بسم الله الرحمن الرحيم

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Evaluation of Biogenic Amines in Cooked Fermented Fish (Fasekh) in Khartoum City

تقييم الأمينات الحيوية فى السمك المخمر (الفسيخ) المطبوخ فى مدينة الخرطوم

By

Ghaydaa Mustafa Ebrahim Niazy

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Supervisor

Dr. Ebrahim Alfaig Alnoor Alfaig

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Abstract

This study was conducted to evaluate the chemical composition and determination of Biogenic Amines (Bas) concentration of Sudanese fermented fish (Fasekh) which were collected randomly from different locations at Khartoum city. Fasekh samples immediately transferred in sterilized container containing ice box. The approximate analysis were conducted in the National Food Research Center NFRC, Shambatt, Sudan, while the biogenic amines analysis and in National Research Center, Al Doki, Cairo by using standard analytical procedures. Chemical composition of Fasekh samples were significantly different ($P<0.05$). The highest moisture content was 64.65%, recorded for Fasekh samples collected from center Khartoum, whereas, the lowest moisture one was 61.34%, reported for Fasekih samples collected from north Khartoum. Fasekh samples collected from north Khartoum, center Khartoum and east Khartoum had protein content of 22.12%, 19.64% and 20.78% respectively. Fasekh samples had fat content ranged between 6.35% to 7.02%. Fasekh samples collected from Khartoum city had pH value with an average 5.81. Biogenic Amines (BAs) concentration of fermented fish (Fasekh) samples were significantly different ($P<0.05$). Fasekh samples collected from north Khartoum recorded higher Terptamine concentration 2.55mg/100g, B-phenyl 1.15mg/100g and Cadaverine 0.49mg/100g. Higher histamine concentration recorded for samples collected from center Khartoum 0.54mg/100g, whereas, the lowest one concentration recorded for samples collected from east Khartoum 0.32mg/100g. Higher Putrescine biogenic amine level 0.30mg/100g, obtained for fasekh samples collected from east Khartoum, while, the lowest one 0.12mg/100g, obtained for samples collected from center Khartoum. All samples under investigation were Tyramine and Sprmidine free. It is worth noting that, the concentration of Biogenic amine of Sudanese fermented fish (Fasekh) within codex standard recommended.

الملخص

صُممت هذه الدراسة لتقييم التركيب الكيميائي وتقدير الأمينات الحيوية للسمك السوداني المخمر (الفسيخ) الذي تم تجميعة عشوائيا من مناطق مختلفة في مدينة الخرطوم : الخرطوم شمال و الخرطوم وسط و الخرطوم جنوب. تم نقل عينات الفسيخ في الحال في حاويات معقمة محتوية على صناديق ثلجية. تم اجراء التحليل الكيميائي في المركز القومي لأبحاث الأغذية، شمبات السودان، بينما تم إجراء التحليل التقديري للامينات الحيوية في المركز القومي للبحوث، الدوقي القاهرة باستخدام طرق التحليل المرجعية. تأثر التركيب الكيميائي للفسيخ تأثيرا معنويا. سجلت عينات الفسيخ التي تم تجميعها من الخرطوم وسط أعلى نسبة رطوبة 64.65%، بينما سجلت عينات الفسيخ التي تم تجميعها من الخرطوم شمال أقل نسبة رطوبة61.34%. سجلت عينات الفسيخ التي تم تجميعها من الخرطوم شمال و الخرطوم وسط و الخرطوم شرق نسبة بروتين 22.12% و19.64% و 20.78% على التوالي. احتوت عينات الفسيخ على نسبة دهن تراوحت بين6.35% إلى 7.02 %. عينات الفسيخ التي تم تجميعها من مدينة الخرطوم احتوت على أس هيدروجيني في متوسط 5.81.

تأثر تركيز الأمينات الحيوية للسمك المخمر تأثراً معنوياُ ($P<0.05$). سجلت عينات الفسيخ التي تم تجميعها من مدينة الخرطوم شمال أعلى تركيز من تربتامين 2.55 وبيتا فينايل أمين 01.150 وكدرفين 49. 0مل جرام \100جرام على التوالي. سجلت عينات الفسيخ التي تم تجميعها من الخرطوم وسط أعلى نسبة تركيز في الهيستامين 0.548مل جرام \100جرام، بينما سجلت العينات التي تم تجميعها من الخرطوم شرق أقل تركيز في الهيستامين 0.497مل جرام \100جرام. تحصلت العينات التي تم تجميعها من الخرطوم شرق على أعلى مستوى من البيوترسسيسين 0.300مل جرام \100جرام، بينما تحصلت العينات التي تم تجميعها من الخرطوم وسط على أقل مستوى من البيوترسيسين 0.211مل جرام\100جرام. خلت جميع العينات تحت التحري من التايرام والاسبيردمين. من الجدير بالذكر، تركيز الأمينات الحيوية في السمك المخمر (الفسيخ) يقع ضمن الحدود الموصى بها من قبل الكوديكس.

Dedication

To my parents

For their true love, constant trust and the principles that guided my life

To my husband

For his devotion, understanding and support during all difficulties.

To my children

For making everything worthwhile

And my sisters and by brother

For their praying, wishing to me all the best

Ghaydaa

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CHAPTER ONE

INTERDUCTION

Biogenic amines are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Askar and Treptow, 1986). Biogenic amines are generated in course of microbial, vegetable, and animal metabolisms (Anli and Bayram, 2009). The decarboxylation process can proceed through two biochemical pathways:decarboxlytion through endogenous decaroboxylase enzymes (naturally occurring) or by exogenous decomposition through enzymes released by microflora, the production of amines by the exogenous process is considered far more significant (Ruiz *et al.*, 2004).

They can also be classified as volatile (phenylethylamine) and nonvolatile (histamine, cadaverine, putrescine, sperm- ine, agmatine, tryptamine, and tyramine). Biogenic amines execute a number of crucial functions in the physiology and development of eukaryotic cells. The most active biogenic amines are histamine and tyramine (Anli and Bayram, 2009). Polyamines such as putrescine, spermine and spermidine also play essential roles in cell growth. Low concentrations of biogenic amine are usually tolerated by the human body, and they are efficiently detoxified by mono- and diamine oxidase in the intestinal tract. However, these compounds can have adverse effects when present at high concentrations and pose a health risk for sensitive individuals (Askar and Treptow, 1986).

Dietary biogenic amines are especially found in aged and fermented food and in foods containing relatively high amounts of free amino acids (Anli and Bayram, 2009). Most notably, ripened cheese, fermented meat, smoked and tinned fish, sauerkraut and fermented yeast and fermented soy

products usually contain significant amounts of histamine. Scombrotoxin fish poisoning (SFP) (often called "histamine poisoning") is caused by ingestion of certain species of marine fish that contain high levels of histamine and possibly other biogenic amines. The fish species involved include tuna, which accounts for 8 percent of globally traded fish. Other pelagic species such as mackerel, sardines and anchovy, which account for significant global fish production, can also be involved (Lehan and Olley, 2000). These fish species contain high levels of free histidine in their tissues and when conditions are favorable for bacteria to multiply in fish, e.g. when fish are subjected to temperature abuse during and/or after harvest, bacterial decarboxylation of histidine leads to histamine formation. Other biogenic amines produced during bacterial growth in fish may potentiate the effect of histamine (Ruiz *et al.*, 2004).

Fermented fish is a traditional preservation of fish. Before refrigeration, caning and other modern preservation techniques became available; fermenting was an important preservation method. Fish rabidly spoils, orgoes rotten, unless some method is applied to stop the bacteria that produce the spoilage. Fermentation is a method which attacks the ability of microbial to spoil fish. It does this by making fish muscle more acidic, bacteria usually cease multiplying when the pH drops below 4.5 (Steinkraus, 2004).

Fermented fish can be notable for their putrid smell. These days are many other techniques of preserving fish, but still fermented because some people enjoy the taste (Mostafa and Salem, 2015).

Fasekh is one of Sudanese fish products. Fasekh is not a truly indigenous Sudanese food product. The technique of its making entered the Sudan during the Turko-Egyptian rule (1821-1885) but its production on large scale was only well established during the Anglo-Egyptian condominium rule (1898-1956).

Therefore, it is acceptable to assume that fasekh production in the Sudan is about a century old. During this period, the Sudanese have brought about some changes in the preparation method. Nevertheless, both local consumption and export trade of the product are today almost completely monopolized by families of Egyptian descent, particularly the ethnic group called Nagada. Other Sudanese dominate in the fishing and manufacturing stages of the business (Dirar, 1993). Fasekh it made from one or both of two morphologically very similar fish Kawara (*Alestes spp*) and Kass (Tiger fish; *Hydrocynus spp).* The major reason given by the processors for the choice of kawara and kass to make fasekh is that these fish types are relatively lean. Both Sudanese and Egyptian consumer demands only little fat in the fasekh (Dirar, 1993).

Since the consumption of Fasekh in Sudan is very high and there is a risk of biogenic amines in popular health specially histamine and there was n researches in biogenic amines on fermented Fasekh in Sudan so this study was conducted to evaluate and determine the biogenic in cooked Fasekh in Khartoum city.

The objectives of the current study were to:

1- Determine and evaluate the biogenic amine in ready to eat fermented fish (Fasekh).
2- Assess the safety of the fermented ready to eat Fasekh regarding their content of the significant biogenic amines.
3- Determine the chemical composition of cooked fermented fish Fasekh in Kartoum city.

CHAPTER TWO

LITERATURE REVIEW

2.1. Classification of biogenic amines (BAs)

The chemical structures of biogenic amines are defined as low molecular weight can either be: aliphatic (putrescine, cadaverine, spermine, and spermidine), aromatic (tyramine, phenylethylamine), heterocyclic (histamine, tryptamine) (Prester, 2011). Several authors had classified cadaverine, putrescine, spermine, and spermidine among polyamines.

Table 1: Classification of biogenic amines (BAs)

Aliphatic	**Aromatic**	**Heterocyclic**
Putrescine	Tyramine	Histamine
Cadaverine	Phenylethylamine	Tryptamine
Spermine		
Spermidine		

Source : Karovicova and kohajdova*a* (2005).

2.2. Function of Biogenic amines (BAs)

Biogenic amines are sources of nitrogen and precursors for the synthesis of hormones, alkaloides, nucleic acids and proteins (Silla-Santos, 1996). They can also influence the processes in the organism such as the regulation of body temperature, intake of nutrition, increase or decrease of blood pressure (Prester, 2011).

In plants, the diamine putrescine and the polyamines spermidine and spermine are implicated in a number of physiological processes, such as cell division, flowering, fruit development, response to stress and senescence (Halasz *et al.,* 1994).

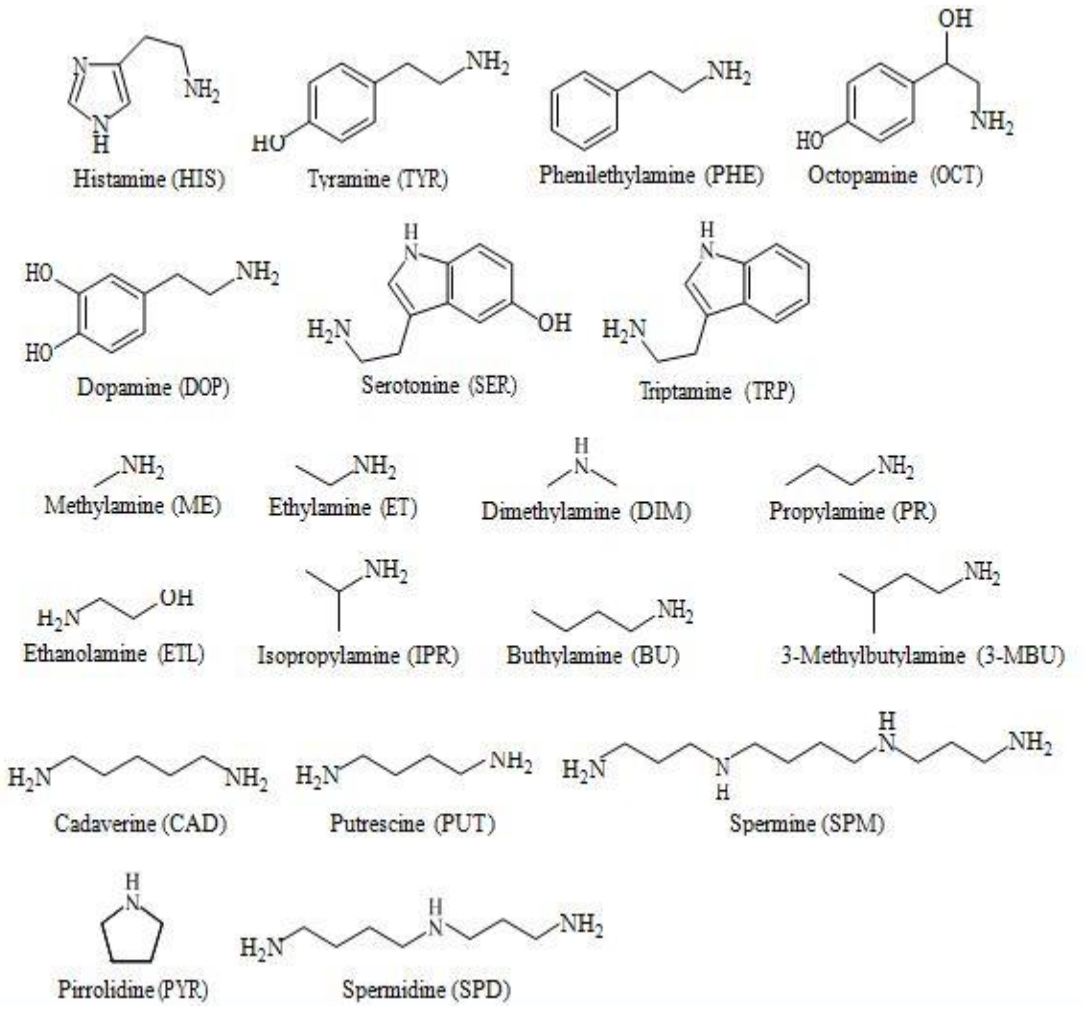

Figure 1: The chemical structure of biogenic amines.

Source: Karovicova and kohajdova (2005).

Polyamines are important for the growth, renovation, and metabolism of every organ in the body and essential for maintaining the high metabolic activity of the normal functioning and immunological system of gut (Silla-Santos, 1996). The roles of polyamines in cellular metabolism and growth, the requirement for polyamines is particularly high in rapidly growing tissues. Indeed, the importance of putrescine, spermidine, and spermine in tumour growth is widely recognized, and the inhibition of polyamine biosynthesis in tumour-bearing individuals is one of the major targets of cancer therapy research (Ruiz *et al.*, 2004).

Biogenic amines are potential precursors for the formation of carcinogenic N-nitroso compound (Prester, 2011). The reaction of nitrosating agents with primary amines produces short-lived alkylating species that react with other components in the food matrix to generate products (mainly) alcohols) devoid of toxic activity in the relevant contents (Mostafa and Salem, 2015). The nitrosable secondary amines (agmatine, spermine, spermidine, etc..) can form nitrosamines by reaction with nitrite, while tertiary amines produce a range of labile N-nitroso products (Halasz *et al.*, 1994).

In fatty foods, such as bacon, at high temperature and in the presence of water, the carcinogen N- nitrosopyrrolidine can be formed from putrescine or spermidine (Silla- santose, 1996). Some biogenic amines such as: putrescine, cadaverine, and spermidine can act as free radical scavengers (Mostafa and Salem, 2015).

Tyramine has a remarkable antioxidative activity, which increases with its content. Thus, inhibiting effect depends on amino and hydroxy groups (Halasz *et al.*, 1994). The spermine is able to regenerate tocopherol from the tocopheroxyl radical through hydrogenic donor from amino group. The spermine radical next binds lipid or peroxide radicals into a lipid complex. Some biogenic amines contribute to the flavor and taste of food (Prester, 2011).

2.3 Hazard Identification

Generally speaking, food hazards fall into three categories

1. Biological contamination: by organic materials present (e.g., animal, bird, insect remains) or from toxins produced from molds and bacteria.
2. Chemical and biochemical contamination: by chemicals deliberately introduced (e.g., pesticides), by accident (e.g., fuel), from cleaning chemicals, or actually produced through a process, and from biochemicals such as toxins produced by molds and fungi.

3. Physical contamination: by physical objects present in the raw materials (e.g., stones, glass, metallic parts), or picked up from plants (e.g., metallic components, glass), or accidentally dropped in by process operators/contractors (e.g., pens, tools) (Lee and Hathaway, 1998).

Hazard identification provides the identification of the types of adverse health effects that can be caused by exposure to some agents as well as the characterization of the quality and weight of evidence supporting this identification (Prester, 2011). It is the process of determining whether exposure to a stressor can cause an increase in the incidence of specific adverse health effects and whether the adverse health effect is likely to occur in humans. The process examines the available scientific data for a given hazard and develops a weight of evidence to characterize the link between the negative effects and the considered agent. Hazard identification is based on experience and has a qualitative reasoning, providing a basis for identifying, evaluating, defining, and justifying the selection (and rejection) of control measures for reducing risk (Lee and Hathaway, 1998; Prester, 2011).

2.3.1 Biogenic amines (BAs) as a biological hazard

Biogenic amines are considered biochemical hazards as their occurrence in food is related with spoilage and fermentation although their presence has also been reported in raw materials of good hygienic quality such as putrescine in meat, fish, milk, and fruits (Ruiz *et al.*, 2004). However, high concentrations of biogenic amine are generally produced in foods during fermentation. The main aim of lactic acid bacteria (LAB) fermentation is the conversion of carbohydrates to lactic acid; therefore, the action of LAB is desirable for the production of fermented sausages, cheese, sour dough bread, and also for malolactic fermentation of wine. However, some of the bacteria involved in fermentation can produce biogenic amines (Prester, 2011). Moreover, spoilage

microorganisms may strongly contribute to biogenic amine formation in fermented foods even though food is not spoild (Lehane and Olley, 2000).

2.4 Conditions for formation of biogenic amines (BAs)

Although amino acid decarboxylases are not widely distributed among bacteria, species of many genera such as *Bacillus, Citrobacter, Clostridium, Klebsiella, Escherichia, Proteus, Pseudomonas, Shigella, Photobacterium* and the lactic acid bacteria *Lactobacillus, Pediococcus, and Streptococcus* are capable of decarboxylating one or more amino acids (Prester, 2011). Because the biogenic amines in food products are mainly generated by decarboxylation of the corresponding amino acids precursors, thought bacterial decarboxylases, for formation of biogenic amines are necessary these conditions are:

- Availability of free amino acid, but not always leading to amine formation,
- Presence of decarboxylase-positive microorganisms.
- Conditions that allow bacterial growth, decarboxylase synthesis and decarboxylase activity (Bodmer *et al.*, 1999).

Free amino acids either occur as such in foods, or may be liberated through proteolysis. Microbial strains with high proteolytic enzyme activity also potentially increase the risk for biogenic amines formation in food systems by increasing the availability of free amino acids.

Removal of the α-carboxyl group from a proteinous amino acid leads to the corresponding biogenic amines. The names of many biogenic amines correspond to the names of their originating amino acids: histamine from histidine, tyramine from tyrosine, β-phenylethylamine from phenylethylalanine, tryptamine from tryptophane (Lee and Hathaway, 1998). In plants and some microorganisms, alternative pathway exists to produce putrescine from arginine via agmatine. Lysine is decarboxylated by lysine decarboxylase to produce cadaverine, although it can also be formed by ornitine decarboxylase if the

content of ornitine is low, but that of lysine is high (Silla- santose, 1996). Amino acid decarboxylase activity is stronger in an acidic environment, the optimum pH being between 4.0 and 5.5 (Halasz *et al.*, 1994).

Furthermore, in such an environment bacteria are more strongly encouraged to produce these enzymes, as a part of their defense mechanisms against the acidity (Ababouch *et al.*, 2007).

The attention is paid to the influence of glucono- δ-lactone (GDL) on the production of biogenic amine in dry sausages. GDL causes decreasing of pH in sausages, which results in increasing decarboxylase activity of bacteria (Ruiz *et al.,* 2004). In these conditions bacteria produced more decarboxylases as part of their protective mechanism. The production of histamine, tyramine and putrescine is increased (Buncic, 1993). The presence of fermentable carbohydrate, such as D-glucose, enhances both growth and amino acid decarboxylase activity in bacteria. D-Glucose content in the range of 0.5-2.0% has been reported to be optimal, while levels in excess of 3% inhibited enzyme formation (Bardocz, 1995). Oxygen supply also appears to have a significant effect on the biosynthesis of biogenic amine. *Enterobacter cloacae* produce about half the quantity of putrescine in anaerobic compared with aerobic conditions, and *Klebsiella pneumoniae* synthesizes significantly less cadaverine but acquires the ability to produce putrescine under anaerobic conditions (Halasz *et al.,* 1994). The redox potential of the medium also influences biogenic amine production. Conditions resulting in a reduced redox potential stimulate histamine production, and histidine decarboxylase activity seems to be inactivated or destroyed in the presence of oxygen (Bardocz, 1995). Amine formation by bacteria is decisively influenced by temperature. Temperature between 20°C and 37°C is optimal for the growth of the most bacteria containing decarboxylases, decreased temperature stops their growth (Ababouch *et al.,* 1991). Presence of sodium chloride activates tyrosine decarboxylase activity

and inhibits histidine decarboxylase activity (Bulushi *et al.*, 2009). At the content of sodium chloride 3.5 % the ability of *Lactobacillus buchneri* to form histamine is partly inhibited and at the content of 5.0% its formation is stopped. The natrium nitrite activates tyrosine decarboxylase activity (Ababouch *et al.*, 2007).

Repression of histidine decarboxylase has also been detected when the amount of histamine was accumulated in the medium. The presence of Histamine has an inhibiting effect on the histidine decarboxylation activity of *Photobacterium histaminum* C-8; histamine, agmatine and putrescine inhibit the histidine decarboxylation activity of *Photobacterium phosphoreum* N-14 (Halasz *et al* , 1994). Application of suitable starter cultures with amino oxidase activity decreases formation of biogenic amines. Addition of *Micrococcus varians* in dry sausages causes decreasing level of tyramine .

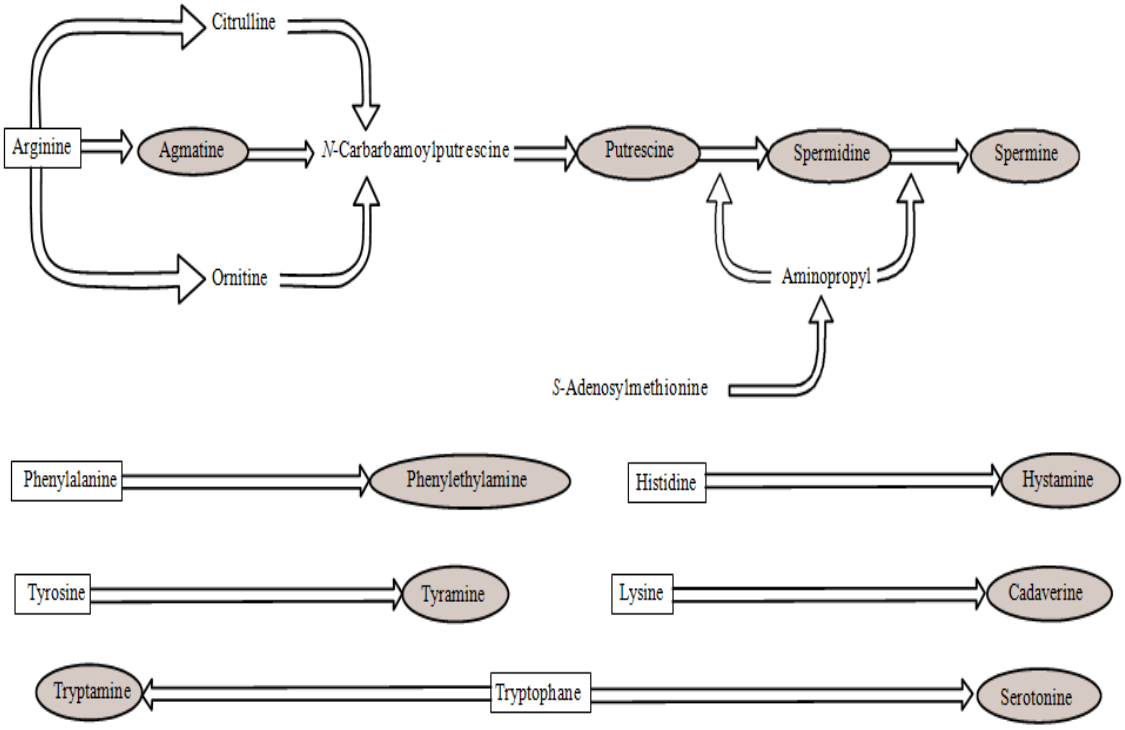

Figure 2: Biogenic amines formation

Source: : Karovicova and kohajdova (2005).

2.5 Toxicology of biogenic amine

Biogenic amines are organic substances present in food, which induce toxicological risks and health troubles (Prester, 2011). The most frequent food borne intoxifcations caused by biogenic amines involve histamine (Bardocz, 1995). An intake of 5-10 mg of histamine can be considered as defecting to some sensitive people, 10 mg is considered as tolerable limit, 100 mg induce a medium toxicity and 1000 mg is highly toxic (Lehane and Olley, 2000).

2.5.1 Histamine

Histamine is also referred to as scombroid fish poisoning because of the association of this illness with the consumption of scombroid fish, such as tuna, mackerel, and sardines. Another phenomenon is the cheese reaction caused by high levels of tyramine in cheese (Mostafa and Salem, 2015) Histamine is contained in mast cells and basophiles, and its biological effects are usually seen only when it is released in large amounts in the course of allergic and other reactions. Histamine exerts its effects by binding to receptors on cellular membranes in the respiratory, cardiovascular, gastrointestinal, and hematological/immunological systems and the skin. There are three types of receptors, H_1, H_2, and H_3. The most common symptoms result from action on the cardiovascular system (Prester, 2011). Histamine causes dilatation of peripheral blood vessels, capillaries and arteries, thus resulting in hypotension, flushing, and headache. Histamine-induced contraction of intestinal smooth muscle, mediated by H_1 receptors, may account for abdominal cramps, diarrhea, and vomiting (Askcar and Treptow, 1986). Gastric acid secretion is regulated by histamine through H_2 receptors located on the parietal cells (Prester, 2011).

The histamine poisoning is possible to overcome by using of antihistaminic drugs (Ruiz *et al.,* 2004). For many years, H_1 -receptor antagonists have been available for the treatment of allergic conditions while

H_2- receptor antagonists have been available for the treatment of gastric ulcers. By contrast, the H_3 -receptor subtype was only discovered in 1983 and H_3-receptor antagonists were used for treatment of central nervous system disorders. The role of H_3 receptors in learning and memory indicates that H_3 -receptor antagonists could have a role in the treatment of memory disorders such as Alzheimer's disease (landete *et al.,* 2006). Humans metabolize histidine to urocanic acid through the activity of L-histidine ammonium lyase, to form glutamate and then α-ketoglutarate, which enters the citric acid cycle, or to histamine through the activity of histidine decarboxylase Fig 3, (Lehan and Olley, 2000).

There are two ways of histamine metabolism in the human body. Nitrogen in the imidazole cycle is methylated by histamine N-methyltransferase at the formation of N-methylhistamine, which is further oxidized by monoamino oxidase to N-methylimidazolylacetic acid (Landete *et al,* .2006).
This enzyme is very selective for histamine detoxification and involves S-adenosylmethionine as donor of methyl group (Mostafa and Salem, 2015).
Histamine is oxidized by diamino oxidase to imidazolylacetic acid, which is bound to ribose (Prester, 2011). N-Methylation is the major process responsible for termination of the neurotransmitter actions of histamine in the brain, it is also the major pathway for the metabolism of histamine in bronchial epithelium (Landete *et al,* .2006).

Histamine is also converted to inactive acetylhistamine in the intestine, presumably by bacterial enzymes. The human kidney has a considerable capacity for removing histamine from blood. When healthy individuals were infused intravenously with histamine, a large proportion was methylated by the kidney and excreted in the urine and a smaller proportion was excreted unchanged in the urine (Prester, 2011).

2.6 Legal Limits

The nutritional codex of the Slovak Republic had determined the maximal tolerable limit for the following two biogenic amines: histamine (20 mg kg^{-1} in beer and 200 mg kg^{-1} in fish and fish products) and tyramine (200 mg kg^{-1} in cheese) (Halasz *et al.,* 1994). The European Union (EU) has established regulations for histamine levels, such that they should be below 100 mg kg-1 in raw fish, and below 200 mg kg-1 in salted fish for species belonging to the Scombridae and Clupeidae families (Prester, 2011).

A recommended upper limit of 100 to 200 mg kg^{-1} for histamine in meat products has been proposed by the Netherlands Institute of Dairy Research and by the Czech Republic (Landete *et al,* .2006).

2.7 Detoxification of Biogenic amine

In the intestinal tract of mammals affects a detoxification system which is capable of metabolizing normal dietary intake of biogenic amine (Silla-Santos, 1996). N-Methylation is the major process responsible for termination of the neurotransmitter actions of histamine. Under normal conditions in humans exogenous amines absorbed from food are rapidly detoxified by the action of amine oxidase or conjugation, but in the case of allergic individuals or if monoamine inhibitors are applied or when too high levels are consumed the detoxification process is disturbed and biogenic amine accumulate in the body (Halasz *et al.*,1994). The enzymes monoamine oxidase (MAO) and diamino oxidase (DAO) play an important role in detoxification process. However, upon intake of high loads of biogenic amine in food, this detoxification system is unable to eliminate biogenic amine sufficiently. MAO and DAO occur in the gut epithel and thus oxidation products of biogenic amines are getting into the blood circulation (Krizek *et al.,* 2002). Polyamines are usually in the first place acetylated and consequently oxidized by DAO or polyamino oxidases (Ascar and Treptow, 1986).

People with gastrointestinal problems (gastritis, irritable bowel syndrome, Crohn's disease, stomach and colonic ulcers) are also at risk because the activity of oxidizes in their intestines are usually lower than that in healthy individuals. In women, there is premenstrual decrease in the activity of B-type MAO and this can also be a problem (Prester, 2011). Patients, who are taking medicines with inhibiting effect to MAO and DAO such as antihistamines, antimalaria agents, psy-chopharmaceutics, might have a changed metabolism of biogenic amine, which can cause healthy problem (Silla-Santos, 1996; Halasz *et al.,* 1994). Amines, especially putrescine and cadaverine, inhibit histamine detoxifying enzymes and thus act as potentiators of histamine toxicity (Halasz *et al.,* 1994). These amines in the intestinal tract preferably react with MAO and DAO that tend to increase the level of histamine in blood (Silla- Santos, 1997). Aminoguanidine, anserine, carnosine, agmatine and tyramine inhibit DAO and phenylethylamine, tryptamine, octopamine have inhibiting effect on N-methyltransferase. Also, injuries of intestinal mucosa can reduce the function of biogenic amine detoxification enzymes (Ababouch *et al.,* 1991).

2.8 Occurrence of biogenic amine in food

In virtually all foods that contain protein or free amino acids and are subject to conditions enabling microbial or biochemical activity biogenic amines can be expected (Prester, 2011). Biogenic amines are present in fermented products (e.g. cheese 5-4500 mg/ kg^{-1}, wine 5-130 mg dm^{-3}, beer 2.8-13 mg dm^{-3}, sauerkraut 110-300 mg/ kg^{-1}) and in improperly kept food (such as fish 2400-5000 mg /kg^{-1}, beef liver about 340 mg kg^{-1}, prepared meats 10 - 700 mg/ kg^{1}) (Krizek *et al.,* 2002). In non fermented foods these compounds were found useful as indicators and markers of food decomposition (Steinkraus, 2004). Spoiled foods are also rich in biogenic amines and usually contain high levels of putrescine and cadaverine (Mostafa and Salem, 2015). The tissues of scombroid fish contain high levels of free histidine, which may be converted to

histamine by associated microorganisms (Bardocz, 1995). Tuna fish and other fish species of the Scombridae and Clupeidae families have been commonly found to contain high levels of histamine, as a result of inadequate handling and preserved (Ruiz *et al.*, 2004). The quantification of histamine in canned products indicates the mostability of the molecule (Prester, 2011).

Odors that normally signal decomposition to the organoleptic analyst may be modified, reduced, or eliminated by thermal processing, therefore histamine is a useful indicator of decomposition in scombroid and certain other fish (Ruiz *et al.*, 2004).

The formation of high levels of histamine in fish products can be fairly rapid and develops on the number of microorganisms present. Several bacteria are involved in toxicity, such as *Proteus morganii, Hafnia alvei, Acromonas hydrophila, Vibrio alginolyticus, Pseudomonas sp., Klebsiella sp.,* etc. These bacteria are capable of producing hazardous amounts of histamine in very short period of time when fish are held at elevated temperatures (Kovacs, 1999). Low storage temperatures are used in the fishery industry to control bacterial histamine production (Ruiz *et al.*, 2004).

The Biogenic Amine Index (BAI) was proposed for measurement of the quality of raw and processed seafood: BAI = (mg/ kg-^{1} histamine + mg/ kg-^{1} putrescine + mg/ kg-^{1} cadaverine) + mg/kg^{-1} spermine + mg/ kg^{-1} spermidine. BAI value exceeds in 10 is regarded as representing some kind of loss in quality (Buncic, 1993). The manufacturing of sauerkraut coursed in three steps characterized by active microorganisms, which produced biogenic amines (Halasz *et al.*, 1994).

1. *Leuconostoc mesenteroides,* producing putrescine in content about 250 mg kg-^{1}
2. *Lactobacillus sp*., producing putrescine and tyramine.
3. *Pediococcus cerevisiae,* producing histamine in content about 200 mg kg-

The biogenic amines, especially putrescine, accumulate in sauerkraut brine (Bardocz, 1995).

The cadaverine, histamine, putrescine, spermidine, and tyramine were found in the lactic acid fermented vegetables (such as carrot and red beet) in content ranging from 1 to 15 mg kg^{-1} (Halasz *et al.,* 1994). Reports on the hygienic status of leafy vegetables indicate the association of high microbial numbers and the presence of biogenic amines, both with the fresh and packed products. Number of the dominant groups, *Pseudomonadaceae* and *Enterobacteriaceae*, typically range between 105 cfu g^{-1} and 107 cfu g^{-1} for fresh salads and up to 108 cfu g^{-1} for processed packed lettuce and salad mixtures (Landete *et al,*.2006).

Fresh and processed pork contains high levels of adrenaline, spermidine and spermine but low levels of noradrenaline, putrescine, histamine, cadaverine, and tyramine (Halasz *et al.*, 1994). Large amounts of cadaverine present in beef have been associated with heavy contamination by *Enterobacteriaceae*. High production of tyramine (100 mg kg^{-1}) in sausages was associated with lactic acid bacterial contamination (Prester, 2011). The occurrence of biogenic amines in fermented sausages may originate from contaminated raw material or from the fermentation process itself. For example, *Carnobacterium divergens* was found responsible for tyramine formation in vacuum-packed meat, the formation of putrescine and cadaverine was caused by *Enterobacteriaceae* or strains of *Pseudomonas* (Mostafa and Salem, 2015). There are three possible origins for biogenic amines in wines. They can be present in the must; can be formed by yeast during malolactic fermentation or result from the action of bacteria involved in malolactic fermentation (Shalaby, 2000).

Table 2: Most common biogenic amines and the produced microorganisms in food

Food	Biogenic amine	Produced microorganism
Fish	Histamine	*Morganella morganii, Klepsiell preumoniae, Hafnia alvei, Proteus mirabilis, Proteus vulgaris, Enerobacter clocae, Enterobcter aerogenes, Serratia fonticola, Serratia liquefacis, Serratia liquefades, Citobacter freuidii, Clostridium sp. Pseudomonas fluoresces,Pseudomonas putida, Aeromonas spp., Plesiomonas shigelloides, Photobacterium spp.*
Cheese	Histamine Tyramine	*Lactobacillus bucheri, Enterococcus faecelis, Enterococcus faectum, Enterococcus durans, Enterococcus hirae, Lactobacillus curvtus, Lactobacillus brevris*
Wine	Putrescine Cadaverine Histamine Tyramine	*Enterobacteriacea Lactobacillus brevis, Enterobacteriacea Oenococcus oeni,Lactobacillus hilgrdii, Pediococcus parvulus, Lctobacillus brevis, Lactobacillus hilgardii, Leuconostoc mesenteroides, Lactobacillus plantrum,*
Meat	Putrescine Histamine Tyramine Putrescine Cadaverine	*Lactobacillus brevis, Lactobacillus hilgadii, Leoconostoc mesenteroides, Lctobacillus plantrum, Oenococcus oeni, Lactobacillus buchneri, Lactobacillus zeao Enterobacteriacea, Staphelococcus capitis, Staphelococcus carnosus, Staphilococcus xylosus,Staphelococcus epidermidis, Staphilococcus saprophyticus, Lactobacillus brevis, Carnobaterius divergens, Carnobaterius pisicolaEnterobacteracea, Morganella morganii, S.liquefacies, Pseudomonas, Lactobacillus curvatus,*

Source: : Karovicova and kohajdova (2005).

2.9 Existing methods for biogenic amine control in food

Biogenic amines formation is temperature dependent, and it is decreased at low temperatures through inhibition of microbial growth and the reduction of enzyme activity (Mostafa and Salem, 2015). Biogenic amines forming bacteria such as *Morganella morganii* and *Proteus vulgaris* in skipjack tuna (Katsuwonus pelamis) were inhibited through chilling (Ruiz *et al.*, 2004). Maintaining the cold chain in foods that already contain high levels of biogenic amines will generally stabilize the levels of biogenic amines, although in some cases there may be slight increase over time (Chen *et al.*, 2010). For example, tuna stored at 0^{o}C and 22 ^{o}C up to 9 day, showed an increase in histamine of 15 ppm at 0 ^{o}C and 45ppm at 22 ^{o}C (Ababouch *et al.*, 2007). Freezing is more effective than cooling in preventing biogenic amines production. High temperature treatments can also be used to extend the shelf life of food. A thermal regime designed to kill the bacterial species responsible for histamine formation and can prevent the subsequent formation of histamine. Although heating can destroy the histamine producing bacteria in food, if recontamination and temperature abuse occurs after thermal processing, histamine formation may still occur in the thermally processed product (Prester, 2011). Histamine is heat stable so applying heat after histamine has formed in the product will not ensure its safety for example, fish paste (Rihaakura, Maldives local dish) is made through prolonged cooking (maximum 100℃ which eliminates all the potential bacteria responsible for histamine formation.

2.10 Emerging method for biogenic amine control

It is possible to control biogenic amine production through temperature alone, since some bacteria produce biogenic amine at temperatures below 5 ^{o}C (Prester, 2011). In addition in some societies, refrigeration is not readily available. In such circumstances, emerging method of control need to be

considered, however, little work has been done on these. Emerging methods as control measures include the addition of starter cultures that degrade histamine, the application of hydrostatic pressures, irradiation, packaging, using food additives and preservatives, and altering conditions based on microbial modeling of histamine producing bacteria. The majority of these methods are not new in terms of food preservation but are not commonly used in controlling biogenic amine (Emborg *et al.,* 2005). The use of enzymes, such as; diamine oxidase (DAO) that degrade biogenic amines, and the use of bacteria that posses this enzyme, are the only potential tools to degrade already formed biogenic mines and are not currently recognized as preservation methods (Emborg and Dalgaard 2008).

The formation of biogenic amines is associated with food spoilage, suggests poor hygienic practices, and may therefore include other food safety issues (Maijala *et al.*, 1993). Any attempts to control biogenic mines must take into account the factors leading to the formation of the biogenic amine and ensure other food safety issues are not being overlooked. Products where a secondary control approach is justified are those that are microbiologically stable. An example is the fish paste product from the Maldives, Rihaakuru, with a maximum water activity 0.8. Temperature abused fish, which has been rejected from fish factories, is used as the raw ingredient for Rihaakuru- a product made through prolonged cooking, that once produced, is stable at temperature (25 ^{o}C to 30 ^{o}C) for over a year. Although Rihaakuru has nutritional benefits, rich bin protein and omega- 3 sources, a health concern is potential for scombroid poisoning due to the high biogenic amines content (Emborg and Dalgard, 2008). Maintaining the cold chain is not a practical solution due to the cost of refrigeration being out of reach for the artisan fishers. One option to ensure the safety of product such as Rihaakuru is to destroy the biogenic amines in the product, but this has not been investigated **(**Maijala *et al.*, 1993). Most

approaches to control histamine in a food such as Rihaakuru focus on delaying biogenic amine formation (Emborg and Dalgaard, 2008).

2.11 Method for delaying biogenic amines accumulation

2.11.1 Additives and preservatives

Additives and preservatives can reduce the formation of biogenic amines in product such as mackerel by inhibiting bacterial growth and amine formation (Prester, 2011). Sodium sorbate may limit the formation of biogenic amines and sodium hexametaphosphate at 2% has been shown to delay histamine production (Prester, 2011). Citric acid, succinic acid, D- sorbitol, and malic acid inhibited decarboxylase activity and the resulted the histamine formation in mackerel stored for 10 at 25 ^{o}C (Shalaby, 2000). Citric acid use 1% during pickled cabbage fermentation produced a slightly decrease in biogenic amines at salt level of 6, 8 and 10% (Yucel, 2008).

Potssium sorbet has also been found to extend the shelf life of seafood (Shalini *et al.,* 2001). Sausage containing potassium sorbet and scorbic acid showed a significant reduction in biogenic amine accumulation (Prester, 2011). Sodium nitrites 45 to 195 ppm in sausage decreased biogenic amines production (Kurt and Zorb, 2009). Sodium nitrite and sodium nitrate inhibit biogenic amine production. The addition of 0% to 1% glucono-delta-lactone (GDL) into meat decreased histamine and putrescine production through a pH drop in meat (Chen *et al.,* 2010). The addition of sugar my also slightly reduce biogenic amines formation (Maijala *et al.,* 1993). When glycine was applied to Myeolchi (a salted and fermented anchovy product) the overall production of biogenic amines was reduced by 63 to 73%, also glycine inhibits the amine forming activity of microorganisms. Biogenic amines in other fermented fish products may be reduced using glycine as food additives (Mah *et al.,* 2009).

Naturally occurring specific inhibitory substances in spices and additives

have also been shown to inhibit biogenic amine formation. Such substances include curcumin (*turmeric*). Capsaicin (red pepper) and piperine (black pepper) (Karovicova and kohajdova, 2005). The disadvantage of these substances is the considerable loss in efficacy that occurs during cooking (Suresh *et al.*, 2007). The most active component of turmeric is to curcumoinds. Curcumin has been as a food additive and spice as a medicinal herb (Bhutani *et al.,* 2009). Curcumin levels 8g/day may be tolerable with approximate consumption being 0.1g/day. It is a potent antioxidant 10 times more powerful than vitamin E (Shishodia *et al.,* 2005).

Components of spices, such as thymol may inhibit biogenic amines formation. Thymol is a phenolic monoterpene, naturally found in essential oils, that has antioxidant and antimicrobial properties. It is a major component of thyme and oregano. However, thymol, having unpleasant pungent flavor, may not be accepted by consumers as an ingredient for food formulation (Lee *et al.*, 2008).

Ginger, garlic, green onion, red pepper, clove, and cinnamon have been shown to delay biogenic amines production in Myeolchi-jeot. The addition of 5% garlic during Myeolchi-jeot ripening reduced the biogenic amines level by 8.7% (Mah *et al.,* 2009). Garlic is one of the most popular herbs in the world used as a flavor agent in food; Allicin is the most active ingredient in garlic, formed from allin by enzyme allinase when the garlic glove is crushed (Batra and Rajeev, 2007). Ginger, lowers blood pressure, may cure hyrtention palpitations (Ghayur *et al.,* 2005), and it possess antibacterial and antifungal activity (Chrubasik *et al.,* 2005). The 6-gengerol, pungent constituent of ginger, is known to enhance gastrointestinal transport (Batra and Rajeev, 2007). The 6-gingerol also been shown to have some inhibitory effect on biogenic amines formation. The effect of spices has been measured on specific bacteria that produce biogenic amines. Ethanol extracts of all spice, sage, clover, cinnamon

and nutmeg were found to delay biogenic amine formation by *Enterbacter aerogenes*. The inhibitory effect was improved with the addition of sodium chloride (NaCl).

Cinnamic aldehyde, a component of cinnamon, and eugenol, a compound of cloves were found to be the most effective inhibitors of biogenic amine formation by specific bacteria *Enterbacter aerogenes* (Wendakoon and Sakaguchi 1995). Histamine formation by *M. morganii* was delayed presence of 0.5% potassium sorbate (Shalaby, 2000) and by the essential oil of lemongrass (Sangcharoen *et al.,* 2009). Histamine formation in *Klebsilla pneumonia* was delayed by sorbate at 0.5% (Shalaby, 2000). *Bacillus licheniforms*, an isolate from Myeolch-jeot, is a strong biogenic amine former. Glycine (10%) was shown to reduce the histamine, cadaverine and putrecine of *B licheniformis* by 93, 78 and 32% respectively and reduce tyramine and spermidine production by 100% (Mah *et al.,* 2009).

Recently, it was found that curcumin inhibits DAO (Bhutani *et al.,* 2009), which may inhibit biogenic amine reduction. When sodium sorbate and sodium hexametaphosphate were applied to sardines, a putrefactive odor was observed within 2day at chill storage (Kang and Park, 1984).

2.11.2 High Hydrostatic Pressure (HHP)

High Hydrostatic Pressure (HHP) is a non thermal preservation method that damages cell membranes of microorganisms resulting in inactivation or sub lethal injury (Rivas *et al.,* 2008). Through inactivation of microorganisms (HHP) extends shelf life while retaining the original flavor and characteristics of food. (HHP) treated food are commercially available in the United States (for example, guacamole, oysters), in Japan (for example, fruit jam) and in Spain (for example, cooked and vacuum packed ham). HHP has been applied to many other foods including cheese (Novella *et al.,* 2002), sausage (Ruiz *et al.,*

Table 3: Biogenic amine reduction through food preservatives

Food Type	Additives Applied	Storage Condition	Storage Tme	Reduction of the biogenic amines formation
Meat	GDL 0%, 0.5% and 1.0%	20 to 22℃	7	Histamine (dropped from 120 to 7ppm) and putrecine (dropped from 236 to 14.7ppm)
Indian mackerel (whole)	10% (weigh of fish) cuecmine (turmeric), capsacin (red pipper), piperine (black pipper)	5℃	8	All spices reduced biogenic amin histamine (dropped from>200 to 13ppm), cadvarene (approximately dropped from200 to 100ppm), putrecine (approximetly dropped from 200 to 25ppm) and tyramine (pproximetly dropped from 0 mm 200 to<100ppm).
Slightly fermented sausage	Sugar (glucose, lactose) between 4000 and 200000ppm	4℃ and 19℃	20 day	Cadvarine –Tyramine
Fomented sausage (sucuk)	Potassium pyrophosphate (2500ppm), dipotassium hydrogen phosphate (2500ppm) alpha-tocopherol (200ppm) ascorbic acid (500ppm), potassium sorbate (200ppm).	Temperature: 20℃, 30℃ and 40℃ relative humidity (RH): 50, 6 and 80	60 day	Histamine dropped from 242 to 35ppm at 80% RH and at 30 ℃, putercsine (dropped from 378 to 12 ppm at65% RH and at 40℃) Trypamine (dropped from 60 to 14 ppm at 50% RH and at 20℃).
Myeolchie-jeot (fermented anchovies)	5% grlic extracted dissolved in ethanol	25℃	10 weak	Histamine and Tyramine reduced by 20.8% and 31.2% respectively. Overall amines reduced by 8.7%.
Myeolchie-jeot (fermented nchovies)	5% glycine (weight bais) NaCl (20%)	25℃	10 weak	Biogenic amines (cadavarine, tyrine, putrescine, histamine and spermidine) reduced between 63% and 73%.

Source: Naila *et al.*,(2010)

2004), fish (Bolton *et al.,* 2009) and sauerkraut (Penas *et al.,* 2010). HHP is applied to raw material or the end products of fermentation, a reduction in the number of bacteria may be inhibit biogenic amine formation , for example, when HHP (200MPAa) was applied to meat batter raw material for sausage fermentation, it inhibited the growth of *Enterobacteria* and simultaneously delayed the accumulation of putrescine and cadverine (Latorre- Moratalla *et al.,* 2007). Inhibition of biogenic amine formation depends on the level of pressure applied. For instance, during cheese ripening, a low-pressure treatment of 50 MPa for 72hrs increased biogenic amine content, while high -pressure treatment of 400MPa for 5min plus 50MPa for 72hrs showed sligh decrease (Novella *et al.,* 2002).

Treating fermented sausage with high pressure (350MPa/15min) reduce lactic acid bacteria (20.1%) and reduced cadaverine (12.5%), putrescine (8.7%), and tyramine (HHP) (Ruiz *et al.,* 2004). Histamine forming bacteria and histidine decarboxylase activity in yellowfin tuna and mahi-mahi fish can be reduced by applying HHP between 300 and 400 MPa without affecting the quality of the fish (Bolton *et al.,* 2009). HHP (300 MPa at 40℃ for 10 m) applied during Saukraut fermentation, extended the shelf life through microbial reduction (Penas *et al.,* 2010).

2.11.3 Irradiation

Irradiation to extend the shelf life of food was introduced in the 1950s (Mbarki *et al.,* 2009). Irradiation has been used in the food industry prolong shelf life and ensure safety of foods, reducing the use chemical preservatives (Prester, 2011). Irradiation may control biogenic amine formation by radiolysis of biogenic amines (Mbarki *et al.,* 2009) and by reducing the number of bacteria responsible of biogenic amine production (Kim *et al.,* 2003). Radiolytic degradiation of biogenic amines was demonstrated in a model system.

Table 4: Effect of High Hydrostatic Pressure HHP on Biogenic amines.

Type of Food	HHP	Storage Condition	Storage Time	Reduction of Biogenic Amine Formation
Goat cheese ripening	400MPa for 5min and 50MPa for 72hrs at 14℃	Ripened at 14℃ and 86% RH	28 day	Tyramine dropped from 10.3 to 1.6ppm
Meat batter, raw material for sausage fermentation	200MPa at 17℃ for 10 min	12℃, RH>95% for 10 day. 80% RH till end of ripening	21day	Putrescine cadaverine level decreased (88% and 98%) reduction compared with the control
Dry-cured sausage (chorizo)	350 MPa for 15 min at 20℃	2℃	160 day	Decreased in tyramine (17%), putrescine (8.7%) and cadaverine (12,5%).
Yellow tuna and mahi	300 to 400 MPa for 5min	4.4℃	12day	Reduced histamine producing bacteria (*Morganellamorganii*) and their histidine decarboxylase activity

Source: Naila *et al* (2010).

Histamine, cadaverine, putrescine, spermidine, spermine, tryptmine, tyramine, and agmatine standards were irradiated at 2.5, 5, 10, 20 and 25 kGy after being dissolved in distilled water at concentration of 100ppm. The degradation observed was between 5 and 100%, overall showing 95% degradation of all amines at 20 kGy. Significant degradation of Spermine, Spermidine and Putrescine accured above 5kGy (Kim *et al.,* 2003). The application to a food system required further investigation. The high dosage use may affect the sensory quality of the food. Irradiation at 10kGy is considered safe to apply to any food product (WHO, 1994), but levels higher than this require studies on the sensory characteristics and safety of treated food. Shelf life extension of food products treated with irradiation has been applied to many foods including pork and beef (Min *et al.,* 2007), sausage (Kim *et al.,* 2003), soya bean paste (Kim *et al.,* 2003), chicken (Min *et al.,* 2007), and fish (Mbarki *et al.,* 2009).

Irradiation delays the formation of some biogenic amines; there are reports of irradiation enhancing the formation of other biogenic amines (Kim *et al.,* 2003). Korean fermented soybean paste treated by irradiation did not have a significant difference in biogenic amine content compared with the control, although the concentration of histamine, tyramine, spermidine, and putrescine decreased, during fermentation. Possible explanations for the latter include a reduction of microorganisms by irradiation, or some of the preformed biogenic amines may have been utilized as substrates by microbes during fermentation (Kim *et al.,* 2003). Biogenic mines in raw chicken breast and thigh meat were reduced using irradiation at a dose of 2 kGy, even though some of the biogenic amines (histamine, spermidine and spermine) were increased, perhaps because irradiation change the structure and physiological properties of enzymes that form biogenic amines (Min *et al.,* 2007). Prior to ripening, Chinese Rugoa ham was irradiated with a dose of 5kGy, producing a degradation of spermine, putrescine, and tyramine, but formtionof tyrptamine, spermidine, phenylethyl-

amine, and cadaverine increased compared to controls after irradiation. The increase of the latter may be due the ham being ripened after irradiation and the growth of decarboxylating microorganisms, during the ripening process (Wei *et al.,* 2009).

The biogenic amine reduction in foods seems to be more effective at high doses of irradiation (Mbarki *et al.,* 2009). High doses are most likely to result in what has been described as "irradiation tastes "It may also be possible that irradiation inhibit the amine decarboylase enzyme activity (Schirmer *et al.,* 2009).

2.11.4 Packaging

Preservation through packaging usually involves changing the gaseous mixture of the environment surrounding the product. This may delay the production of biogenic amines, due to inhibition of the microorganisms or the enzymes producing biogenic amines. The histadine decarboxylase enzyme was reported to be more effective in the absence of oxygen O_2, while histaminases (such as DAO), the enzyme that oxidizes histamine, were found effective, only in the present of O_2 (Kapeller, 1941). However, both anaerobic and aerobic bacteria are capable of producing biogenic amines, and as well as degrading biogenic amines so finding a balance that will control microbial growth and enzyme activity may be difficult. Successful control of biogenic amines through packaging. This include vacuum packing of salmon (Mbarki *et al.,* 2009), MAP of fish (Emborg *et al.,* 2005), sausage (Kim *et al.,* 2003), and active packing of seer fish (Mohan *et al.,* 2009). In active packing, different gas scavengers are used (O_2, carbon dioxide CO_2 to control the environment within the pack O_2 scavengers eliminate O_2 in the headspace and product <0.01% (Mohan *et al.,* 2009).

Table 5: Effect of Irradiation on Biogenic Amine

Type of Food	Irradiation Condition	Storage Condition	Storage Time	Biogenic Amine reduced
Distilled water containing 100ppm biogenic amines	Applied doses 0, 2.5, 5,10, 15, 20 and 25KGy (best reduced at 25KGy) Source strength 100KCi:dose rate 5KGy/hrs at12℃	-	-	At 20KGy putrscine, sperimidine, histamine, spermine, phenylethyleamine were completely destroyed. At 25KGy the remaining amines cadaverine, tryptamine, tyramine and agmatine were completely destroyed.
Pepperoni sausage fermented	Applied doses 0, 5, 10 and 20KGy (best reduced at 20KGy) Source strength 100KCi:dose rate5KGy/hrs at12℃	Air packed and stored at 4℃	4week	Decreased amine at 20KGy: putrescine (from 2.6ppm to completely destruction), tyramine (from 0.9ppm to0.2ppm), speramine (dropped from 9.6 to 4.2ppm) and spermidine (dropped from 11.8 to 8.4ppm).
Law-salt fermented soy-bean paste with (6% and 8% salt)	Applied doses 5,10, 15 KGy (best reduced at 15KGy) Source strength 100KCi:dose rate 5KGy/hrs at13℃	25℃	12week	Putrescine (dropped from 3124 to 797.3ppm at 8% salt and 15KGy)
Beef and pork	Applied doses: 0. 0.5 and 1.2KGy (best reduced at 2) Source strength:100KCi dose rate: 83.3Gy/min at 12℃	4℃	20hrs	Decreased amines in 2Gy:putescine (dropped from 4.7 to 2ppm in beef, and 2.3 to 0.3 in pork).tyramine (dropped from 24.7 to 9.3 in beef, and1.3 to 0.8ppm in pork) and spermine (dropped from 28.4 to 22.4 in beef and 31.3 and 25.9ppm in pork)
Vacuum packed chub Mackerel (*Scomber japonicas*)	Applied dose:1.5KGy	At 1℃ on air circulation	14day	Significant reduction of histamine (dropped from 50.91 to 2.87ppm).

Source: Naila *et al* (2010).

Mohan (2009) found that, the presence of O_2/air increased biogenic amine production in seer fish (*Scomberomorus commerson*) steaks and by removing O_2 (99%) with O_2 scavengers (active packing), biogenic amines in the fish were lower and shelf life was extended from 12day (air) to 20day. biogenic amine producers were apparently from aerobic bacteria that possess the decarboxylase activity. Thus removal of O_2 inhibited aerobic bacteria and delayed biogenic amine accumulation.

Vacuum packing extends the shelf life of food compared to air packing (Gonzeález *et al.,* 2007). Recently a novel packing method was developed (Schirmer *et al.,* 2009) that involves combining organic acids with $C0_2$ from the head space dissolving into the product until a vacuum is formed ("CO_2- vacuum packed" products). This was used on salmon as an effective method to inhibit microbial growth and extend shelf life. Microbes that were reduced included *Photobacterium phosphorium* that has reported s an active histamine former able to grow under normal MAP conditions producing more than 1000ppm histamine (Emborg *et al.,* 2005). MAP extends shelf life of foods longer than vacuum packing (Ozogul *et al.,* 2004). Histamine content in vacuum packed tuna was >7000ppm, and the bacteria responsible for were suspected to be either *P phosphorium* or *M morganii_M. Psychrotolerance.* Histamine production was controlled when MAP with a gas mix of 40% CO_2/60% O_2 was applied to tuna stored for 28day at 1.0℃ (Emborg *et al.,* 2005). This method may have controlled histamine formation by the inhibition of the growth of the psychrotrophic histamine producing bacteri *P phosphorium* and or *M morganii_M. Psychrotolerance.* Therefore, it was suggested to use MAP with the above gas mixture for lean fish, such as tuna loins, to void possible scombroid poisoning (Dalgaard *et al.*, 2006) demonstrated the synergistic effect of MAP with a gas mixture of 40% CO_2/ 60% N_2 and freezing and thawing to control histamine production in chilled garfish by *P.phosphrium* that had

produced histamine > 1000ppm at chilled storage under air and MAP. When the garfish was frozen, thawed and stored at 5℃, the shelf life was 70% longer under the MAP gas mix and histamine production was reduced compared with storage and air (Emborg *et al.*, 2005).

2.11.5 Microbial modeling

Microbial molding can be used to study the growth and inactivation of microorganisms with the aim of controlling growth and predicting risk factors (Seo *et al.*, 2007). Molding microorganisms responsible for biogenic amine formation and has used to explore option for biogenic amine control (Gardini *et al.*, 2008).

Temperature, time and pH affect biogenic amine production, and these could be modeled for particular microbial species in specific food. Such models may help design conditions to limit amine production. However, the drawback of this method is that there are many known bacterial species capable of producing biogenic amines already known and probably others yet to be found therefore generic modeling to be account for all these species would be complex, time consuming and tedious (Gardini *et al.*, 2008). Emborg and Dalgaard (2008) stated that, developed a mathematical model for histamine formatting bacteria, *M. Psychrotolerans* and *M morganii* and identified the conditions to inhibit the growth of these bacteria through heat in canned tuna meat, thawed garfish meat, tuna juice and broth. The mathematical equations of the model (equation 1to 3) have subsequently been incorporated into freely available software (Seo *et al.*, 2007).

Table 6: Effect of Packing on Biogenic amines.

Type of Food	Packaging Condition	Storage Condition	Storage Time	Reduction of Biogenic Amines
Yellowfin tuna	MAP (40% $C0_2$ and 60% O_2)	0℃	28day	No histamine formed/strong inhibitory effect to histamine production and growth of *Mor*ganella *morganii* and Photobactrium phosphorium.
Garfish Breast chicken meat	MAP (40% CO_2 and 60%N_2) MAP (30% CO_2 and 70%N_2)	1and 5℃ 4℃	38day 17day	Reduced histamine formation in thawed MPA garfish. Slightly decreased in cadaverine (223.7ppm in MPAand 252.7ppm in air packing).
Precooked chicken meat	MAP (30% CO_2 and 70% N_2)	4℃	23day	Reduced putrescine (90.4 under MPA at 23rd day, 20.6 at 23rd day under air.
Chub mackerel	Vacuum packed	1℃	7day	Slighty reduction (on 7th day on storage) of biogenic amines, histamine (dropped from 57.22 to 47.44ppm).
Seer fish	Packed in pouches (a multilayer film of ethyline-venyle alcohol with O_2 scavenger sahet	0 to 2℃	30day	Delayed formation of putrescine (15th day on air pack condition.

Source: Naila *et al* (2010).

$$\log Nt = \log N0 \qquad t < t\,tag$$

$$\log N = \log\ (Nmax/(1 + ((\frac{Nmax^{m}}{N0}\ ) - 1)$$

$$\sqrt{\mu_{max}} = b.(T - T_{min})\,.\left(1 - exp\left(c.(T - T_{max})\right)\right)$$

Wherer μmax =maximum specific growth rate, N_{max} =the maximum cell density.$^{y_{His}}/_{CUF}$ =yield factor for histamine formation, N0= actual initial concentration, t_{lag}=2.55 ln (2)/$\mu_{max} = lag\ time, Nt = cell$

Concentration at time t, m=parameter to characterize growth dampening when the cell concentration. Nt approaches the maximum cell concentration (N_{max} His t and His 0= concentration of histamine (ppm) at time t and 0 and N_1 and N_0 (cfu/g or cfu/ml)= corresponding cell, b and c= constants, t= temperature, and T min and T max = the theorical minimum and maximum respectively .

2.11.6 Starter culture

Starter culture used in fermentation can also delay the formation of biogenic amines (Mah *et al.,* 2009). Starter used for fermented foods are either amine negative (not able to decarboxylate amino acid into biogenic amines) or amine oxidizing bacteria (oxidize biogenic amine into aldehyde, hydroden peroxide, and ammonia) (Bover-Cid *et al.*, 2000). These bacteria require optimal growth condition to dominate over biogenic amine producing and other contaminant bacteria (Hu *et al.*, 2008).

A number of bacteria have been found to have negative decarboxylase activity or enzymes that oxidize biogenic amines in food (amine-negative bacteria), Artisanal Manchego chesse isolate of *Lactobcillus plntarum* and *Lactobacillus paracasei subsp*. *paracasei* were found to be amine-negative

bacteria except for one isolate from the latter, found producing tyramine. These amine-negative organisms were suggested as potential starters for cheese production (Mah *et al.,* 2009). Amine-negative starter, *Staphylococcus xylosus* and Lactobacillus *caravatus* delay putrescine and cadaverine formation during the ripening and storage of dry fermented sausage (Bover-Cid *et al.,* 2000). The inoculation of amine-negative mixed starters, *Pediococcus acidilactici, Staphylococcus carnosus, Lactobacillus sake, Sxylosus* into cold smoked fish, can help control biogenic amines (Petaja *et al.,* 2000). Amine-negativemixed starter of *S carnosus, Lactobacillus sakei, and Sxylosus* have also been used during the fermentation of dry sausage and were found to suppress biogenic amine accumulation (Brover-Cid *et al.,* 2000). Mixed starters of *L. plantarum, Pediococcus pentosceus, Sxylosus, Lctobacillus casei* inhibited formation of biogenic amines and suppressed the contaminant microorganisms in silver carp sausage (Hu *et al.*, 2008).

Mixed starters produce a synergistic effect in the control of biogenic amines. The use of mixed starters results in a large pH decrease that may be an additional factor contributing to reducing biogenic amine accumulation (Hu *et al.,* 2008).

Effective control of biogenic amines may require a combination of several factors. For example, the control of biogenic amines with starters is likely to be most effective with good quality raw material (Bover-Cid *et al.,* 2000).

2.12 Method for oxidizing degrading biogenic amines

There are few methods are available for degradation biogenic amines. Such methods include the use of oxidizing microorganisms, such as biogenic amine oxidizing bacteria, and enzymes such as DAO. Biogenic amine degrading bacteria could be introduced into a food processing step to degrade the biogenic amines in the food, or the bacteria could be used as a starter for fermented

foods. Bacteria described as biogenic amine oxidizers include *Micrococcus varians*, *Natinema gari*, *Brevibacterium linen*, *Vergibacillus sp* SK33, *L. Sakei*, *Lactobcillus curvtaus* and *Sxylosus* (Mah *et al.*, 2009). *Arthrobacter crystallopietes* contains the amine oxidizing enzyme (DAO) that is specific to histamine oxidation. *Micrococcus Varians*, having tyramine oxidase, degraded tyramine during sausage fermentation. *Natrinema gari*, an extremely *halophilic archaea* isolated from anchovy fish sauce, was reported to degrade histamine in high-salt media. The optimum temperature and pH for the degradation was between 6.5, 8.3 and 40 and 55℃ respectively and the NaCl concentration was 3.5 to 5 M (Tapinkage *et al.*, 2010).

DAO is another option for biogenic amines degradation, the ability of DAO to degrade histamine in both phosphate buffer (pH 7.0), and ensiled fish slurry (pH4.5) (Mah *et al.*, 2009). DAO was investigated by applying the similar conditions found in fish silage to fish slurry, 2% NaCL 12% Sucrose, 0.05% cysteine. DAO degraded histamine in fish slurry incubated at 30℃ with starting pH of 6.4. The addition of 0.05 % cysteine decreased histamine degradation and degradation did not occur at pH 4.5. The optimum temperature for DAO activity is 37℃ DAO activity needs to be investigated in a variety of foods to determine the effectiveness of the enzyme in degrading biogenic amines in different food matrices. A factorial designed experiment combining key factors such as temperature, pH, and DAO concentration on the degradation of biogenic amines in food will be useful in recommending DAO for use in specific foods (Tapinkage *et al.*, 2010).

The use of bacteria with amine oxidizing activity to reduce biogenic amine levels in foods is a potential control measure where it is difficult to control biogenic amine levels through the traditional means of refrigeration, and to eliminate already formed biogenic amines in foods (Dapkevicius *et al.*, 2000).

2.12 Determination of Biogenic Amines

For determination of biogenic amines numbers of analytical methods were developed. The complex matrix sample, the presence of potentially interfering compounds, and the occurrence of several biogenic amines simultaneously are typical problems encountered in the analysis (Silla-Santos, 1996).

Preclean-up includes extraction of sample with suitable extracting reagent (Silla-Santos, 1996). The following solvents have been suggested for the extraction of biogenic amine 0.6 M-perchloric acid, 5-10 % trichloroacetic acid and 0.1M-HCl (Bardocz, 1995). For milk product is suitable extraction of biogenic amine with methanol at the increased temperature (60). Some authors suggest extraction with butanol or butanol-chloroform at basic pH for the clean-up of samples. Some parameters significantly influence the extraction and recovery of biogenic amine (pH and the degree of saturation of the extracting solution by salts) (Silla-Santos, 1996).

The relative extraction efficiencies of these solvents depend on type and nature of the biogenic amine and the food from which they are being extracted. The solid-phase extraction (SPE) has provided a more efficient choice than classical liquid-liquid extraction by virtue of the wide availability of sorbent materials and of the fact that the need to dispose of organic solvents is avoided (Bardocz, 1995).

The analytical methods used for separation and quantification of biogenic amine are mainly based on chromatographic methods: gas chromatography (GC), thin-layer chromatography (TLC), and high performance liquid chromatography (HPLC) with precolumn or postcolumn derivatization techniques (Greif *et al.*, 1997). Aliphatic biogenic amines do not show pronounced absorption bands in the UV VIS region, so that usual spectrometric detectors cannot be used (Seiler, 1986). The direct analysis of biogenic amine

without derivatization by means of ionpair chromatography has been suggested using octylamine or heptanesulfonate as ion-pair reagents (Cobo and Silva, 1999). For the separation of ion pairs of the biogenic amine the usual reversed-phase columns with C_{12} - $C1_8$ aliphatic chains phenyl residues bound to a silica core are suitable (Bockhardt and Klostermeyer, 1996).

The HPLC procedures involve pre- or postcolumn derivatization step (Pacheco *et al.*, 1998). Deferent chemical reagents have been used for the biogenic amine analysis, for example ninhydrine and phthalaldehyde, as a postcolumn derivatization reagent, dansyl and dabsyl chloride, benzoylchloride, fluoresceine, 9-fluorenylmethyl chloroformate with precolumn derivatization (Greif *et al.*, 1997).

Dansyl chloride has been the most widely used reagent for derivatization of biogenic amine prior to HPLC. Light sensitivity and limited stability of dansyl chloride lead some authors to the use of deferent derivatization agents. Benzoyl chloride is an inexpensive, stable, easily accessible chemical and its purity is less critical than that of dansyl chloride. Benzamides are not sensitive to light; reaction proceeds at room temperature in alkaline media and no buffers are required (Pacheco *et al.*, 1998). For the detection fluorescence, UV, and electrochemical detectors are used. Electrochemical detectors are based on the oxidation of amino groups (Beljaars, 1998).

TLC method is especially popular in plant biochemistry (Pacheco, 1998). The TLC procedure is of value for semi quantitative screening of food (Shalaby, 2000). TLC with preclean-up of sample and derivatization of biogenic amine can be used to detect chlorides, 3, 5-dinitrobenzamides dansyl and fluorescein derivatives of biogenic amine (Askar and Treptow, 1986). Dansyl chloride reacts with primary and secondary amino groups and fluorescein reacts only with primary amino group (Karovica and Kohajdova 2005).

Dansylated biogenic amines emit the energy of absorbed long- wave UV

light as fluorescent light, enabling the analyst to detect these compounds at low levels on the chromatogram. The natural fluorescence (under UV light) of the separated spots of dansylated biogenic amines from sample extract can be compared with that of standard spot by eye (Shalaby, 2000). The fluorescent dansyl derivative zones are visualized and marked with the aid of a suitable UV-light source (360 nm) (Shalaby, 2000). TLC one- dimensional developing techniques enable to give sufficient separation of biogenic amine, it is because other interfering compounds, such as amino acids also moved along with the analyzed biogenic amine (Costa *et al.*,2015).

The multidimensional developing technique improved resolution of biogenic amine from each other and from interfering materials, and compact and intense spots were obtained. For visual detection of eluated biogenic amine, various systems of detecting agents such as ninhydrin, o-phthalaldehyde (for chloride of biogenic amine) solution of ethanol and naphthylamine can be used (Shalaby, 2000).

The biogenic amines are determined in derived forms as trifluoroacetyl, trimethylsilyl or 2, 4-dinitrophenyl derivatives (Askar and Treptow, 1986). The columns used in the GC are capillary or filling. The capillary columns allowed better separation of biogenic amine. The detectors for the determination of biogenic amines by GC are healthy conductivity, flame ionization, and electron capture detector (Askar and Treptow, 1986).

Reports dealing with separation of biogenic amines by capillary electrophoresis (CE) are not numerous to data. There are three possible approaches to solve this task (Pacheco, 1998).

1. Aromatic or heterocyclic biogenic amine can be separated in selected buffer systems without derivatization.
2. Polyamines are determined either derivatized (usually in electrokinetic capillary chromatography mode).

3. Their detection must be indirect. CE has several advantages: it is simple, rapid, cost elective, and reliable, making it a very useful tool for screening a large number of samples in a short period of time (Wei, 2009).

Capillary isotachophoresis is used to quantification of histamine in fish (Smela *et al.*,2003). And deferent biogenic amines in the lactic Acid fermented vegetable juices (Karovicova and kohajdova, 2005).

Fluorometric methods are used owing to fluorescence of biogenic amine at some pH and reaction of biogenic amine with suitable agents to the fluorescence derivatives. The histamine can be determined by o-phthalaldehyde and tyramine by β-naphthol (Askar and Treptow, 1986). For determination of biogenic amine it is possible to use amino acid analyzer, when at the suitable chosen conditions not only biogenic amine, but also their precursors amino acids are determined (Halasz *et al.*, 1994).

Due to the commercial availability of enzymes like MAO and putrescine oxidase several groups tried to couple the enzymatic reactions with electrochemical sensors in order to obtain simple and reproducible biosensors. In some cases the biogenic amines have been coupled with oxygen sensors or hydrogen peroxide sensors. The biosensor procedure has advantages, such as low cost, short analysis time, simplicity of use and it can be used outside an organized laboratory. The biosensors show a low detection limit with lifetime estimated at one month with a 10-30% loss of sensitivity (Karovicova and kohajdova, 2005).

2.1.2 Fish production and consumption patterns in Sudan

Marine and fresh fish resources are considered important for food security and socio-economic development in Sudan. The country has a high potential for the development of aquaculture, given its rich biodiversity of fish and a favorable environment with many cultivable species and its land and water resources (Agab and Sahfi, 1998). Table (2.1) shows the total fish catch of Sudan distributed by sector.

Table 7: Sudan total fish production by sector in 2006

Fishery sub-sector	Total production tons/year
Marine	5 550
Inland	57 000
Aquaculture	2 000
Recreation	-
Total	64 550

Source: FAO (2008).

Sudan per capita fish consumption was estimated in 2008 at 1.6 kg per annum, which is considered low compared to other parts of the world (FAO, 2008). However, with current population growth rates of 2.5 per annum and very high urbanization patterns, especially the influx to the capital city (Khartoum) region, it is expected that demand for fish will rapidly overtake current supplies, placing serious pressures on fisheries 'ecosystems and hiking fish prices in the country. Fish consumption in Khartoum has increased markedly since 2005 when the influx of displaced people began. More than 70 % of the actual fish production is consumed fresh (basically caught from the White Nile-JAR and the upper southern reaches of the White Nile and the Sudd Region). Fish processing is very common (by salting, drying, fermenting and smoking) (FAO, 1999). Growth value of fisheries output reached US$ 1.2 billion in 2006 (FAO, 2008). Inland fisheries contribute 88.3% of the estimated production potential of Sudan. The main fishing localities are: The Sudd

swamps in the south and the man-made lakes on the White Nile (JAR), the Blue Nile (Roseries and Sinnar Reservoirs), the Atbara River (Khashm El Girba Reservoir) and the main Nile River (Lake Nubia) (FAO, 2008). Most inland fisheries in Sudan are operated as small-scale artisanal systems. Different ethnic groups harvest these waters with relatively primitive equipment.

2.1.3 Nutritive value of fish

1. Fish is generally considered as high in nutritional value due to its cheap and high quality protein with a higher biological value of 15-23%. Interest in fish consumption increased of late due to the high content of health significant omega-3 PUFAs, particularly eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid 22:6n-3) fishs are well known to contain highly valued fatty acids including omega-3 fatty acids.
2. Acids such as Docosahexaenoic Acid (DHA and Eicosapentaenoic Acid (EPA), enzymes for examples; pepsin, trypsin and chymotrypsin), collagen.
3. Further, fish has a good complement of the essential amino acids, particularly lysine which is low in cereals, thus providing a nutritional balance in the overall quality of a mixed diet (Algab and shafi, 1998). Fish are important sources for many other nutrients namely vitamins such as vitamin A, D and E as well as minerals including calcium, iodine and selenium etc... (Ahmed, 2006).

2.1.4 Health benefit of fish

Fish consumption reduces the risk of coronary heart disease (CHD) and overall cardiovascular mortality, especially in high-risk populations. Fish can decrease blood triglyceride concentrations, lower blood pressure and increase HDL cholesterol levels (Dural *et al.,* 2007).

Consumption of LC n-3 PUFAs delays disease progression. Furthermore, hypertension and dyslipidemia are common co morbidities of type II diabetes and major risk factors for cardiovascular complications (Basheer, 2017).

Beneficial effects of high consumption of LC n-3 PUFAs in patients with rheumatoid arthritis, a chronic inflammatory disease, by reducing the production of inflammatory mediators (eicosanoids, cytokines and reactive oxygen species) and the expression of adhesion molecules. However, evidence of clinical efficacy of LC n-3 PUFAs is weak in other inflammatory diseases such as asthma and inflammatory bowel diseases (Ahmed, 2006).

Consumption of fish and fish oil in pregnancy has been positively associated with higher birth weight, which is associated with lower risk of hypertension later in life. However, a very high intake of fish oil was associated with decreased infant size at birth, possibly due to three fold consumption of the recommended dietary allowance of vitamin A (Basheer, , 2017). Seafood have to a nutritionally balanced energy-restricted diet may increase weight loss, since the inclusion of either lean or fatty fish, or fish oil as part of an energy-restricted diet resulted in 1kg significantly more weight loss after eight weeks than did an isocaloric diet without seafood (Basheer, 2017). However, the weight loss effect cannot only be contributed to LC n- 3 PUFAs since lean fish (cod), which contains only small amounts of these fatty acids (0.3 g/day), resulted in similar amount of weight loss as fatty fish (salmon, 3.0 g LC n-3 PUFAs/day) and fish oil (1.5 g LC n-3 PUFAs/day), or 5.4-5.5 kg (Dural *et al.*, 2007).

2.1.5 Fish spoilage

- Spoilage and freshness are the two qualities that have to be clearly defined. A fresh product is defined as the one whose original characters remain unchanged. Spoilage therefore is the indicative of post harvest change. This change may be graded s the change from absolute freshness to limits of acceptability to unacceptability (Prabjeet ,*et al.*,2018).

Spoilage is usually accompanied by change in physical characteristics; color, odor, texture, color of eyes, color of gills and softness of the muscle. Spoilage is caused by the action of enzymes, bacteria and chemicals present in the fish. In addition ,there are many factors contribute to spoilage in fish such as; moisture , fat and protein contents, weak muscle tissue, ambient temperature and unhygienic handling (Burt,1976).

2.1.6 Fermented fish

Fermented fish products are important sources of nourishment, they contain great amount of a high quality protein. These products are used sometimes as seasoning, and at other times as the only source of animal protein in various dishes to replace meat and fresh fish (Dural *et al.*, 2007). In the Sudan, special types of traditional fermented fish products have long been made. Dirar (1993), described the most significant fermented fish items, namely, " Fasekh " (wet salted fish), which was introduced into Sudan from Egypt during the 19th century, " Tarkeen" (fermented fish sauce), which is a true Sudanese fermented food, "kejiek" (dried- fish), an African product; "Mindeshi" (fermented fish paste) possibly another true Sudanese food, and Batarikh (fermented roe fish), is a household fermentation, of Mediterranean origin (Agab and Shafi, 1989).

2.1.6.1 Fasekh

Fasiekh is the most popular fish food in Sudan has witnessed a large increase in the number of small industries supplying Fasekh that is sold either wet or dried and distributed all over the country where local demand is very high. Due to the very high local market demand of Fasekh, its price highly exceeds the price of fresh *Tilapia* (Agap and Shafi, 1989).

Fasekh is not a truly indigenous Sudanese food product. The technique of its making entered the Sudan during the Turko-Egyptian rule but its production on large scale was only well established during the Anglo-Egyption condominium rule. Therefore, it is acceptable to assume that fasekh production in the Sudan is about a century old. During this period, the Sudanese have brought about some changes in the preparation method. Nevertheless, both local consumption and export trade of the product are today almost completely monopolized by families of Egyptian descent, particularly the ethnic group called Nagada (Dirar, 1993). Fish products other than Fasekh that are consumed in Sudan include Terkeen (fermented fish), Mandasha (smoked and sun-dried), and *Seer*, a very small fried fish sold cheap. The home made Terkeen encourages the use of destructive gear, since the smaller the fish size the higher the market value of this product (FAO, 2008).

2.1.6.2 Preparation of Fasekh

Fasekh is not a truly indigenous Sudanese food product. It is made and widely consumed in Egypt where it is known by the same name. Fasekh making is a seasonal activity, which start in October to November and ends in May-June with a peak in February and March (Dirar, 1993).

Fasekh is almost made from one or both of two morphologically. Very similar fish Kawara (*Vlestes spp*), and Kass (Tiger fish; *Hydrocynus spp)*The major reason given by the processors for the choice of kawara and kass to make fasekh is that these fish types are relatively lean. Both Sudanese and Egyptian consumer demands only little fat in the fasekh (Dirar, 1993).

Fasekh is best made from small young fish of 6-8cm in length. Nevertheless, all fish sizes and ages are actually used in the preparation of the

product. Young fish are preferred, it is said, because they are tastier and have concentrated fat. The ratio of salt to fish used for fasekh is about 1:5.

In one preparation method, the individual fish are salted as stack shape layer fish and layer salt. This initial stage of curing takes between nine and fifteen days before the fish matures. The end product is completely liquefied and homogeneous, thick slurry or soft paste, full of bones (Hassan, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Food materials

Fermented fish (Fasekh) cooked samples were obtained randomly from different super markets located in Khartoum city (A-B-C) this samples were prepared in small factories. The fasekh samples were stored in deep freezer till transferred to National Food Research Center (NFRC), Shambatt, Sudan for a proximate analysis and National research center, Cairo, Al Doke, Egypt for evaluation the biogenic amines.

3.1.2 Chemical materials

Standard amines including: Tryptamine hydrochloride, 2-phenylamine hydrochloride, puterscine dihydrochloride, cadaverine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, histamine dihdrochloride, tyramine hydrochloride and agmatine sulfate were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (LC grade) and dansyle chloride (GR grade), another chemicals were purchased from E.Merck (Darmstadt, Germany). All Chemicals and reagents used were of analytical grade.

3.2 Methods

3.2.1 Analytical methods

The proximate analysis was determined according to the standard method of the Association of Official Analytical Chemists (AOAC, 2005).

3.2.1.1 Moisture determination

A sample of 5g was weigh into a dre- dried and cared dish. Then ,the sample was placed into an oven (Kat-NR.2851,Electrohelios,Swedden) and let to dry at 105℃ until a constant weigh was obtained. After drying, the recovered sample was transferred into a discator and cooled to room temperature before reweighing. Triplicate results were obtained for each sample and the mean value was reported to two decimal points according to the following formula:

Calculation:

Moisture content%

$$\text{Moisture content\%} = \frac{w_1 - w_2}{w_1} \times 100$$

Where w_1: origen weight of sample

W_2: weight of sample after drying

3.2.1.2 Crude protein determination

The crude protein content was determined in all samples by micro-Kjeldahl method using a copper sulphate or sodium sulphate catalyst according to the AOAC (2005).

Principle:

The principle of the method consists of sample oxidation and conversion of nitrogen to ammonia, which reacts with the excess amount of sulphuric acid forming ammonium sulphate. The solution is made alkaline and the ammonia is distilled into a standard solution of boric acid (2%) to form the ammonia-boric acid complex, which is titrated against a standard solution of HCl (0.1N). Accordingly, the crude protein content is calculated by multiplying the total N % by 6.25 as a conversion factor for protein.

Procedure: 0.5g sample was accurately weighed and transferred together with 2-3 glass pellets, kjeldahl catalyst and 20ml concentrated sulphuric acid into kjeldahl digestion flask. After that, the flask was placed into a kjeldahl digestion unit (Tecator, Sweden) for about 3hours, until a colorless digest was obtained. Following, the flask was left to cool to room temperature. The distillation of ammonia was carried out in 30 ml boric acid (2%) by using 40 ml distilled water and 60 ml sodium hydroxide solution (33 %). Finally, the distillate was titrated with standard solution of 0.1N HCl in the presence of 2-3 drops of indicator (Bromocreasol green and methyl red) until a brown reddish color was observed. The total nitrogen and protein were calculated using the following formula:

$$N\% = \frac{\text{volum of HCl} \times N \times 14}{\text{weight of sample} \times 1000} \times 100$$

P%= N% ×6.25 (factor)

Where:

N% = crude nitrogen.

P% = crude protein.

N = normality of HCl.

14 = equivalent weight of nitrogen.

3.2.1.3 Fat content

The crude fat in the product was determined according to the standard analytical method of AOAC, (2005).

Principle: The method determines the substances which is soluble in petroleum ether (B.P, 40 - 60ºC) and extractable under the specific conditions of Soxhlet extraction method. The dried ether extract is weighed and reported as percentage of the dry matter as crude fat.

Procedure: A sample of 5g was weighed into an extraction thimbles and covered with cotton that previously extracted with petroleum ether. Then, the sample

and a pre-dried and weighed Erlenmeyer flask containing about 100 ml petroleum ether were attached to the extraction unit (Electrothermal, England) and the temperature was adjusted to produce about 150 to 200 drops of the condensed solvent per minute for 16 hours. At the end of the distillation period, the flask was disconnected from the unit and the solvent was redistilled. Later, the flask with the remaining crude ether extract was put in an oven at 105 ºC for 3 hours, cooled to room temperature in a desicator, reweighed and the dried extract was registered as crude fat according to the following formula:

Calculation:

$$\text{Fat \%} = \frac{(W2 - W1)}{W} \times 100$$

Where:

W_1 = weight of empty flask.

W_2 = weight of flask with oil.

W = weight of sample in g.

3.2.1.4 pH measurement

Ten grams of the samples were placed in blender gar and 90 ml of distilled water was added the mixture was blended at high speed for 1 min. The pH of the mixture was measured using a recalibrated pH meter model (HI 8521 microprocessor bench pH / MV / ˚C meter). This has been calibrated using two standard buffers (6.8 and 4.0).

3.2.2 Biogenic amines determination

3.2.2.1 Sample preparation

Samples was blended in a Waring blender for 3min. the blended samples (5g) were transferred to 50 ml centrifuge tubes and homogenized with 20 ml of 6% trichloroacetic acid (TCA) for 3min. the homogenates were centrifuged (10,000g,10 min, 4℃) and filterd through Whatman No.2 filter paper

(Whatman, Maidstone, England). The filtrates were then placed in volumetric flasks, and TCA was added to bring to a final volume of 50 ml. One milliliter aliquot of the fasiekh extracts was derivatized with dansyl chloride using the same procedure as for the dansylation of standard amine solution.

3.2.2.3 Prepareation, dansylation and the calibration curve of standard amine solution

Tryptamine hydrochloride (61.4mg), 2-phenylethylamine hydrochloride (65.1mg), putrescine dihydrochloride (91.5mg), cadavaerine dihydrochloride (85.7mg), spermidine tetrahydrochloride (87.7mg) spermine trihydrochloride (86.0mg), histamine dihydrochloride (82.8mg), tyramine hydrochloride (63.4mg), and agmatine sulfate (87.7mg) were dissolved in 50 ml of a 0.1 M HCl and used the working solution. The final concentration of each amine (free base) was 1.0mg/ml.

The dansyl derivatives of amine were prepared according to the method of Eerola,*et al.*, (1993) with minor modifications. To 1ml of mixed amine solution containing 0_20 μg of each amine, 0.2 ml of 2 M sodium hydroxide and o.3 ml of saturated sodium bicarbonate were added. The solution was added 2 ml of 1% dansyl chloride solution dissolved in acetone, mixed by using a vortex mixer, and allowed to stand at 40 ℃ for 45min. after the reaction, 100 μl of ammonia was added and allowed to stand for 30 min .Acetonitrile was added to a final volume of 5ml and the solution was centrifuged (10,000g, 5 min, 4℃). The supernatant was filterd through a 0.45-μm filter, and then used for HPLC.

3.2.24 Determination biogenic amines by HPLC

High Perform Liquid Chromatography HPLC was used to determine the biogenic amine. The HP1100 system was equipped with auto-sampler, quaternary pump, on-line degasser and DAD detector at 254nm, controlled with ChemStation software (Hewlett Packard, Waldbronn, Germany) and Column : Zorbax Eclipse XBD C18, 5u (150 x 4.6 mm, id).

CHAPTER FOUR

RESULTS AND DESCUSSION

4.1 Chemical composition of Sudanese fermented fish (Fasekh) at Khartoum city

The proximate composition of ready to eat Fasekh there were significant ($P<0.5$) differences of moisture content among Fasekh samples. The highest moisture content 64.65%, recorded for samples (B) collected from center Khartoum, whereas, the lowest one 61.34%, recorded for samples (A), collected from north Khartoum . Osman *et al.,* (2012) reported that, fermented fish (Tarkeen) had moisture content of 40.08%.

Eltom (1989) stated that, salting treatment significantly decreased ($p\leq0.05$) the moisture content of fish due to adding coarse salt which results to be drawn out of the fish tissues causing slight dehydration.

The moisture content preference and limits of microorganisms causing spoilage of cured. The current results of moisture content in all "fasekh" samples were higher than 44%, the recommended safe moisture level given by FDA (1996).

There were significant ($P<0.05$) differences of protein content among Fasekh samples which were collected from Khartoum city. The highest protein content 22.12%, recorded for samples (A), whereas, the lowest one 19.64%, recorded for samples (B). The current results supporting with that stated by Eltom (1989) who reported that, fish had contains consider amount of protein. Lower protein content 18.48% was reported by Osman et al., (2012).

Table 8: Chemical composition of cooked fermented fish (Fasekh) at Khartoum city

Samples Code	Moisture %	Protein %	Fat %	pH
A	61.34^{c} (±0.01)	22.12^{a} (±0.02)	6.98^{b} (±0.01)	5.67^{c} (±0.02)
B	64.65^{a} (±0.04)	19.64^{c} (±0.22)	6.35^{c} (±0.05)	5.95^{a} (±0.06)
C	63.07^{b} (±0.05)	20.78^{b} (±0.04)	7.02^{a} (±0.04)	5.82^{b} (±0.05)
$Lsd_{0.05}$	0.0006217*	0.0006217*	0.0006217*	0.0006217*
SE±	0.0001924	0.0001924	0.0001924	0.0001924

Values are means ± SD.

* Means in the same column bearing the same superscript small letters are not significantly different ($P<0.05$).

Where:

A: Samples collected from north Khartoum

B: Sample collected from center Khartoum

C: Samples collected from east Khartoum

Higher than that was reported by Mostafa and Salem (2015) who found that, fasekh samples had protein content in ranged of 18.12 and 28.52%. The current results were agreed with that stated by Ahmed (2006) who reported that, protein content of fresh and salted fish ranged between 16.54% and 20.5%. These variations could to attribute to, some factors affected chemical composition of fish such as; fish species, feeding design, breeding system, genetic factors, and type of fermentation and storage conditions as stated by Ahmed (2006). .

Regarding fat contents, there were significant differences ($P<0.05$) among Fasekh samples. The highest fat content was 7.02%, reported for samples (C), whereas, the lowest one 6.35%, reported for Fasekh samples (B). The current results were lower than that reported by Mostafa and Salem (2015) who found that, fasekh samples had fat content of 9.67%. Osaman *et al.*, (2012) found that, Fasekh samples were 8.09% fat content. Hassan (2010) reported that, fermented fish (Terkeen) was fat content of 5.70%.

Furthermore, Ahmed (2006) stated that, lipids are an important component in fish product and human diets, both as energy and fatty acids (FA) sources. The fats are also needed in diets to absorb fat-soluble vitamins A, D, E and K for tapregulating body cholesterol metabolism.

Regarding pH there were significant differences ($P<0.05$) were noticed for pH values for Fasekh samples. The maximum mean of pH value of Fasekh samples were 5.95, obtained for samples (B), while, the minimum one were 5.67, obtained for samples (A). The current results were lower than that reported by Mostafa and Salem (2015) who found that Mullet samples had pH value with an average mean of 6.34. Similar results were reported by Sangcharoen (2009) who found that, the pH values in Thai-fermented fish with an average mean 6.3. The current results were disagreement with that results

found by Hassan (2010) who found that, fermented fish (Terkeen) was pH value of 6.15. These variations could be attributed to, the production of volatile basic components such as ammonia, trimethylamine and total volatile nitrogen by fish spoilage bacteria significantly affected on pH value as stated by Ahmed (2006).

Furthermore, Mostafa and Salem (2015) stated that, pH value is considered an important intrinsic factor related to post-mortem changes of fish flesh.

4.2 Biogenic amine of cooked fermented fish (Fasekh)

4.2.1 Terptamine contents of Fseikh samples:

Fig.3 show there were significant differences ($P<0.05$) for Terptamine concentration among Fasekh samples. The highest Terptamine was recorded for Fasekh samples (A) 2.55mg/100g, whereas, the lowest Terptamine for Fasekh samples (B) 1.27mg/100g. The current results of Terptamine concentration were higher than that results obtained by Prester (2011) who found that, Sardine (*S. pilchardus*) samples had Terptamine concentration of 0.16mg/100g. Mostafa and Salem (2015) reported that, salted fermented fish had Terptamine in low concentrations 0.0326 mg/100g. These variations of Terptamine concentration could be attributed to, the fact that, some factors affected on Terptamine concentration such as; Species, season, storage conditions, bacteria and intrinsic enzyme activity as reported by Yuecel (2008). The current results supported by that stated by Halasz *et al.*, (1994) who stated that, fermented fish contained eight biogenic amines, namely; Treptamine, -phenylethylamine, Putreseine, cadaverine, histamine, tyramine, spermidine and Spermine.

Mostafa and Salem (2015) stated that, Terptamine content is often used as a biochemical index to assess keeping quality and shelf-life of fish.

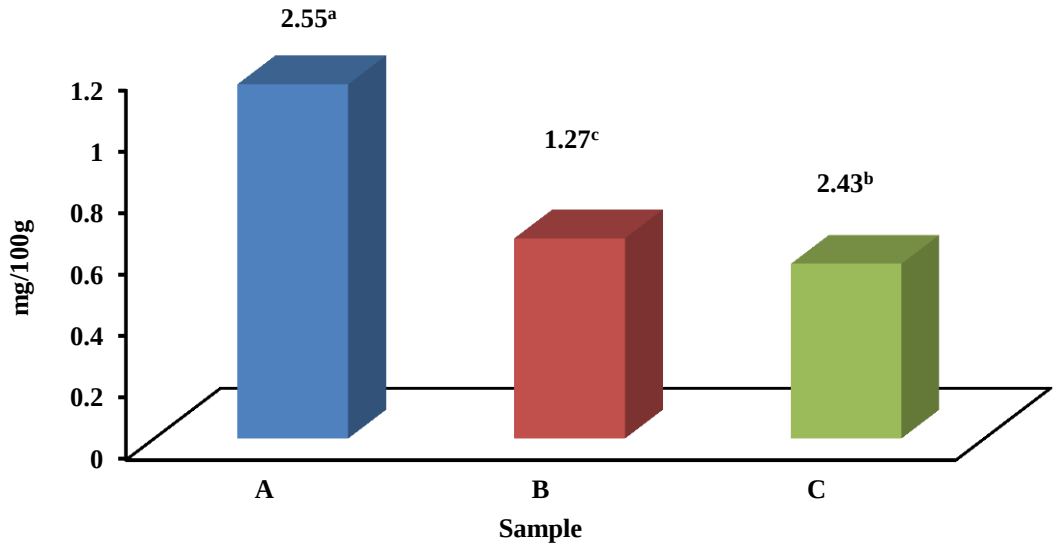

Fig. 3 terptamine content of Fasekh

Key:

A ≡ Fasekh obtain from center Khartoum

B ≡ Fasekh obtain from north Khartoum

C ≡ Fasekh obtain from east Khartoum

4.2.2 B.Phenyleamine content of Fasekh samples:

Fig 4 show there were significant (P<0.05) differences of B. Phenyl concentrations among Faseikh samples. The maximum B.Phenyl 1.15mg/100g, observed for Fasekih samples (A), whereas, the minimum one 0.57mg/100g, observed for Fasekih samples (C). Mostafa and Salem (2015) found lower amount of B.Phenyl for Mullet 0.036mg/100g. In contrast, Ruiz *et al.,* (2004) observed that, no B. phenyl in minced beef and pork samples.

Furthermore, Yuecel (2008) mentioned that, the formation of high levels of biogenic amines in fish products depends on the number of microorganisms present.

4.2.3 Putrescine content of Fasekh samples:

Fig 5 there were significant differences (P<0.05) . Detected among samples for Putrescine level. Fasekh samples (A), (B), and (C) had Putres concentrate of 0.289, 0.122 and 0.300mg/100g, respectively. The current results of Putrescine were higher than that results obtained by Prester (2011) who found that, Sardine (*S. pilchardus*) samples had Putrescine concentration of 0.11mg/100g. Mostafa and Salem (2015) observed that, slightly increment in putrescine throughout storage period from 0.17mg/100g to reach 0.66mg/100g after 60 day of storage.

Ruiz *et al.,* (2004) sin fitated that, Putrescine and cadaverine, considered a toxic individually and can enhance the effect of histamine and tyramine by interacting with the aminooxidases and interfering with the detoxifying mechanism. Yuecel (2008) stated that, Putrescine amine has been considered as a spoilage indicator.

Furthermore, Mostafa and Salem (2015) evaluated the quality of meat and found a high correlation coefficient between the content of putrescine and carp meat spoilage as assessed by sensory evaluation.

In addition, Ruiz *et al.*, (2004) set a value of 0.5 mg/kg of putrescine as the limit of acceptance of canned skipjack tuna.

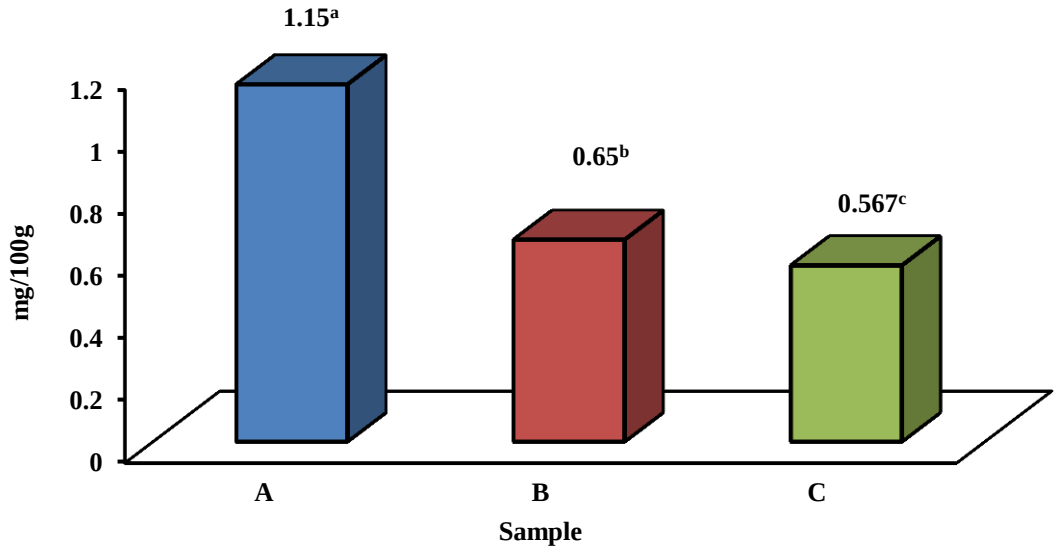

Fig. (4): B-phenylamine content of Fasekh

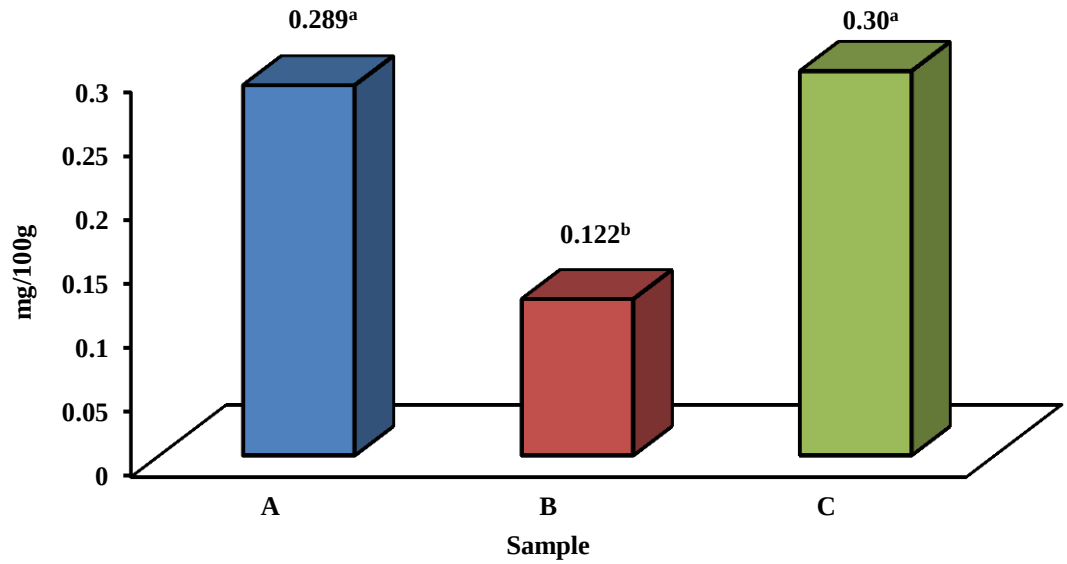

Fig. (5): Putrescine content of Fasekh

Key:

A ≡ Fasekh obtain from north Khartou

B ≡ Fasekh obtain from center Khartoum
C ≡ Fasekh obtain from east Khartoum

4.2.4 Cadaverine content of Fasekh samples:

Figure 6 demonstrated that, the concentration of Cadverine of Fasekh samples were significantly ($P<0.05$). The ranged of Cadverine of Fasekh samples ranged between 0.14 and 0.49mg/100g. Lower result was obtained by Yuecel (2008) who found that, fish. Samples had cadaverine with an average 0.14mg/kg. Prester (2011) found that, Sardine (*S. pilchardus*) had cadaverine concentration of 0.10mg/100g.

Ababouch *et al.*, (2007) stated that, the early cadaverine formation in marine fish has been explained by the activity of fish enzyme.

In addition, cadaverine was found to be the main biogenic amine in Egyptian salted-fermented fish during ripening. Cadaverine originates from the decarboxylation of lysine and has been associated with *Enterobacteriaceae*.

It is worth to mention that, Cadaverine has been found to be a useful index for evaluating fish microbial spoilage in a wide range of fish including non-pelagic fish.

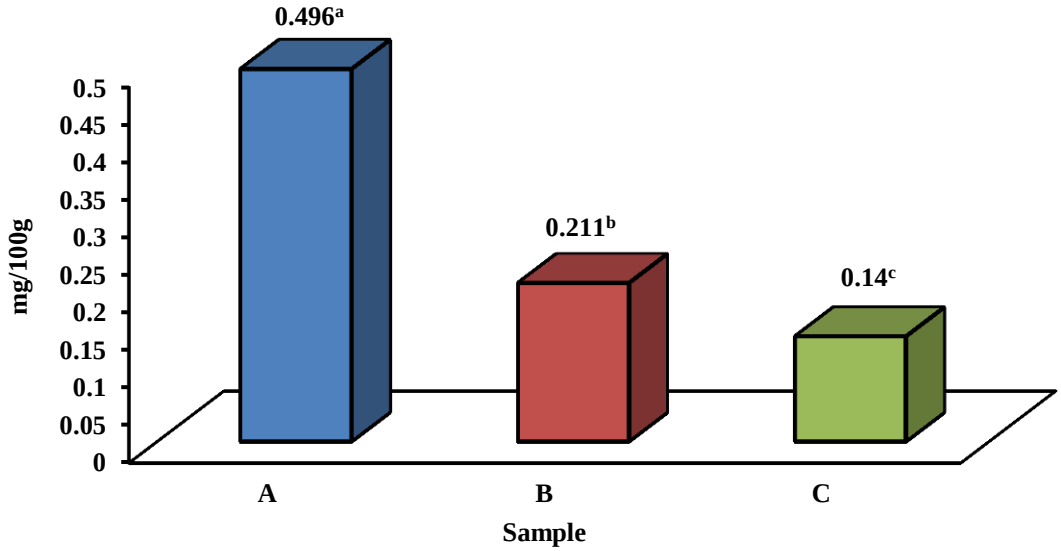

Fig. (6): Cadaverine content of Fasekh

Key:

A ≡ Fasekh obtain from north Khartoum

B ≡ Fasekh obtain from center Khartoum

C ≡ Fasekh obtain from east Khartoum

4.2.5 Histamine content in Fasekh samples:

Significant differences (P<0.05) were noticed for Histamine levels among Fasekh samples. The highest level of Histamine were reported for Fasekh samples (B) 0.548mg/100g, while, the lowest level of Histamine were reported for Fasekh samples (C) 0.325mg/100g. Prester (2011) found that, Sardine (*S. pilchardus*) had histamine content of 0.20mg/100g(figure 7). The current results of histamine concentration near the American Food and Drug Administration FDA (1996) , which recommend that, histamine concentration in fish must not exceed than set 100mg/kg (10mg/100g).

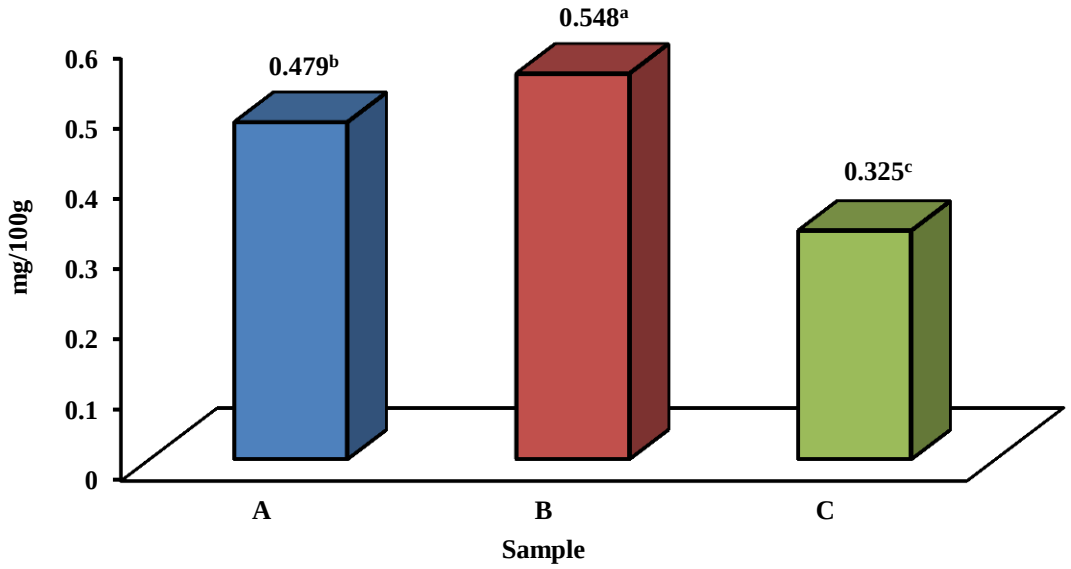

Fig. (7): Histamine content of Fasekh

Key:

A ≡ Fasekh obtain from north Kartoum

B ≡ Fasekh obtain from center Khartoum

C ≡ Fasekh obtain from east Khartoum

Prester (2011) mentioned that, among amines, histamine is important from the toxicological point of view as it is the causative agents of scombroid fish poisoning and food intolerance.

Generally, the concentrations of some biogenic amines such as; Tyramine, putrescine, and cadaverine) normally increase during the processing and storage of meat and meat products as stated by Ruiz *et al.*, (2004).

Furthermore, FDA (1996) recommends that, quantification of biogenic amines associated with fish decomposition.

4.2.6 Tyramine and Spermidine content of Fasekh samples

The results show that, Fasekh samples were free from Tyramine and Spermidine . The current results were matching with that results obtained by Mostafa and Salem (2015) who found that, all fish samples under investigation wereTyramine free. Same observation was found by Ruiz *et al.,* (2004) who observed all beef samples under investigation was Tyramine and Spermidine free. In contrast, Yuecel (2008) found that, Tyramine in low concentration 0.032mg/100g and Spermidine free.

The implementation of HACCP programmes (hazard analysis and critical control point) in the fishery industry has reduced failure costs, improved quality and control of seafood, and therefore prevents fish safety problems as mentioned by Prester (2011).

CHAPTER FIVE

CONCLUTION AND RECOMMEDATIONS

5.1 Conclusion

Biogenic amine play an important role in the physiological function of animals including fishes and in normal conditions the exogenous amines are detoxified by amine oxidase or by conjugation. But if it ingested high levels it leads to intoxication. The maximum acceptable limit for the histamine in seafood as per FDA regulation is 100mg/kg. Hence estimation of biogenic amines is important from the point of view of the toxicity and freshness of product. In this study the concentration of biogenic amines of cooked Fasekh within codex standard in all samples under investigation .

5.2 Recommendations

- It is recommended that, implementation of good manufacturing practice in Fasekh small industry to reduce the biogenic amine formation .
- Further research is needed to control the biogenic amine of Sudanese cooked fermented fish.
- The individual intake of ready to eat Fasekh always not exceeds of 250_300g per day, the biogenic amine in this quantity is in tolerable limit. For example, histamine in Fasekh was 0.548mg/100g; in the individual intake will be 1.664mg/300g.

REFERENCES

Ababouch, L. ;Afilal, M.E. ;Benabdeljelil, H. and Busta, F.F. (2007). Quantitative changes in bacteria, amino acids and biogenic amines in sardine (*Sardina pilchardus*) stored at ambient temperature and in ice. International Journal of Food Science and Technology. 26 (3): 297-306.

Agab, M.A. And Shafi, R. B. (1989). Traditionally salt fermented fish (Fasseikh). Cited in: Dirar (1993).

Ahmed, I. O. (2006). Comparison of the nutritive value of Faseikh using *Hydrocynus spp*.and *schilbe .spp.* Ph.D. Thesis, Al Neelain University. Sudan.

Anli, R. E. and Bayram., M. (2009). Biogenic amines in wines. International Journal of food review. Vol. 25: 86-102.

AOAC. (2005). Official Methods of Analysis of the Association of Analytical Chemists International, 18th ed. Gathersburg, MD U.S.A Official methods, 2005.

Askar, A. and Treptow, H. (1986). Biogene Amine in Lebensmitteln.Vorkommen,Bedeuting and Bestimmung ,Eugen Ulmer Verlag, Stuttgart. ISBN 3800121328,p:664

Bardocz, A. (1995). Polyamines in food and their consequences for food quality and human health. Trends Food Science and Technology. 6, 3 46.

Basheer,E. O. (2017). Development and Quality Evaluation of Beef Frankfurter Enriched with Omega-3 Unsaturated Fatty Acids. Ph.D Thesis, Faculty of Agric, University of Khartoum. Sudan.

Batra, Y. K . and Rajeev, S. (2007). Effect of common herbal medicines on patients undergoing. International Journal of Clinical Anaesth. 51(3):184-192.

Beljaars, P.R.(1998). Liquid Chromatoghraphic Determination of Histamine in fish,sauerkraut and wine. Journal of AOAC,81(5): 991_998

Bhutani, M. K. ; Bishnoi, M. and Kulkarni, S. K. (2009). Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress induced behavioral, biochemical and neurochemical changes. Pharmacol Biochemistry Behavior Journal. 92 (1): 39-43.

Bockhardt, A. K. I. and Klostermeyer, K. (1996). Determination of biogenic amine by HPLC. Lebensm- Unters. Forsch. 203, 270.

Bodmer, S.; Imark, C. AND Kneubuhl, M. (1999). Biogenic amines in foods: histamine and food processing. Journal of Inflamentation Research. 48 (6): 296-300.

Bolton, G. E. ;Bjornsdottir, K. ;Nielsen, D. ; Luna, P. F. and Green, D. P. (2009). Effect of high hydrostatic pressure on histamine forming bacteria in yellowfin tuna and mahi-mahi skinless portions. Institute of Food Technologists (IFT) Conference. USA 006-05.

Bover-Cid, S.; Hugas, M. ; Izquierdo, M. and Vidal, C. (2000). Reduction of biogenic amine formation using a negative amino acid-decarboxylase starter culture for fermentation of Fuet sausages. Journal of Food Protocol. 63: 237-243.

Bulushi, H. K. ; Zarohate, K. P.; Norahomater, H. K. (2009). Formation of biogenic amines in meat packed in oxygen permeable foil. In: Proceedings of the 42nd International Congress of Meat Science and Technology., pp. 552-553. Lillehammer, Norway.

Buncic, S. (1993). Heat resistance of *Listeria monocytogenes* in vacuum packaged pasteurized fish fillets. International Journal of Food Microbiology. 50, 148.

Burt, J. R.1976. Hypoxanthine a biochemical index of fish quality.Process Biochemestry.11(10).

Chen, C. H.; Huang, Y. R ; Hsia, H. H.; Lind, S. C.;Min, M. M. and Tsai, Y.H. (2010). Determination of histamine and biogenic amines in fish cubes (*Tetrapturus angustirostris*) implicated in a food-borne poisoning. Food Control Journal.21 (1): 13-18.

Coba, M. and Silva, M.J. (1999). Continuous Solid- Phase extraction dansylation of low molecular mass amine on –line with liquid chromatoghraphy and peroxoxalate chemituminine scenc. Journal of Chromatoghraphy841,115

Costa,M.P. ; Balthazar,C.F. and Conte-Juniur,C.A.(2015). Determination of biogenic amine by HPLC-DADin probiotic cow and goat fermented milk and acceptance. Journal of Food Science and Nutrition.3(3):172_178.

Chrubasik, S.; Pittler, M. H. and Roufogalis, B. D. (2005). Zingiberis rhizome: a comprehensive review on the ginger effect and efficacy profiles. Journal of Phyto medicine. 12 (9): 684-701.

Dalgaard, P.; Madsen, L.; Samieian, N.; Emborg, J. (2006). Biogenic amine formation and microbial spoilage in chilled garfish (Belone belone belone)-effect of modified atmosphere packaging and previous frozen storage. Journal of Applied Microbiology. 101(1): 80-95.

Dapkevicius, M.; Nout, M.; Rombouts, F.; Houben, J. H. and Wymenga, W. (2000). Biogenic amine formation and degradation by potential fish silage starter microorganisms. International Journal of Food Microbiology. 57 (1-2): 107-114.

Dirar, H. A. (1993). The indigenous fermented foods of the Sudan. A study in Africa food and nutrition. CAB International. Wallingford.

Dural, M.; Goksu, M. L. and OZak, A. A. (2007). Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food Chemistry Journal. 102: 415-421.

Eerola, S.; **Hinkkanen, R.; Lindfors, E. and Hirvi, T. (1993).** Liquid Chromatoghraphic Determination of Biogenic amines in Dry Sausages. Journal of AOAC International, 76,575_577.

Eltom, A. M. (1989). Microbiology and Biochemistry of Fassiekh Fermentation. M.Sc. Thesis, Faculty of Agric. University of Khartoum, Sudan.

Emborg, J. and Dalgaard, P. (2008). Growth inactivation and histamine formation of *Morganella psychrotolerans* and *Morganella morganii* development and evaluation of predictive models. Journal of International Food Microbiology 128 (2): 234-243.

Emborg, J.; Laursen, B. and Dalgaard, P. (2005). Histamine formation in tuna (*Thunnus albacares*) at -2°C effect of vacuum and modified atmosphere-packaging on psychrotolerant bacteria. Journal of Food Microbiology. 101 (3): 263-279.

FAO (1999). Fermented fish in Africa. A study on processing, marketing and composition. FAO Fishers Technical paper No.329, Rome, Italy.

FAO (2008). Fishery country profile. Food and Agriculture Organization of the Untied Nations.

Food and Drug Administration (FDA),Fish and fisher products hazards and control guidance .Washington DC:Food and Drug Adminstration Office of Seafood;1996.

Gardini, F.; Bover-Cid, S.; Tofalo, R.; Belletti , N. Gatto, V.; Suzzi, G. and Torriani, S. (2008). Modeling the aminogenic potential of *Enterococcus faecalis EF37* in dry fermented sausages through chemical and molecular approaches. Applied Envieonment Microbiology. 74 (9): 40-50.

Ghayur, M. N.; Gilani, A. H.; Afridi, M. B. nd Houghton, P.a J. (2005). Cardiovascular effects of ginger aqueous extract and its phenolic constituents are mediated through multiple pathways. Vascular Pharmacology. 43 (4): 234-241.

Gonzałez, B.; Capita, R.; Guevara, F,.; Prieto, M. and Alonso, C. (2007). Influence of oxygen exclusion and temperature on pathogenic bacteria levels and sensory characteristics of packed ostrich steaks throughout refrigerated storage. Meat Science. 76 (2): 201-209.

Greif, G.; Greifova, M. and Drdak, M. (1997). Biogenic Amine Production in Olomouc Curd Cheese (*Olomoucké tvarůžky*) at Various Storage Conditions. Journal of the University of Veterinary and Pharmaceutical Sciences. 115, 129.

Halasz, A,.;Barath, A.; Simon, L. and Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. Trends Food Science and Technology. 5:42-49.

Hassan, H. M. (2010). Nutritive value of fresh and salted fermented fish (*Alestes dentex*) Terkin. M.Sc. Thesis, Faculty of agriculture. University of Khartoum. Sudan.

Hu, Y.; Xia, W. and Ge, C. (2008). Characterization of fermented silver carp sausages inoculated with mixed starter culture. LWT. Journal of Food Science and Technology. 41(4): 730-738.

Kang, I. J. and Park, Y. H. (1984). Effect of food additives on the histamine formation during processing and storage of mackerel. Bull Korean Fisheries Society. 17: 383.

Kapeller-Adler, R. (1941). Histidine metabolism in toxaemia of pregnancy. Isolation of histamine from the urine of patients with toxaemia of pregnancy. Biochemistry Journal. 35: 213- 218.

Karovicova, J. and Kohajdova, Z. (2005). Biogenic amine in food**.** Journal of Slovak University. 59: 70-79.

Kim, J. H.; Ahn, H. J.; Kim, D. H.; Jo, C.; Yook, H. S.; Park, H. J. and Byun, M. W. (2003). Irradiation effects on biogenic amines in korean fermented soybean paste during fermentation. Journal of Food Science. 68(1): 80-84.

Kovács, A. (1999). Determenation of biogenic amines by capillary electrophoresis. Journal Chromatography 305- 313.

Krizek, M.; Pavlicek, T. and Vacha, F. (2002). Formation of selected biogenic amines in carp meat. Journal of Food Science and Agriculture. 82: 1088-1093.

Kurt, S. and Zorba, Ơ. (2009). The effects of ripening period, nitrite level and heat treatment on bio- genic amine formation of "sucuk" – A Turkish dry fermented sausage. Meat Sci 82(2):84.-179

Landete, J. M.;Pardo,I. and Ferrer,S. (2006). Tyramine and phenylethylamine production among lactic acid bacteria isolated from wine. M.Sc, Thesis. Faculty of biological, University of Valencia, Spain

Latorre-Moratalla, M. L.; Bover-Cid, S.; Aymerich, T.; Marcos, B.; Vidal-Carou, M. C. and Garriga, M. (2007). Aminogenesis control in fermented sausages manufactured with pressurized meat batter and starter culture. Meat Science. 75 (3): 460-469.

Lee, J. A. and Hathaway, S. (1998). The challenge of designing valid HACCP plans for raw food commodities. Journal of Food Control (9): 111-117.

Lee, S. P.; Buber, M.. T.; Yang, Q.; Cerne, R.; Cortes, R. Y.; Sprous, D. and G, Bryant, R ..W (2008). Thymol and related alkyl phenols activate the hTRPA1 channel. Britich Journal of Pharmacol. 153 (8): 1739-1749.

Lehane, L. and Olley, J. (2000). Histamine fish poisoning revisited. International Journal of Food Microbiology, Vol. 58: 1-37.

Leuschner, R. G. and Hammes, W. P. (1998). Degradation of histamine and tyramine by *Brevibacterium linens* during surface ripening of Munster cheese. Journal of Food Protocol. 61(7): 874-878.

Mah, J. H.; Kim, Y. J. and Hwang, H. J. (2009). Inhibitory effects of garlic and other spices on biogenic amine production in Myeolchi-jeot, Korean salted and fermented anchovy product. Food Control Journal. 20 (5): 449-454.

Maijala, R.; Eerola, S,.; Aho, M. and Hirn, J. (1993). The effect of GDL-induced pH decrease on the formation of biogenic amines in meat. Journal of Food Protocol. 56 (2): 125-129.

Mbarki, R.; Miloud, N. B.; Selmi, S.; Dhib, S. and Sadok, S. (2009). Effect of vacuum packaging and low-dose irradiation on the microbial, chemical and sensory characteristics of chub mackerel (*Scomber japonicus*). Journal of Food Microbiology 26 (8): 821-826.

Min, J. S.; Lee, S.; Jang, A.; Jo, C. and Lee, M. (2007). Control of microorganisms and reduction of biogenic amines in chicken breast and thigh by irradiation and organic acids. Poultry Science 86 (9): 2034-2041.

Mohan, C. O.; Ravishanka., C. N.; Gopal, T. K.; Kuma, K. A . and Lalitha, K. (2009). Biogenic amines formation in seer fish (*Scomberomorus commerson*) steaks packed with O_2 scavenger during chilled storage. Food Research International Journal. 42 (3): 411-416.

Mostafa, A. and Salem, R. (2015). Characterization of Microbiological and Nutritional Variations in Processed Mullet *(Mugil cephalus)* Fish. International Journal of Microbiological Research. 6 (2): 108-122.

Naila,A.; Flint, S.;Fletcher,G.;Bremer, P.and Meerdink,G.(2010). Control of biogenic amines in food-existing and emerging approaches.Journal of food science.75(7):141_145.

Novela, R . S.; Veciana, N.; Saldo, J. and Vidal, M. (2002). Effects of high hydrostatic pressure treatments on biogenic amine contents in goat cheeses during ripening. Journal of Agriculture Food Chemistry. 50 (25): 7288-7292.

Osman, A. O.; Abdel Moneim E. S. ; Elkhalifa, E. A. and Mustafa, A. W. (2012). Chemical and microbiological characteristics of fermented fish product fassiekh. Food and Public Health. 2 (6): 213-218.

Ozogul, F.; Polat, A,. andOzogul., Y. (2004). The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). Food Chemistry. 85 (1): 49-57.

Pabo, M.C.; Perezc- Camino, M.C.;Montero,V.Q. and Ruiz-Gutierrez,V.(1998). Determination of the molecular species compostion of diacylgyceols in human adipose tissue by solid- phase extraction and gas chromatoghraphy on a polar phase . Journal of chromatoghraphy.714(2),127_132.

Pennas, E.; Frias, J.; Gomez, R. and Vidal-Valverde, C. (2010). High hydrostatic pressure can improve the microbial quality of sauerkraut during storage. Food Control Journal. 21 (4): 524-528. .

Petaja, E. ; Eerola, S. and Petaja, P. (2000). Biogenic amines in cold-smoked fish fermented with lactic acid bacteria. European Food Research Technology. 210(4): 280-285.

Prabjeet,S. ;Mohd,D. and Amita,S. (2018). Spoliage of fish- process and its preventation. Department of Fishery Biology, College of Fishers, G.B.

Pant University of Agriculture and Technology,Pantnagar, Uttrakhand, India,

Prester, L. (2011). Biogenic amines in fish, fish products and shell fish**.** Journal of Food Additives and Contaminants**.** Vol. 28: 1547-156

Rivas, B.; Gonzaĺez, R.; Landete , J. M. and Muñoz, R. (2008). Characterization of a second ornithine decarboxylase isolated from *Morganella morganii.* Journal of Food Protction 71(3): 657-661.

Ruiz, C. ;Capillas, S.; Francisco, L. and Jimenez, C. (2004). Biogenic Amines in Meat and Meat Products. Journal of Critical Reviews in Food Science and Nutrition. 44: 489-499.

Sangcharoen, N.; Ruksakultha., N. ; Malaphan , W. and Wilaipu., P. (2009). Effect of some essential oils and indigenous plant extracts on the growth inhibition of histamine forming bacteria. Proceedings of the 47th Kasetsart University Annual Conference. Kasetsart University. Thailland. 608-617.

SAS. (2002). SAS User Guide, Release 6- 03. Edition SAS. Institute Inc. Gray, NC, USA.

Schirmer, B. C.; Heiberg, R.; Eie, T.; Mororeto, T.; Maugesten, T. Carlehogh, M. and Langsrud, S. (2009). A novel packaging method with a dissolving CO_2 headspace combined with organic acids prolongs the shelf life of fresh salmon. International Journal of Food Microbiology. 133: 154-160.

Seiler, J.(1986). Ahighly sensitive accurate and reproduce HPLC method. Journal of chromatoghraphy.157-176.

Seo, K.; Y, Heo, S. K,.Lee, C.; Chung, D. H.; Kim, M. G.; Lee, K .; Kim, K. S.; Bahk, G. J.; Bae, D.; Kim, K.; Kim, H. and Has, S. (2007). Development of predictive mathematical model for the growth kinetics

of *Staphylococcus aureus* by response surface model. Journal of Micro and Biotechnology. 17(9): 1437-1444.

Shalaby, A. R. (2000). Changes in biogenic amines in mature and germinating legume seeds and their behavior during cooking. Journal of Nahrung/Food. 44 (1): 23-27.

Shalini, R.; Jasmine, G.; Shanmugam, S,. and Ramkumar, K. (2001). Effect of potassium sorbate treatment in vacuum packaged Lethrinus lentjan fillets under refrigerated storage. Journal of Food Science and Technology 38:12-16.

Shishodia, S.; Sethi, G. and Aggarwal, B. B. (2005). Curcumin: Getting back to the roots. Ann New York Academy Science. 1056 (1): 206-217.

Silla Santos, M.H. (1996). Biogenic amines: their importance in foods. International Journal of Food Microbiology. 29: 213-231.

Singh, V. K.: Sanjay, S.; Sushma, S. and Singh, K. (1999). Effect of active molluscicidal component of spices on different enzyme activities and biogenic amine levels in the nervous tissue of Lymnaea acuminate. Physiotherapy Research. 13 (8): 649-654.

Smella, D.;Pechora,P.;Komprda, T.;Klejdus,B. and Kaban, V.(2003). Liquid chromatoghraphic determination of biogenic amines in meat products during fermented and long – term store .Gzech Journal of food science 21(5):167-175.

Steel, R.G. and Torrie, J.H. (1980). Principles and procedures of statistics, MC Grow Hill, New York, USA.

Steinkraus, S. K. (2004). Industrialization of indigenous fermented food. Woodhead Publishing Limited and CRC Press, Boca Raton, FL, USA.

Suresh, D.; Manjunatha, H. and Srinivasan, K. (2007). Effect of heat processing of spices on the concentrations of their bioactive principles: turmeric (*Curcuma longa*), red pepper (*Capsicum annuum*) and black

pepper (*Piper nigrum*). Journal of Food Composition Analysis. 20: 346-351.

Tapingkae., W, Tanasupawat., S, Parkin., K., L, Benjakul., S, Visessanguan., W. (2010). Degradation of histamine by extremely *halophilic archaea* isolated from high salt-fermented fishery products. Enzyme Microbial Technology. 46 (2): 92-9.

Wendakoon, C. N. and Sakaguchi, M. (1995). Inhibition of amino acids decarboxylase activity of *Enter- obacter aerogenes* by active components in spices. Journal of Food Protection. 58: 280-283.

Wei, F.; Xu, X.; Zhou, G. ;Zhao, G.; L., C.; Zhang, Y.; Chen, L. and Qi, J. (2009). Irradiated Chinese rugao ham: Changes in volatile N-nitrosamine, biogenic amine and residual nitrite during ripening and post-ripening. Meat Science. 81(3): 451-455.

WHO (1994). World Health Organization. Safety and nutritional adequacy of irradiated food. Geneva.

Yuecel, U. A. (2008). Biogenic amines in Turkish type pickled cabbage: effects of salt and citric acid concentration. Acta Aliment Journal. 37(1): 115-122.