual	8	9322399.33		116299.917		

Dependent TOTAL VIABLE COUNT

APPENDIX VII

Type III Sums of Squares

Sirrce		df	Sum of Square	Mean Square	F-Value	P-Value
[SAMPLE		6	2090122850.00C	348241.424	4.032	0.014
(Residual		LT		• 7£		

Dependent: HISTAMINE

Scheffe's S
Effect: SAMPLE
Dependent: HISTAMINE
Significance level:.05

	Count	Mean
Element 1	3	14.801
Element 4	3	14.872
Element 3	3	14.667
Element 2	3	14.383
Elemeot 5	3	21,740
Element 6	3	21.942
Element 7	3	40.151

None were sigificantl+A4y dlffrent at the this level
Type III sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SIMPLE	6	1286.624	214.437	314.224	0.0001
Residual	14	9.42	0.871		

Dependent Nacl

Scheffe's S
Effect SAMPLE
Dependent: Nacl
Significance levet.05

	Count	Mean
Element 5	3	0.700
Element 7	3	0.700
Element 6	3	0.800
Element 3	3	15.610
Element 4	3	15.682
Element 2	3	18.151
Element 1	3	18.320

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SIMPLE	6	29.194	4.866	1.366	0.0001
Residual	14	48.177	3.441		

DependentMC

Scheffe's S
Effect SAMPLE
Dependent MC
Significance level:.05

APPENDIX VII

	Count	Mean
Element 7	3	37.000
Element 1	3	46.210
Elemest"	3	49S1G
Element 3	3	49.540
Element 4	3	49.540
Element 5	3	60.491
Element 6	3	62.130

Type ill Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	0.006	0.001	3.571	0.233
Residual	14	0.U04	3.000 E4		

DepenendentWATER

Scheffe's S
Effect SAMPLE
Dependent: WATER
Significance ievel:.05

	Count	Mean
Element 7	3	0.560
Element 1	3	0.620
Element 2	3	0.620
Element 4	3	0.620
Element 3	3	0.630
Element 5	3	0.670
Element 6	3	0.670

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	5041668.557	828811.111	1.822	1684
Residual	14	6444333.33:	480308.524		

DepenendentTOTAL VIABLE COUNT

Scheffe's S
Effect: SIMPLE
Dependent HISTAMINE
Significance level:,05

	Count	Mean
Element 1	3	79.000
Element 2	3	218.000
Element 3	3	262.000
Element 4	3	2.340 E-3
Element 5	3	1.240 E-5
Element 6	3	8.300 E-5
Element 7	3	1.42 E-7

None were significantly diffrent of this level.

APPENDIX VIII

Type III Sum of Squares

Source	df	Sum of Squares	Mean Square		F-Value P-Value
SAMPLE	6	20901118.85;	3483519.810		4.075| 0.0142
Reb&aal	14	I SS92CS.^	' S:i947???		I

DependentHISTAMINE

Scbeffe's S
Effect: SAMPLE
Dependent HISTAMINE
Significance level:.05

	Count	Mean	
Element 3	3	14.610	a
Element 2	3	14.620	
Element I	3	14.670	
Element 4	3	15.250	
Element 6	3	20.170	b
Element 5	3	20.330	b
Element 7	3	42.800	e

Type III Sum of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	1495.868	249.311	726.845	0.0001
Residual	14	4.802	0.343		

DependentNacl

Scbeffe's S
Effect SAMPLE
Dependent Nacl
Significance level:.05

	Count	Mean	
Element 5	3	0.800	
Element 6	3	0.800	
Element 7	3	0.800	
Element 4	3	15.010	c
Element 3	3	15.321	b
Element 2	3	16.611	b
Element 1	3	17.359	b

Type III Sum of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	123.628	205.271	20.004	0.0001
Residual	14	143.659	10.261		

DepenendentMC

Scbeffe's S
Effect: SAMPLE
Dependent MC
Significance level:.05

APPENDIX IX

Type III Sums of Square

Sume-p	df	"v:s:fSi«.j:es	jfem S	,-M	" Value	P-Valas
SAMPLE	6	1534.982	255.830		184.165	0.0001
Residual	14	19.448	1.389			

BependentHIST AMINE

Scheffe's S
EffectSAMPLE
Dependent; HISTAMINE
Significance level:.05

	Count	Mean	
Element 1	3	16.630	a
Element 2	3	11.660	a
Element 3	3	13.340	b
Element 4	3	13.511	b
Element 6	3	15.122	b
Element 5	3	15.671	b
Element 7	3	20.552	c

Type HI Sums of Square

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	1495.815	249.303	1810.413	0.0001
Residual	14	1.928	0.138		

DepenendentNaci

Scheffe's S
Effect SAMPLE
Dependent: Nacl
Significance level:.05

	Count	Mean	
Element 6	3	0.700	
Element 5	3	0.800	
Element 7	3	0.800	b
Element 3	3	15.741	b
Element 4	3	15.802	b
Element 2	3	17.552	c
Element 1	3	17.691	c

Type III Sums of Square

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	1368.092	228.015	39.090	0.000!
Residua	14	81.663	5.833		

DepenendentMC

APPENDIX IX

Scheffe's S

Effect SAMPLE

Dependent MOISTURE

Significance level:.05

	Count	Mean	
Element 1	3	65.021	
Element 3	3	66.140	a
Element 2	3	66.400	a
Element 4	3	67.000	a
Element 6	3	68.000	a
Element 5	3	68.152	b
Element 7	3	70.011	b

Type III Sums of Square

Source	df	Sum of Squares	faean square	[--value	P-Value
SAMPLE	6	0.240	0.004	30.250	0.0001
Residual	14	0.002	1.333 E -4		

DependentWATER

Scheffe's S

Effect SAMPLE

Dependent WATER

Significance level:.05

	Count	Mean	
Element 2	3	0.620	a
Element 3	3	0.640	a
Element 4	3	0.640	a
Element 1	3	0.650	a
Element?	3	0.650	a
Element 6	3	0.670	a b
Element 5	3	0.680	a b

Type III Sums of Square

Source	dl	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	5.062	8.436	1.630	2112
Residual	14	7.246 E 14	5.175		

DependentTOTAL VIABLE COUNT

Scheffe's S

Effect SAMPLE

Dependent TOTAL VIABLE COUNT

Significance level;.05

	Count	Mean
Element 1	3	0.000
Element 2	3	0.000
Element 3	3	262.000
Element 4	3	2340.000
Element 7	3	3.530 E5
Element 5	3	1.940 E6
Element 6	3	3.50 E6

None were singniflcantly different at this level

APPENDIX X

Type III of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6				0.0001
Residual	14				

D ependent HISTAMIN E

Scheffe's S
Effect SAMPLE
Dependent HISTAMINE
Significance level:.0S

	Count	Mean	
Element 1	3	11.021	
Element 3	3	11.140	
Element 2	3	11.152	a
Element 4	3	11.422	
Element 6	3	11.981	b
Element 5	3	12.133	b
Element 7	3	13.114	c

Type U Sums of Squares

Source	df	Sura of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	14.95.815	249.303	1810.413	0.0001
Residual	14	1.928	1.39		

DependentNacI

Scbeffe's S
Effect SAMPLE
Dependent Nacl
Significance IeveL.05

	Count	Mean	
Element 5	3	0.800	
Element 6	3	0.800	a
Element 7	3	0.800	
Element 4	3	14.850	b
Element 3	i	14.880	b
Element 2	3	16.G02	c
Element 1	3	16.722	c

Type II Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	1356.032	720.015	99.06	0.0001
Residual	14	81.663	58.33		

DependentMC

APPENDIX X

	Count	Mean	
Element 1	3	rr non	
Element 7	3	66.841	a
Element 3	3	67.400	
Element 4	3	67.621	
Element 3	3	67.702	
Element 5	3	68.SSI	a b
Element S	3	69.112	a b

Type 11 Sums of Squares

Source	df	sum or Squares	Mean Square	F-Value	P-Value
SAMPLE	6	0.24	0.004	1.63	2112
Residual	14	0.02	5.175 E 3		

Dependent'WATER

Scheffe's S
Effect SAMPLE
Dependent: WATER
Significance level:.05

	Count	Mean	
Element 7	3	0.610	a
dement 2	3	0.640	a b
Element 1	3	0.650	b
Element 3	3	0.650	b
Element 4	3	0.650	b
Element 6	3	0.660	b
Element 5	3	0.670	b

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
SAMPLE	6	5041668.557	840278.1	1.822	0.314
Residual	14	6444333.33C	460309.52		

DependentVLABLE COUNT

Scheffe's S
Effect: SAMPLE
Dependent: TOTAL VIABLE COUNT
Significance level:.05

	Count	Mean
Element 7	3	0.000
Element 2	3	0.000
Element 1	3	0.000
Element 4	3	0.000
Element 3	3	330.660
Element 6	3	353.000
Element 5	3	1400.000

None were singnlflcantly different of this level

APPENDIX XI

TABLE 4.1

	HISTAMINE	Nacl	MC	WATER	TOTAL VIABLE COUNT
HISTAMINE	1				
Nacl	■0.044932223	1			
MC	0.5410307-?	0.1714949	I		
WATER	0.303590215	0.2946111	0.7738388 I		
TOTAL VIABLE COUNT	•0.025917079			0.074338828 t	
		0.0730393	0.2274096		

TABLE 4.2

	H1ST4MIMK	SC	MC	WATER T!'TAL V.'ANLE	COUNT
HISTAMINE	I				
Nacl	-0.573959619	1			
MC	0.88460896		1		
		0.8033363			
WATER	0.747089372		0.8579887 1		
		0.8379757			
TOTAL VIABLE COUNT	■0.315075058	0.5644945		-0.571536302 1	
			0.3934225		

TABLE 4.3

	HISTAMINE	Nacl	MOISTURE	WATER	TOTAL VIABLE COUNT
HISTAMINE	1				
Nacl	0.156043965	1			
MOISTURE	-0.437468811	0.0313378	1		
WATER	-0.013145583	0.5990394	0.1424638 1		
TOTAL VIABLE COUNT	-0.100641969	0.2041624	0.2281653	-0.383649043 I	
		0.6006735			

APPENDIX XII

TABLE 4.4

	HISTAMINE MC	WATER	TOTAL VIABLE COUNT
HISTAMINE	1		
MG	*0.889215626	1	
WATER	■0.124713797	0.5293623 1	
TOTAL VIABLE COUNT	0.757856812	0.2100017	1
	0.5588987		
			TOTAL VIABLE COUNT

TABLE 4.5

	BATCH HC	MC	WATER	TOTAL VIABLE COUNT
BATCH	1			
HC	0.843964981	1		
MC	•0.38726682		1	
	0.4037656			
WATER	•0.166666667		0.1945298	1
	0.6087889			
TOTAL VIABLE COUNT				1

TABLE 4.6

	HISTAMINE MC WATER			TOTAL VIABLE COUNT
HISTAMINE	1			
MC	•0.685319784 1			
WATER	■0.743516914 0.9220026 1			
TOTAL VIABLE COUNT	0.410842092			1
		0.3599345	0.3430510	

APPENDIX XIII

	HISTAMINE Nacl		WATER	TOTAL VIABLE COUNT
HISTAMINE	•0.944104 IBS	I		
Nacl	0.851609479		I	
MG		0.952242		
WATER	0.830801089		0.8851095	I
		"AДIISVM		
TOTAL VIABLE CcWr	0-56545*5044		0.5365708	0.535564493
		0.5679925		

TABLE 4.9

HISTAMINE				
Nacl	0 9535188	I		
ac	•}.»£ i’MlM*7		1	
		0.9458408		
WATER	0.7:13788997		0.8812863	1
		0.8224167		
TOTAL VIABLE COUNT	0.532113449		0.5253979	0.392453066
		0.5548719		

TABLE.4 10

	HISTAMINE Naci	MC	TOTAL VIABLE COUNT
HISTAMINE			
Nad	•0.796651204		
MC	0-45127104	I	
		0.4260272	
WATER	0.706374098	0.4686571	I
		0.71695660	
TOTAL VIABLE COUNT	0.339906096	0.1884563	0.385862356
		0.5142650	

u

	HISTAMINE Nacl			TOTAL VIABLE COUNT
HISTAMINE				
Nacl	-0.7584024			
MG	0.444061466			
WATER	0.122573249		0.1230989	
		0.5289693		
TOTAL VIABLE COUNT	0.552429743			0.50619478

APPENDIX XIV

Type III Sums of Square

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TEMPERATURE	3|	398.185	132.728	22.858	0.0001
Residual	Ouj	394.810	USO''		

Dependent:HISTAMINE

Scheffe's S
Effect TEMPERATURE
Dependent: HISTAMINE
Significance level:,05

	Count	Mean	
FSKEZER	18	11.472	a
FRIDGE	18	13.486	a
SOLAR	18	16,606	b
AMBIENT	18	17.268	b

Scheffe's S
Effect: TEMPRETURE
Dependent: Nacl
Significance level:.05

	Count	Mean	
FREEZER	18	10.508	a
FRIDGE	18	i 1.130	a
SOLAR	18	11.293	a
AMBIENT	18	11.293	a

None were significantly different at this level

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TEMPERATURE	3	7.516	2,505	0.038	0.9900
Residual	68	4482.964	65.926		

DependeutNacl

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	, F-Value	P-Value
TEMPERATURE	3	7200.146	2400.049	69.567	0.0001
Residual	68	2345.997	34.500		

DependentMC

Scheffe's S
Effect: TEMPERATURE
Dependent: MC
Significance level:.05

	Count	Mean	
SOLAR	18	43.944	a
AMBIENT	18	52.334	b
FRIDGE	18	66.785	c
FREEZER	18	67.673	c

Scheffe's S
EffectTEMPERATURE
Dependent: WATER
Significance level:.05

	Count	Mean
SOLAR	18	0.626
AMBIENT	18	0.638
FRIDGE	18	0.648
FREEZER	18	0.653

Type III Sums of Squares

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TEMPERATURE	3	0.008	0.003	3.580	0.0182
Residua,	68	0,052	0.091		

Dependent:WATER

APPENDIX XIV

Types Ill Sums Of SV'i-f"

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TEMPRETURE	3	8.666 E12	2.889E 12	1.645	0.1872
Residual	68	1.194 E14	1.756 E12		

DspendentTOTAL VIABLE COUNT

Scheme's S
Effect TEMPERATURE
Dependent TOTAL VIABLE COUNT
Significance level:,05

	Count	Mean	
SOLAR	18	341.667	a
AMBIENT	18	1.598 E5	a
FRIDGE	18	2.261 E5	a
FREEZER	18	9.072 E5	a

None were significantly different at this level.