The GOOD
The BAD & UGLY
of Food Safety
From MOLECULES to MICROBES

PROFESSOR DR. FATIMAH ABU BAKAR

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Contents

ABSTRACT

Food safety is about ensuring that our food is safe to eat. Food quality concerns ensuring that it is nutritious and acceptable. Food safety is the absolute priority. It must be assured to protect the consumer, and to maintain and expand food markets.

Like all disease, foodborne illness seems to strike at random. When people are exposed to foodborne microbes, some of them get sick while many others will suffer few or no ill effects.

Foodborne diseases are largely preventable, though there is no simple one-step prevention measure like a vaccine. Instead, measures are needed to prevent or limit contamination all the way from the farm to the table.

- A variety of good agricultural and manufacturing practices can reduce the spread of microbes among animals and prevent the contamination of foods.

- Careful review of the whole food production process can identify the principal hazards, and the control points where contamination can be prevented, limited, or eliminated.

- A formal method for evaluating the control of risk in foods exists is called the Hazard Analysis Critical Control Point, or *HACCP* system. This was first developed by *NASA* to make sure that the food eaten by astronauts was safe. HACCP safety principles are now being applied to an increasing spectrum of foods, including meat, poultry, and seafood.

For some particularly risky foods, even the most careful hygiene and sanitation are insufficient to prevent contamination, and a definitive microbe-killing step must be included in the process.

The main concern is the safety hazards associated with the poultry and aquaculture food animals especially bacterial pathogens.

Currently, the most fail-safe method is to consistently monitor the bacterial level in the food animals from the point immediately after obtaining them to just before it is sold to the consumer. However, this has done very little to reduce food poisoning among consumers, as it is time-consuming and lacks reinforcement in some improvised areas of the world. Therefore, research is ongoing worldwide on finding a way to reduce, if not, remove the hazards present in the food animals, either by prevention (before or during its growth), or production.

This publication discusses the importance of food safety. It analyzes issues related to food safety, such as spoilage, food-borne pathogens and the microbiological quality of food including some of the examples of work carried out by our research group. It then discusses the significance of contaminants such as biogenic amines with regards to food safety during handling and storage. Our findings provide significant information regarding histamine degrading bacteria and factors influencing its activity. In addition, the findings also emphasized the effectiveness of using bacteria with amines oxidase activity in reducing histamine accumulation in fermented food products. Lastly, the publication looks at the development of rapid enzyme-based biosensor technology in detecting histamine and formaldehyde in fish and seafoods.

INTRODUCTION

Like all disease, foodborne illness seems to strike at random. When people are exposed to foodborne microbes, some of them get sick while many others will suffer few or no ill effects.

Now it appears that the randomness extends to the public response. Some outbreaks of disease are quickly traced to their sources while other epidemics — probably the great majority — will take longer to be detected, if at all.

Making food safe in the first place is a major effort, involving the farm and fishery, the production plant or factory, and many other points from the farm to the table. Many different groups in public health, industry, regulatory agencies, and academia have roles to play in making the food supply less contaminated. Consumers can promote general food safety with their dollars, by purchasing foods that have been processed for safety.

- For example, milk pasteurization was a major advance in food safety that was developed 100 years ago. Buying pasteurized milk rather than raw unpasteurized milk still prevents an enormous number of foodborne diseases every day.

- *V. cholerae* is the etiological agent of cholera which is spread by contaminated food, water or direct fecal contact with food handlers (Suzita et al., 2010)

- Now juice pasteurization is a recent important step forward that prevents *E. coli O157:H7* infections and many other diseases. Consumers can look for and buy pasteurized fruit juices and ciders.

- In the future, meat and other foods will be available that has been treated for safety with irradiation. These new technologies are likely to be as important step forward as the pasteurization of milk.

Malaysia fishery products export is valued at RM 2610 million per year. About 50% of Malaysia fishery products are exported to the European Union (EU) and United States of America (USA). However, in 2008, Malaysia had voluntary delisted 58 fishery establishments from the EU approved third country approval list in section VIII for fishery products. A total of 6 were re-listed after the mission inspection in May, 2009 and total 19 are listed as of December, 2010. The main microbiological non-compliance are due to food safety hazards such as *Salmonella* and *E. coli*; while chemical non-compliances are histamine, nitrofuran, malachite green, leuco-malachite green, chloramphenicol, and banned drugs. The voluntary delisting of fishery establishments in 2008 had caused major economic impact to Malaysia fishery industry and economic losses to the country and has yet to recover.

What is Foodborne Illness (Disease, Infection)?

Foodborne illness (sometimes called "foodborne disease," "foodborne infection," or "food poisoning) is a common, costly—yet preventable—public health problem. Each year, 1 in 6 individuals gets sick by consuming contaminated foods or beverages. Many different disease-causing microbes, or pathogens, can contaminate foods, so there are many different foodborne infections. In addition, poisonous chemicals, or other harmful substances can cause foodborne diseases if they are present in food.

More than 250 different foodborne diseases have been described. Most of these diseases are infections, caused by a variety of bacteria, viruses, and parasites that can be foodborne.

Other diseases are poisonings, caused by harmful toxins or chemicals that have contaminated the food, for example, poisonous mushrooms.

These different diseases have many different symptoms, so there is no one "syndrome" that is foodborne illness. However, the microbe or toxin enters the body through the gastrointestinal tract, and often causes the first symptoms there, so nausea, vomiting, abdominal cramps and diarrheas are common symptoms in many foodborne diseases (Haryani et al., 2007).

Many microbes can spread in more than one way, so we cannot always know that a disease is foodborne. The distinction matters, because public health authorities need to know how a particular disease is spreading to take the appropriate steps to stop it.

These are some of the important definitions associated with food safety:

- **Foodborne illness –** any illness carried to humans by food.
- **Foodborne infection** – an illness caused by the ingestion of live bacteria in or on food.
- **Foodborne intoxication** – an illness caused by ingestion of toxins in food, regardless of the presence or absence of live bacteria.
- **Toxins** – compounds produced by living organisms that cause harm to other organisms.
- **Microbes** – living organisms only visible by microscope (e.g. bacteria, yeasts, molds).
- **Spores** – produced by some types of microbes and are very resistant to heat and dehydration (bacterial survival kits, so to speak).

- **Pathogens** – disease-causing microorganisms such as those responsible for foodborne illnesses

Foods are biological materials and are therefore subjected to many changes during handling, processing, and storage.

POTENTIAL HAZARDS TO FOOD SAFETY

Food safety hazards are divided into three categories: biological hazards, chemical hazards, and physical hazards.

- **Biological hazards** include certain bacteria, viruses, parasites, and fungi, as well as certain plants, mushrooms, and fish that carry harmful toxins.
- **Chemical hazards** include pesticides, food additives, preservatives, cleaning supplies, and toxic metals that leach from cookware and equipment.
- **Physical hazards** consist of foreign objects that accidentally get into the food, such as hair, dirt, metal staples, and broken glass.

By far, biological hazards pose the greatest threat to food safety. Disease-causing microorganisms are responsible for the majority of foodborne illness outbreaks**.**

Biological Hazards: Microbes

Microorganisms are tiny living organisms. Bacteria, yeasts, and molds are microbes that are important in the food industry. Desirable, controlled growth of selected microorganisms provides us with delightful, flavourful products such as cheeses, yogurts, sauerkraut, bread, and wines.

The most common cause of food contamination is the unwanted, undesirable growth of yeasts, molds, and bacteria. When we use

the term "growth" we mean an increase in numbers or population. Furthermore, improperly handled or stored food can carry and transmit diseases as a result of growth of organisms such as *Staphylococcus aureus*, *Salmonella*, and *Clostridium botulinum* (Robin et al., 2007).

We live in a microbial world, and there are many opportunities for food to become contaminated as it is produced and prepared.

- Many foodborne microbes are present in healthy animals (usually in their intestines) raised for food. Meat and poultry carcasses can become contaminated during slaughter by contact with small amounts of intestinal contents.
- Similarly, fresh fruits and vegetables can be contaminated if they are washed or irrigated with water that is contaminated with animal manure or human sewage.
- Some types of *Salmonella* can infect a hen's ovary so that the internal contents of a normal looking egg can be contaminated with *Salmonella* even before the shell in formed.
- Oysters and other filter feeding shellfish can concentrate *Vibrio* bacteria that are naturally present in sea water, or other microbes such as norovirus that are present in human sewage dumped into the sea (Abu Bakar, 1997).

Later in food processing, other foodborne microbes can be introduced from infected humans who handle the food, or by cross contamination from some other raw agricultural product.

- For example, *Shigella* bacteria, hepatitis A virus and norovirus can be introduced by the unwashed hands of food handlers who are themselves infected.
- Cross-contamination is the scientific word for how bacteria can be spread from one food product to another. This is especially

true when handling raw meat, poultry, and seafood, so keep these foods and their juices away from ready-to-eat foods.

- In the kitchen, microbes can be transferred from one food to another food by using the same knife, cutting board or other utensil to prepare both, without washing the surface or utensil in between (Chai et al., 2008).
- A food that is fully cooked can become re-contaminated if it touches other raw foods or drippings from raw foods that contain pathogens (Chai et al., 2007).

Thousands of species of microorganisms exist in nature. Microorganisms only become visible when growing in masses containing millions of cells. For example, more than 250,000 of them will fit onto the head of a pin! Because microorganisms are so widely distributed in the soil, water, and air, it is normal to find many types and species on the surfaces of fresh foods. Their vegetative cells are easily killed by heating food to a boiling water temperature. However, the vegetative cells of some bacteria are capable of forming spores that resist death during heating. Spores are a form of the organism that survives in a resting (or hibernating) state. When a favorable environment occurs, the spores will germinate and produce vegetative cells. The growth of vegetative cells on food is influenced by temperature, the availability of moisture, nutrients, oxygen, acidity, and growth inhibitors (Abu Bakar and Motohiro, 1996).

Bacteria

Bacteria come in many shapes including rods, balls, or spirals. They grow and increase in number by the splitting of a single cell.

Some cause diseases and are known as pathogens. Foodborne means the pathogens can be transmitted by food. Many non-pathogenic bacteria cause spoilage when they grow on food.

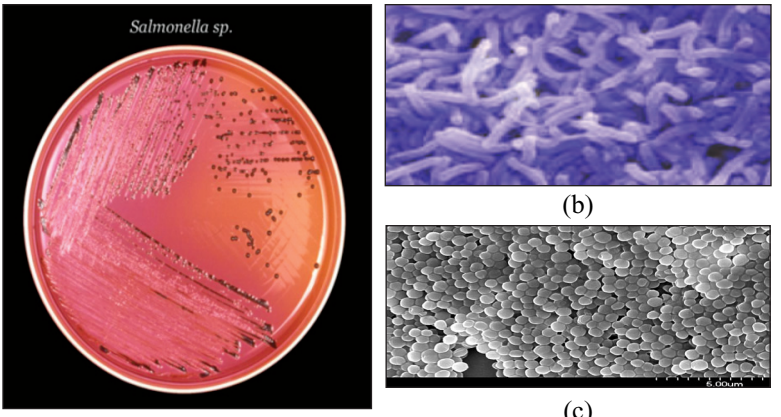
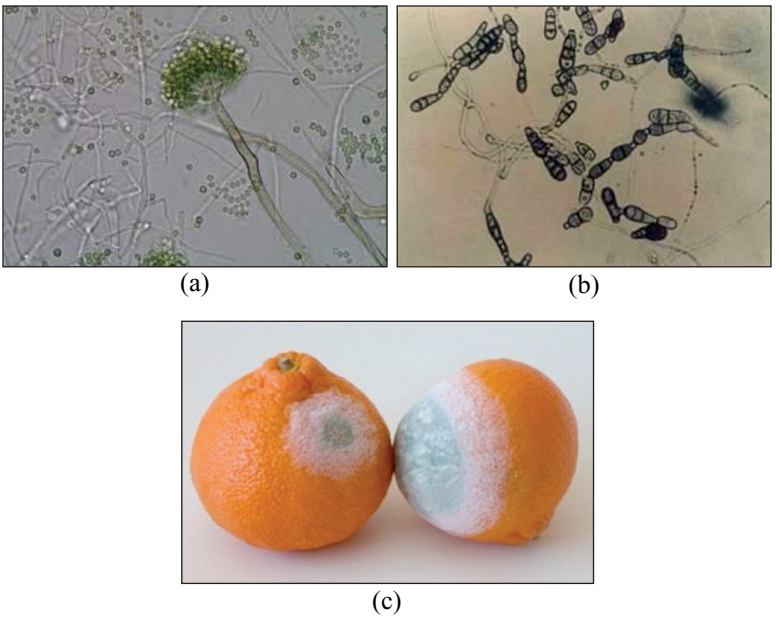


Figure 1 Plating media showing the growth of *Salmonella* sp. (a), electron micrograph of rod-shaped bacteria (b) and electron micrograph of coccus-shaped bacteria (c)

Molds

Molds grow on most foods and require air and water, although they need less water than bacteria. In appearance, their masses of growth usually are fuzzy and can be nearly any color. Some molds produce toxins that are carcinogenic (cancer causing). These molds commonly grow on fruits, fruit products, grains, grain products,



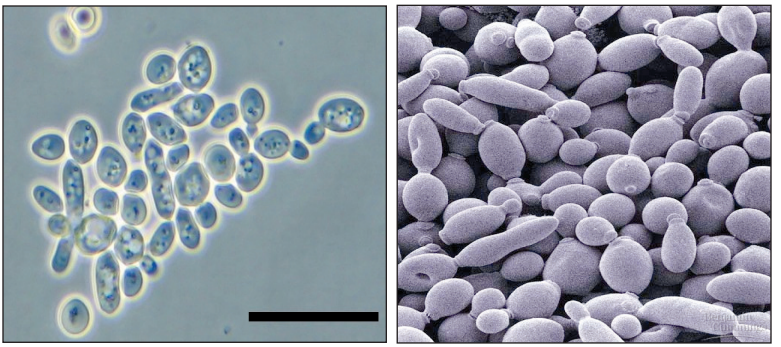
(a) (b)

(c)

Figure 2 Microscopic pictures of *Aspergillus* sp. (a) and *Alternaria* spp. (b) and fruit contaminated with *Aspergillus* sp. (c)

Yeasts

Yeasts can grow with or without air and require more water than molds. Their masses in or on food appear as slime, scum, or murkiness. Yeast fermentation in food is recognized by gas bubbles, froth, or foam, which result from the fermentation activity and the production of carbon dioxide gas. Depending on the specific growth conditions, yeasts convert sugars to ethanol and then bacteria convert the ethanol to vinegar, alcohol (e.g., beer and wines), or carbon dioxide (e.g., raised bread) during fermentation (Law et al., 2011).



(a) (b)

Figure 3 Yeast cells as observed under light microscope (a) and electron microscope (b)

Chemical Contamination

The contamination of food by chemical hazards is a worldwide public health concern and is a leading cause of trade problems internationally. Contamination may occur through environmental pollution of the air, water and soil, such as the case with toxic metals, PCBs and dioxins, or through the intentional use of various chemicals, such as pesticides, animal drugs and other agrochemicals.

Chemicals are routinely added to processed foods to preserve them and to enhance flavor and appearance. These chemicals have been tested and are believed to be safe. However, some additives, such as artificial sweeteners or fat substitutes, carry warnings about potential bad effects of eating them (Suwaibah et al., 2009). Boric acid, a banned preservative is still occasionally found in many products such as yellow noodle and fish ball (See et al., 2010). Although many consumers worry about artificial chemicals, naturally occurring chemicals can be more dangerous. Allergy-producing substances, called allergens, are a major concern.

Some people have dangerous, potentially fatal allergic reactions to chemicals found in peanuts, tree nuts (e.g., walnuts, pecans, and almonds), shellfish, eggs, milk, soy, fish, and wheat. Other naturally-occurring chemicals are added to foods as dietary supplements, but there may not be proof that these are either effective or safe. In rare cases, chemicals get into food accidentally and the potential exists for pesticides, animal drug residues, or cleaning substances to show up in foods. Other chemical hazards may arise from environmental pollution.

Physical Contamination

A physical contaminant is anything that can be visibly seen and is not part of the food originally. Physical contamination is foreign bodies in food. The contamination could occur at any stage of food production. It could be contaminated during production from raw materials; it could be contaminated during preparation or during storage and service. Common items found include stones, wood, bones, pests, plastic, screws, wire and glass.

Through these means, improperly preserved foods can lose their aesthetic appeal, nutritional value and worse, may become hazardous. By investigating the various causes of spoilage and the effects of various food handling, preparation, packaging, and storage techniques on food quality, we can learn to distinguish between safe and hazardous preservation methods (Suzita et al., 2009).

What Foods are Most Associated with Foodborne Illness?

- Raw foods of animal origin are the most likely to be contaminated; that is, raw meat and poultry, raw eggs, unpasteurized milk, and raw shellfish (Suzita et al., 2009)

- Because filter-feeding shellfish strain microbes from the sea over many months, they are particularly likely to be contaminated if there are any pathogens in the seawater (Suzita et al., 2010)
- Foods that mingle the products of many individual animals, such as bulk raw milk, pooled raw eggs, or ground beef, are particularly hazardous because a pathogen present in any one of the animals may contaminate the whole batch

Figure 4 Several food products at risk of food-borne illness

PRACTICAL STERILIZATION APPROACH TO CONTROL MAJOR PATHOGENIC BACTERIA ON FOOD

Food can harbour a large number of pathogenic and spoilage microorganisms during primary and further processing, therefore appropriate and safe antibacterial agents able to decontaminate food surfaces have long been big concern of food industry. Organic acids are weak acids that are commonly found in fruit juices and fermented foods. Organic acids have a long history of being applied as food additives and preservatives for preventing food deterioration and extending the shelf-life of perishable food ingredients. Organic acids are Generally Recognized As Safe (GRAS) antimicrobial agent and the dilute solutions of organic acids (1-3%) are generally without effect on the desirable sensory properties of meat when used as a carcass decontaminant (Raftari et al., 2009).

Various researchers indicated the antibacterial effect of different types of organic acids. Previous studies focused on limited treatments for controlling bacteria in which results were inconsistent because of the extensive variations in conditions of experiments. Studies carried out on spray-wash treatments utilizing concentrations (1, 1.5 and 2%) of acetic, lactic, propionic and formic acids, alone or in combination of two acids, were performed to reduce numbers of *Salmonella typhimurium* and *Listeria monocytogenes* on meat tissues at 4±1ºC. *Salmonella typhimurium*- and *L. monocytogenes*-inoculated meat was spray-washed with treatments for 15 sec separately. The populations of *S. typhimurium* and *L. monocytogenes* significantly ($p<0.05$) decreased after being spray-washed with all treatments. The lethality effect of all organic acids according to the concentration was 2>1.5>1% concentration ($p<0.05$). The antibacterial effect of formic acid was > lactic acid > acetic acid > propionic acid ($p<0.05$). Moreover, the antibacterial effect of organic acids increased when used in combinations. Lactic and

formic acid combination showed the highest lethal effect (Raftari et al., 2012).

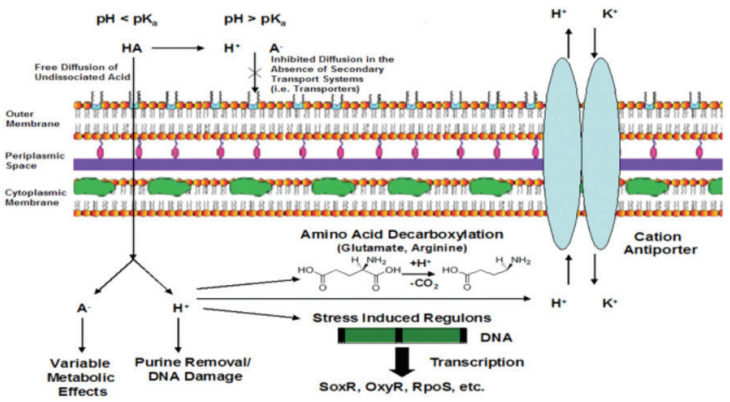


Figure 5 Mode of action of organic acids on bacteria (eg. *E. coli, Salmonella sp., Listeria sp.* etc.)

SIGNIFICANCE OF BIOGENIC AMINES TO FOOD SAFETY

The primary relevance of biogenic amines (BA) is that intake of foods or beverages containing high concentrations of biogenic amines can present a health hazard through the direct toxic effect of these compounds and their interaction with some medical treatments; but also they may have a role as indicators of quality and/or acceptability in some foods (Shalaby, 1996). As food safety is the main focus of this document, attention will primarily be on the former aspect. As neither the most toxic (histamine and tyramine) nor other BAs are significantly affected by normal cooking or other processing of food or beverages makes food safety assurance in respect to BA more challenging. Biogenic amines (BA) are natural antinutrition factors and are important from a hygienic point of view as they have been implicated as the

causative agents in a number of food poisoning episodes, and they are able to initiate various pharmacological reactions. Histamine, putrescine, cadaverine, tyramine, tryptamine, βphenylethylamine, spermine, and spermidine are considered to be the most important biogenic amines occurring in foods. These amines are designated as biogenic because they are formed by the action of living organisms. Histamine has been implicated as the causative agent in several outbreaks of food poisoning, while tyramine and βphenylethylamine have been proposed as the initiators of hypertensive crisis. The toxicity of biogenic amines to chicks in terms of loss of weight and mortality was also reported. The toxicity of histamine appeared to be enhanced by the presence of other amines such as cadaverine, putrescine, and tyramine. Biogenic amines may also be considered as carcinogens because of their ability to react with nitrites to form potentially carcinogenic nitrosamines (Jamilah et al., 2010).

Formation of biogenic amines can occur during food processing and storage as a result of bacterial activities. Consequently, higher amounts of certain amines may be found in foods as a consequence of the use of poor quality raw materials, microbial contamination and inappropriate conditions during food processing, and microbial contamination and inadequate conditions during storage. There is evidence that as the hygienic quality of the product decreases, the biogenic amine content increases. Therefore, a number of published studies explored possibilities of using the amine concentrations as a parameter of process hygiene and food spoilage/quality (Abu Bakar et al., 2008a).

The biogenic amine content of various foods and feed have been widely studied and found in cheese, fish and meat products, eggs and mushrooms. Food substances that have been prepared by a fermentative process, or have been exposed to microbial

contamination during aging or storage, are likely to contain amines. Alcoholic beverages such as beers can contain biogenic amines, as do some other fermented foods such as sauerkraut and soy bean products. Amines were also considered as endogenous to plant substance that is commonly used for food, where some fruits and vegetables were found to contain high concentrations of various amines (Law et al., 2011).

The biogenic amine content (particularly) of some foods has been widely studied because of their potential toxicity. Histamine has been implicated as the causative agent in outbreaks of food poisoning where intoxication results from the ingestion of foods containing excessive amounts of histamine. Although commonly associated with the consumption of scombroid-type fish, other foods such as cheese have also been associated with outbreaks of histamine poisoning (Abu Bakar et al., 2008a).

What are Biogenic Amines and How they are Formed in Food?

Biogenic amines are basic nitrogenous compounds with low molecular weight formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Halasz et al., 1994; Santos, 1996). Their chemical structure can either be aliphatic (putrescine, cadaverine, spermine, spermidine, agmatine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) (Santos, 1996). Biogenic amines are formed and degraded during the normal metabolism in the cell of animals, plants, and microorganisms. Metabolic pathway for the formulation of biogenic amines is presented on Figure 6 (Halasz et al., 1994).

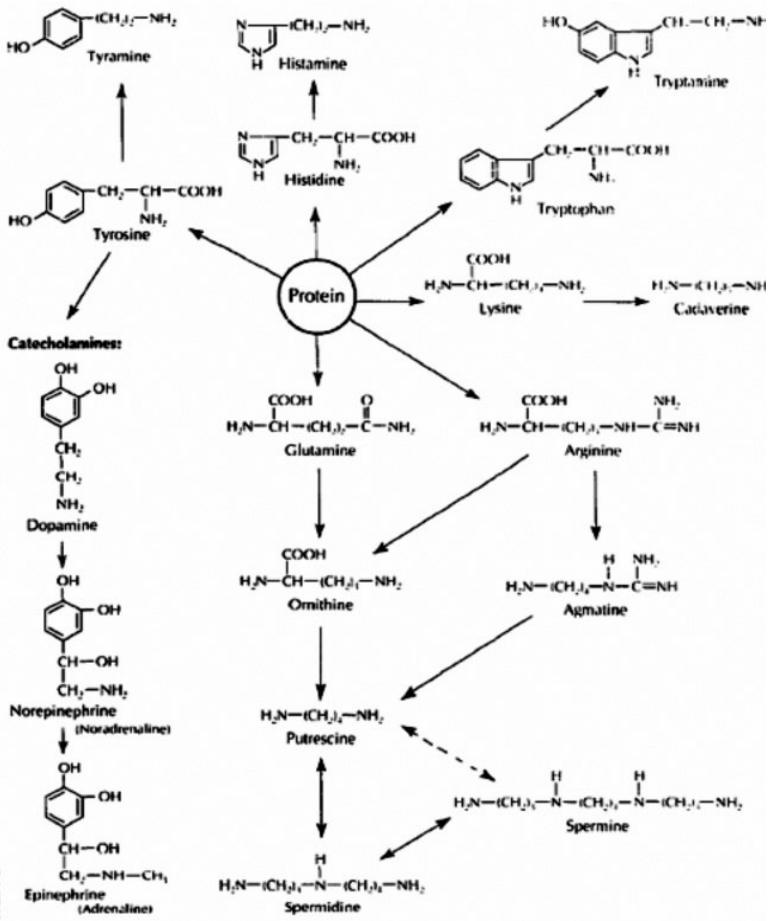


Figure 6 Metabolic pathways for the formation of biogenic amines (Halasz et al., 1994)

Biogenic amines in food are formed mainly through microbial decarboxylation of free amino acids and favourable conditions for their growth. Decarboxylation of free histidine resulting in the formation of histamine, which is the most common amine related to food poisoning incidence by biogenic amines (Abu Bakar

et al., 2008b). Tyrosine, tryptophan, lysine and phenylalanine are decarboxylated to tyramine, tryptamine, cadaverine and phenylethylamine, respectively (Halasz et al., 1994; Shalaby, 1996; Teti et al., 2002). Arginine is directly decarboxylated to agmatine in the presence of arginine decarboxylase. On the other hand, arginine is converted to ornithine by arginase, followed by the decarboxylation of ornithine to putrescine, catalyzed by ornithine decarboxylase. Putrescine can also be produced from agmatine through agmatine deamination process catalyzed by agmatine amidinohydrolase (Halasz et al., 1994; Teti et al., 2002). Spermidine and spermine are formed biochemically from putrescine by attachment of an aminopropyl moiety catalyzed by spermidine and spermine synthase (Halasz et al., 1994; Teti et al., 2002). High concentration of biogenic amines in foods and beverages can be produced by microbial decarboxylation of corresponding amino acids during food spoilage. The concentrations of biogenic amines have also been proposed to be the indices of the bacterial contamination of food (Rezaei et al., 2007).

The relationship between the production of these amines and spoilage of fish has been extensively studied (Zaman et al., 2009; Chong et al., 2011). As the amines are produced by spoilage bacteria, these amines only appear in significant amount towards the end of the shelf life of a fish, their concentrations are more likely to be of value as indices of spoilage, rather than freshness. Several biogenic amines (e.g.,histamine) are considered toxic if these exceed a certain level and consequently accused of causing poisoning (Paleologos et al. 2004). However, histamine is currently the only biogenic amine which has established maximum legal level in fish. For example, 100 mg/g is the maximum average level established in tuna and other fishes of the Scombriade and Scomberesocidae families, in the European Union (EEC 1991). More recently, the Food and Drug

Administration (FDA) has lowered the histamine defect action level from100 to 50 mg/g and has recommended the use of other biogenic amines related to fish spoilage evaluation (FDA1995). Putrescine and cadaverine may cause nitrosoamine-based carcinogenic effect if these react with nitrite (Shalaby, 1996). Although high levels of putrescine and cadaverine have been shown to potentiate histamine/ tyramine toxicity, there has been no recommendation on the level of putrescine and cadaverine. Both putrescine and cadaverine may also be proposed as indicators of spoilage. Despite the potential biological relevance of biogenic amines in freshwater fish, there are limited numbers of studies on the biogenic amines content in freshwater fishes compared with sea marine fishes (Abu Bakar et al., 2008a).

Fish muscle has the ability to support the bacterial formation of a wide variety of amine compounds which results from the direct decarboxylation of amino acids. Most spoilage bacteria possessing decarboxylase activity do so in response to acidic pH, and these bacteria may raise the pH of the growth medium through the production of these amines. The combined level of putrescine and cadaverine has been suggested to serve as an index of food acceptability as the increased concentrations of these amines correlate well with protein spoilage. Furthermore the concentrations of these amines increase considerably prior to spoilage and correlate well with the microbial load (Abu Bakar et al., 2008b)

The amount and type of biogenic amines formed in foods is strongly influenced by the intrinsic food characteristics including pH, water activity, composition, microbiota and by extrinsic parameters such as storage time and temperature, which allow bacterial growth during food processing and storage.

Impact of Biogenic Amines on Human Health

Biogenic amines are required for many physiological functions in human and animals. However, consumption of food containing high concentration of biogenic amines can lead to toxicological effects. Histamine which is most active and common amine found in seafood is also known as a mediator of allergic disorder. Histamine exerts its toxic effect by interacting with receptors (H_1, H_2 and H_3) on cellular membranes which are found in the cardiovascular system and in various secretory glands (Sellers et al*.,* 2006; Shalaby, 1996). Histamine causes dilatation of peripheral blood vessels, capillaries and arteries, resulting in hypotension, urticaria, flushing and headache. Histamine also induced contraction of intestinal smooth muscle, mediated by H_1 receptor, causes abdominal cramp, diarrhea, nausea and vomiting. Moreover, stimulation of sensory and motor neuron by histamine causes pain and itching (Lehane and Olley, 2000).

Putrescine and cadaverine can cause hypotention and potentiate toxicity of other amines, particularly histamine (Shalaby, 1996). Several biogenic amines are also precursor of carcinogenic compounds (Halasz et al*.*, 1994). Putrescine and cadaverine can be converted into pyrrolidine and piperidine, respectively, from which carcinogenic nitrosopyrrolidine and nitrosopiperidine are formed by heating (Shalaby, 1996).

Nitrosamines from polyamines may not necessarily pose a health risk as toxicity is reached only after consumption of large amounts, more than expected in a daily meal. Tyramine, 2- phenylethylamine, and putrescine are versoactive amines and increase blood pressure that can lead to heart failure or brain hemorrhage (Abu Bakar et al., 2008a).

Histamine poisoning (scombroid poisoning) is a worldwide problem that occurs after the consumption of food containing

biogenic amines, particularly histamine at concentrations higher than 500 ppm (Abu Bakar et al., 2008b). Histamine poisoning manifests itself as an allergen-type reaction characterized by difficulty in breathing, itching, rash, vomiting, fever, and hypertension. People having deficient natural mechanisms for detoxifying biogenic amines through genetic reasons or through inhibition due to the intake of antidepression medicines, such as monoamine oxidase inhibitors (MAOIs) are more susceptible to histamine poisoning (Zaman et al., 2009).

Occurrence of Biogenic Amines in Seafood

Fish and fish products have paid greatest attention regarding high concentration of biogenic amines. The so called "scombroid poisoning" are typical phenomena due to the consumption of fish from family of *Scombridae* such as tuna (*Thunnus* spp.) and mackerel (*Scomber japonicus*) containing high level of histamine. However, non-scombroid fish species are also implicated in histamine poisoning incidence worldwide such as sardines (*Sardinella* spp.), anchovies (*Engraulis* spp.), mahi-mahi (*Coryphaena* spp.), pilchards (*Sardina pilchardus*), bluefish (*Pomatomus* spp.), swordfish (*Xiphias gladius*) and garfish (*Belone belone*). Tao et al. (2001) had conducted a survey on the occurrence of histamine in seafood including tuna, mackerel, mahi-mahi, sardine, herring, fish sauce and dried fish sold in market of nine countries. Histamine was found in 35 of 159 fish samples tested with a detection rate of about 21%. Nine percent of fish sample contain histamine more than 50 ppm with 5 samples exceeding 500 ppm of which 2 were above 1000 ppm.

Figure 7 Various seafood products potentially containing biogenic amines

Fresh fish normally contain low amount of histamine or other amines, but the concentrations progressively increase if it is highly temperature abused (more than 20 °C). Histamine content in pacific mackerel reach 2830 mg/kg after stored at 25 °C for 48 h (Kim et al., 2001). After stored at 25 °C for 24 h, histamine content (mg/kg) reaches 2240 in sailfish, 3390 in milkfish (Tsai et al., 2005), 1465 in anchovies, 1106 in pilchard (Visciano et al., 2007), 2124 in mackerel, 1776 in saury and 189 in Spanish mackerel (Kim et al., 2009). Skipjack tuna contain 1533 mg/kg histamine after storage at 21 °C for 48 h (Rossi et al., 2002). However, it is observed that histamine formation is slower in low temperature abuse (less than 7 °C). Ozogul et al. (2002) reported that histamine concentration in herring was 271 mg/kg after storage at 0 °C for 16 days. Histamine content in sardine was 203 mg/kg after storage at 4 °C for 15 days (Ozogul and Ozogul, 2006). Freshwater fish also contain considerable amount of biogenic amines. The concentration of

amines is increased during storage of fish. Initial histamine content in catfish (*Mystus nemurus*) of around 30.1 ppm may increase to more than 150 ppm after storage at ambient temperature for 24 h or at ice temperature for 20 days (Widjaja et al., 2011).

Biogenic amines content are also abundant in processed or fermented fish products. Histamine content can reach 1220 ppm in Taiwan fish sauce (Tsai et al., 2006) and 1380 ppm in Korean anchovy sauce (Sanceda et al., 1996). Putrescine and cadaverine are also dominant amines in some commercial fish sauce and might reach up to 1257 ppm and 1429 ppm, respectively (Stute et al., 2002). Histamine content in 549 commercial fish sauce samples in Thailand was observed within the range 0f 200-600 ppm (Brillantes and Samosorn, 2001). While fish sauce samples consumed in Malaysia contain around 62.5-393.3 ppm of histamine, 5.6-242.8 ppm of putrescine and 187.1-704.7 ppm of cadaverine (Zaman et al., 2010). The quality of raw materials and hygienic condition during fermentation are thought to be most important factors in determining biogenic amines content in fish sauce products (Zaman et al., 2009). Naila et al. (2011) reported that the highest histamine content among twenty eight samples of *Rihaakuru* (fish paste of Maldives) was detected at 5487 mg/kg. Samples of Taiwan fermented fish products namely fish sauce, fish paste and shrimp paste contain highest histamine concentration of 394, 263 and 382 mg/kg, respectively (Tsai et al., 2006). The authors also revealed that 7.4% of tested samples contain more than 1000 mg/kg of histamine.

Regulation of Biogenic Amines Content in Food

Many countries have different regulation on acceptable level of biogenic amines in foods. As regulated in manufacturing standard 1992 of United Kingdom, acceptable content of histamine in foods is 100 ppm. For control of histamine in fish belonging to the

Scombridae and Clupeidae families, as stated by Luten et al. (1992) and Lehane and Olley (2000), European Union has established the regulation that nine independent samples from each batch should correspond to:

1. An average of histamine concentration lower than 100 ppm.
2. No more than two samples out of the nine with the concentration of histamine between 100 and 200 ppm.
3. No sample with histamine content higher than 200 ppm.

Australia New Zealand Food Authority (ANZFA) regulated that histamine content must not exceed 200 mg/kg in a composite sample of fish and fish products, other than crustaceans and mollusks (Lehane and Olley, 2000). The US Food and Drug Administration set value of 500 ppm histamine as the toxicity level and 50 ppm as the defect action level (Lehane and Olley, 2000). The Canadian Fish Inspection Agency set up the maximum limit for histamine content in fish sauce at 200 ppm (Brillantes and Samosorn, 2001). Brink et al. (1990) reported a legal upper limit for histamine in food (100 ppm) and alcoholic beverages (30 ppm). Furthermore, level of 100-800 ppm for tyramine and 30 ppm for phenylethylamine are considered potentially hazardous to human health (Brink et al., 1990; Halasz et al., 1994). However, the exact toxicity threshold of biogenic amines is not precisely determined because it depending on many factors such as individual acceptability and the presence of other components as toxicity potentiators (Brink et al., 1990; Halasz et al., 1994; Lehane and Olley, 2000; Santos, 1996). Furthermore, the threshold levels for other biogenic amines are not yet established.

The determination of biogenic amines is important not only from the view point of their toxicity, but also because they can be used as spoilage indicators of food, in particular fish, meat and their products (Brink et al., 1990; Vinci and Antonelli, 2002).

The concentration of histamine, putrescine and cadaverine are generally increased during spoilage of food, whereas concentration of spermine and spermidine are decreased during this process. The relationship between these amines was defined as Biogenic Amine Index (BAI):

$$BAI = \frac{\text{histamine} + \text{putrescine} + \text{cadaverine}}{1 + \text{spermine} + \text{spermidine}}$$

The concentrations of amines are expressed as ppm. Fish or meat with BAI value below 1 is considered as the first quality, whereas BAI values above 10 indicate a very poor microbiological quality. The index score based on the formula above compared favorably to organoleptic values score (Brink et al., 1990). Positive correlation between these two scores have been reported both for fish (Ozogul and Ozogul, 2006; Paleologos et al., 2004) and meat (Vinci and Antonelli, 2002).

Factors Influencing Biogenic Amines Formation In Seafood

Biogenic amines formation in food products is either a result of endogenous amino acids decarboxylase activity in raw food material or the growth of decarboxylase positive microorganisms under conditions favorable for enzyme activity (Brink et al., 1990; Halasz et al., 1994; Shalaby, 1996). Therefore, prerequisites for the formation of biogenic amines in foods including:

1. The availability of free amino acids as precursor of amines formation.
2. The presence of microorganisms which possess decarboxylase activities.

3. Environmental condition enabling growth of microorganism's growth and their enzymatic activities.

Biogenic amines formation was also possibly altered by the presence of proteolytic enzymes as they play an important role in the release of free amino acids from protein tissues which offer the substrates for decarboxylation activity. Many factors such as microorganisms, pH, temperature, salt content and oxygen level are considered to contribute significant effect on biogenic amines formation.

Microorganisms

Biogenic amines profile in seafood is characterized by different type of microorganisms present in the product because they exhibit different decarboxylase activities. *Enterobacteriaceae* are generally recognized as dominant biogenic amines producers in fish and its products, particularly histamine, putrescine and cadaverine. *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* have been isolated from fish incriminated in scombroid poisoning (Halasz et al., 1994). Histamine forming enzyme was found in *Proteus mitabilis* (Yosinaga and Frank, 1982), *Klebsiella oxytoca* (Hernandez-Herrero et al., 1999), *Enterobacter cloacae*, *Pantoea* sp., *Pantoea agglomerans* (Tsai *et al.*, 2005) and *Morganella psychrotolerans* sp. nov. (Emborg et al., 2006). *Enterobacter cloacae* and *Pantoea agglomerans* are producer of putrescine and cadaverine (Tsai et al., 2005). *Paenibacillus tyramigenes* isolated from salted and fermented anchovy was a strong tyramine producer (Mah et al., 2008). *Pantoea* sp and *Pantoea agglomerans* can produce spermine and spermidine in low amount (Tsai et al., 2005). *Bacillus licheniformis* isolated from *Myeolchi-jeot*, a Korean salted and fermented anchovy is reported as spemidine producer (Mah and Hwang, 2009). *Photobacterium posporeum* isolated from garfish

is found to produce phenylethylamine and agmatine (Dalgaard et al., 2006). Table 1 shows many bacteria which attributed to the formation of biogenic amines in fish products.

Table 1 Bacteria responsible for biogenic amines formation in fish products

Biogenic amines	Microorganisms	Reference
Histamine	*Tetragenococcus muriaticus*	(Kimura et al., 2001)
	Tetragenococcus halophilus	(Satomi et al., 2008)
	Enterobacter cloacae, Pantoea sp., *Pantoea agglomerans*	(Tsai et al., 2005)
	Bacillus coagulans, Bacillus megaterium	(Tsai et al., 2006)
	Morganella morganii, Klebsiella pneumoniae, Hafnia alvei	(Halasz et al., 1994)
	Morganella psychrotolerans	(Emborg et al., 2006)
	Lactobacillus sp., *Lactobacillus sakei, Leuconostoc mesenteroides*	(Dapkevicius et al., 2000)
	Staphylococcus epidermidis, Staphylococcus capitis	(Hernandez-Herrero et al., 1999)
	Photobacterium phosphoreum	(Dalgaard et al., 2006)
Putrescine	*Enterobacter cloacae, Pantoea agglomerans*	(Tsai et al., 2005)
	Bacillus megaterium	(Tsai et al., 2006)
	Photobacterium phosphoreum	(Dalgaard et al., 2006)
	Lactobacillus hilgardii	(Alberto et al., 2007)
	Bacillus licheniformis	(Mah et al., 2003)

Cadaverine	*Enterobacter cloacae, Pantoea agglomerans*	(Tsai et al., 2005)
	Bacillus megaterium	(Tsai et al., 2006)
	Photobacterium phosphoreum	(Dalgaard et al., 2006)
	Bacillus licheniformis	(Mah et al., 2003)
Tyramine	*Paenibacillus tyraminegenes*	(Mah et al., 2008)
	Lactobacillus brevis	(Coton and Coton, 2009)
	Photobacterium phosphoreum	(Dalgaard et al., 2006)
	Enterococcus faecium	(Capozzi et al., 2011)
Spermine	*Pantoaea* sp., *Pantoea agglomerans*	(Tsai et al., 2005)
Spermidine	*Pantoaea* sp., *Pantoea agglomerans*	(Tsai et al., 2005)
	Bacillus coagulans	(Tsai et al., 2006)
Phenylethylamine	*Photobacterium phosphoreum*	(Dalgaard et al., 2006)
Agmatine	*Photobacterium phosphoreum*	(Dalgaard et al., 2006)

Amino acid decarboxylase activity is also found in some species belonging to genera of *Micrococcus* and *Staphylococcus*. *Staphylococcus epidermidis* and *Staphylococcus capitis* are strong histamine producers from salted anchovy (Hernandez-Herrero et al., 1999). *Staphylococcus carnosus* isolated from salted mullet roe products was also found to have histamine forming enzyme (Feng Kung *et al.*, 2008). *Micrococcus luteus* was reported as histamine former during salting of sardine (Lakshmanan et al., 2002a).

Lactic acid bacteria are generally recognized as non toxinogenic, although some species isolated from fish and its products can produce biogenic amines. Histidine decarboxylase enzyme was observed in halophilic *Tetragenococcus muriaticus*

and *Tetragenococcus halophilus* isolated from fish sauce (Kimura et al., 2001; Satomi et al., 2008). Some strains of *Lactobacillus* sp., *Lactobacillus sakei* and *Leuconostoc mesenteroides* isolated from fish silage also exhibit ability to produce histamine (Dapkevicius *et al.*, 2000).

Biogenic amines forming enzyme are widely distributed in many bacterial species. Histamine forming bacteria belong to the genus of *Bacillus* isolated from fish including *Bacillus coagulans*, *Bacillus megaterium* and *Bacillus pumilus* (Hernandez-Herrero et al., 1999; Tsai et al., 2006). *Bacillus megaterium* can also produce putrescine and cadaverine, while *Bacillus coagulans* can produce spermidine (Tsai et al., 2006). Some *Pseudomonas* species isolated from fish sauce and fish paste were found to decarboxylate histidine. Another histamine forming bacteria are including *Pseudomonas cepaceae* (Hernandez-Herrero et al., 1999), *Clostridium perfringens* and *Vibrio alginolyticus* (Dalgaard et al., 2006; Yosinaga and Frank, 1982). *Photobacterium phosphoreum* was found to be a producer of many amines including histamine, putrescine, cadaverine, tyramine, phenylthylamine and agmatine (Dalgaard et al., 2006).

pH

Biogenic amines formation by bacteria has been considered as a physiological mechanism to counteract acid environment (Gardini et al., 2001; Halasz et al., 1994). Hence, activities of bacterial amino acids decarboxylase are generally optimum in low pH (Gardini et al., 2001; Kimura et al., 2001; Masson et al., 1997). Kimura et al. (2001) stated that *Tetragenococcus muriaticus* produce more histamine at pH 5.2 (668.6 ppm) than at pH 7.1 (15.8 ppm). In a model system, *Carnobacterium divergens* produce high level of tyramine when the initial pH is less than 5.0 (Masson et al., 1997). The highest formation of spermidine by *Oenococcus oeni* T65 in

vitro was also detected at pH 3, however, tyramine formation by this strain was reduced with the decrease of pH (Gardini et al., 2005).

Temperature

Formation of biogenic amines in fish is generally increased with the increase of temperature and exposure time (Emborg et al., 2005; Halasz et al., 1994; Paleologos et al., 2004). *Enterobacter cloacae* can produce putrescine at 20 °C but not at 10 °C. Cadaverine formation by *Klebsiella pneumonia* was also detected more extensive at 20 °C than at 10 °C (Halasz et al., 1994). Histamine formation is optimum at 37.8 °C and is very dependent upon microbial activity (Shalaby, 1996). Furthermore, higher temperature might favor proteolytic activity which provides substrate for decarboxylase enzyme resulting in increment of biogenic amine level. However, psychrotolerant *Photobacterium phosphoreum* and *Morganella morganii* can produce high concentration of histamine in chilled tuna and garfish (Dalgaard et al., 2006; Emborg et al., 2005). Formation of histamine, putrescine and cadaverine in rainbow trout was also detected in high rate during ice storage (Rezaei et al., 2007). During the ice storage of fish and shrimp, amine forming bacteria including genera of *Photobacterium*, *Aeromonas* and *Micrococcus* could survive and proliferate rapidly between 9 and 12 days and contribute to the amine formation (Lakshmanan et al., 2002b). Abu Bakar et al. (2008a) also revealed that formation of histamine, putrescine and cadaverine in freshwater shrimp (*Macrobrachium rosenbergii*) were delayed during storage at lower temperature. Icing temperature was effective to delay formation of those three amines in freshwater shrimp to a level below 100 mg/g after storage for 16 days (Figure 8).

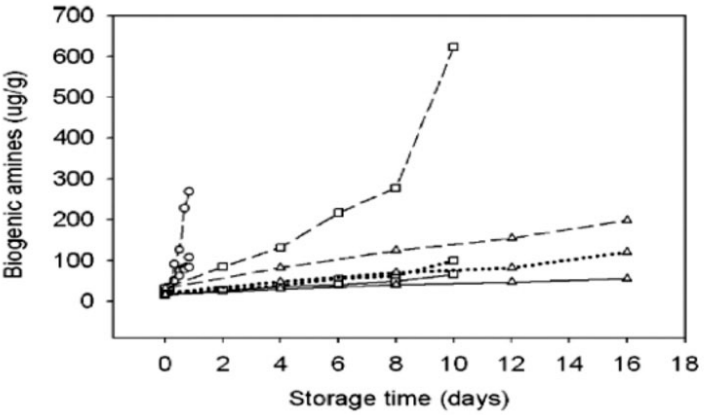
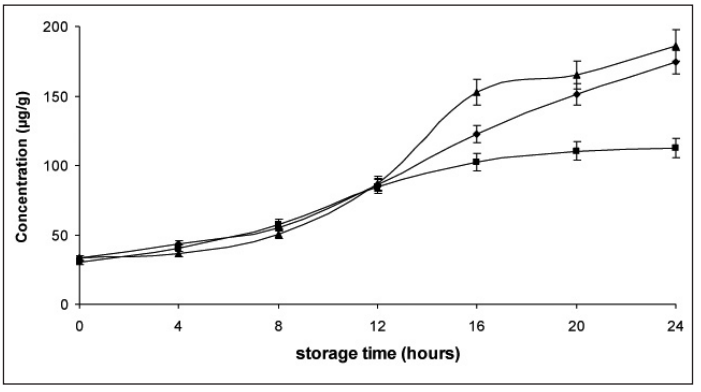
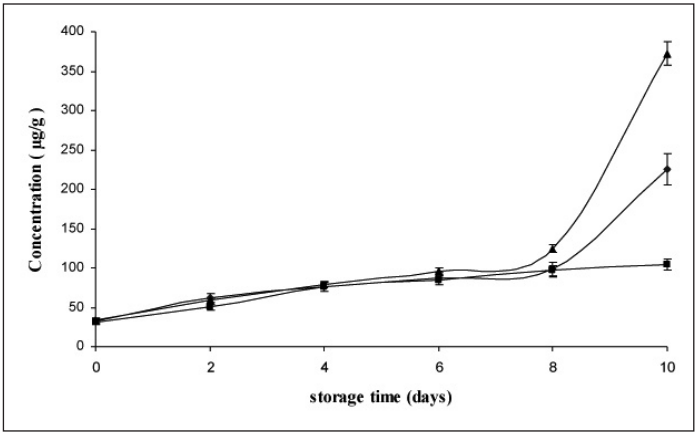


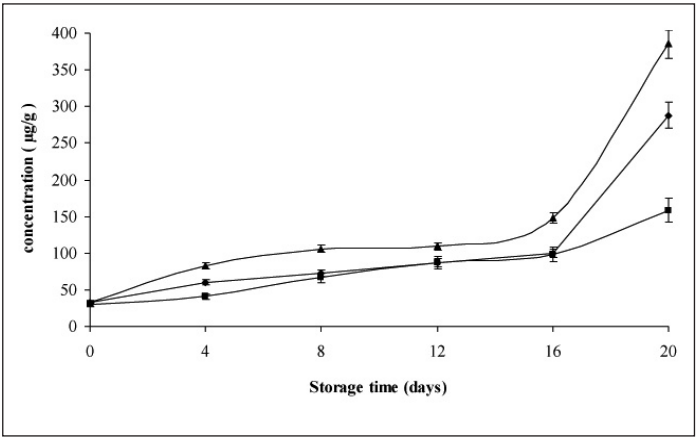
Figure 8 Biogenic amines profile of *M. rosenbergii* during storage. (···o···) putrescine at ambient storage; (—o—) cadaverine at ambient storage; (---o---) histamine at ambient storage; (···□···) putrescine at 10° C storage; (—□—) cadaverine at 10° C storage; (---□---) histamine at 10° C storage; (···Δ···) putrescine at icing storage; (—Δ—) cadaverine at icing storage; (···Δ···) histamine at icing storage



(a)



(b)



(c)

Figure 9 Change in biogenic amine histamine (♦), putrescine (■), and cadaverine (▲) of *Mystus nemurus* during storage at ambient temperature (28±2 °C) (a), chilled temperature (10±2 °C) (b) and iced temperature (2±1 °C) (c). Error bars indicate the standard deviation of three replications.

Widjaja et al. (2011) studied effect of temperature on biogenic amines formation during storage of river catfish (*Mystus nemurus*) which is locally known as 'ikan baung' in Malaysia. They found that formation of biogenic amines in catfish is faster in higher temperature. Histamine concentration is increased sharply exceeding the level of 100 ppm after stored for 12 hours at ambient, 8 days at chilled and 16 days at iced temperature (Figure 9). Putrescine levels increased and exceeded 100 mg/g after 16 hours at ambient, 8 days at chilled and 16 days at iced temperature.

Sodium Chloride Concentration

Sodium chloride has a significant influence on bacterial growth and therefore influences their amino acids decarboxylase activity. The rate of histamine production by *Tetragenococcus muriaticus* was considerably reduced when salt concentration increased from 5-20% (Kimura et al., 2001). Histidine decarboxylase activity of *Staphylococcus capitis*, *Enterobacter cloacae* and *Pantoea agglomerans* were also retarded by the high concentration of salt (Feng Kung et al., 2006; Tsai et al., 2005). This phenomenon can be attributed to reduced cell yield obtained in the presence of high sodium chloride concentration and to a progressive disturb of the membrane located microbial decarboxylase enzymes (Suzzi and Gardini, 2003). Zaman et al. (2010) had observed that fish sauce with low salt level containing higher histamine concentration than fish sauce with high salt level. On the contrary, Hernandez-Herrero et al. (1999) reported that sodium chloride enhanced activity of histidine decarboxylase of halotolerant *Stappylococcus* spp. isolated from salted anchovies. Hence, it can be assumed that the influence of sodium chloride in either inhibiting or stimulating biogenic amines production is strains specific.

Oxygen

Oxygen appears to have a marked effect on the formation of amines which is dependent on the producing species. *Enterobacter cloacae* produce about twice putrescine quantity in aerobic compared to anaerob condition, while *Klebsiella pneumonia* produce considerably less cadaverine but acquired the ability to produce putrescine under anaerobic condition (Halasz et al., 1994). Modified Atmosphere Packaging (MAP) with 40 CO_2 /60% O_2 can reduce histamine formation by *Photobacterium phosphoreum* during chilled storage of tuna for 28 days (Emborg et al., 2005). MAP with higher concentration of CO_2 also reduced histamine and cadaverine accumulation in Indian mackerel (Chong, 2012). Biogenic amines content of sardine were highest in sardine stored in air, followed by vacuum packaging and modified atmosphere packaging (Ozogul and Ozogul, 2006). In contrast, production of histamine by *Tetragenococcus muriaticus* was higher in oxygen limiting than aerobic condition although the growth rate was similar under both condition (Kimura et al., 2001).

Other Factors

The presence of fermentable carbohydrate such as glucose can increase growth and amino acids decarboxylase of bacteria (Halasz et al., 1994). *Tetragenococcus muriaticus* produced histamine for only 82.1 ppm in a medium without glucose, but addition of 1-3% (w/v) glucose in the medium significantly increase histamine level (410.6 -773.0 ppm) (Kimura et al., 2001). Halasz et al. (1994) reviewed that optimum glucose concentration for biogenic amines formation was in the range of 0.5-2.0% (w/v), while level in excess of 3% inhibited decarboxylase activity. Furthermore, the presence of arabinose was also influences the formation of tyramine and

spermidine by *Oenococcus oeni*, the values that able to maximize the production of tyramine could minimize spermidine production. The formation of biogenic amines by *Oenococcus oeni* was influenced by the presence of ethanol in the medium. The highest production of tyramine was obtained in the presence of 8% ethanol, whereas spermidine at 12% ethanol level. Moreover, the presence of SO_2 and pyridoxal 5-phosphate also influence production of amines by this bacteria (Gardini et al., 2005). Repression of histidine decarboxylase activity has been known when the amount of histamine is accumulated in the medium (Alberto et al., 2007; Halasz et al., 1994).

Several phenolic compounds may also influence the formation of biogenic amines. Putrescine formation from agmatine by *Lactobacillus hilgardii* X_1B was diminished in the presence of protocatechuic, vanillic and caffeic acids and the flavonoid catechin and rutin (Alberto et al., 2007). This indicated that beside their inherent beneficial properties to human health, phenolic compounds seem to be potential to diminish biogenic amines formation. Cinnamic aldehyde (a component of cinnamon) and eugenol (a compound of clove) are effectively inhibiting the formation of histamine, putrescine and cadaverine by *Enterobacter aerogenes* (Wendakoon and Morihiko, 1995). Histamine formation by *Klebsiella pneumonia* and *Morganella morganii* are inhibited by potassium sorbate at 0.5% (Shalaby, 1996).

CURRENT AND EMERGING APPROACHES IN CONTROLLING BIOGENIC AMINES CONTENT IN FOODS

Application of Food Additives or Preservatives

Food additives and preservatives had been used to inhibit accumulation of biogenic amines in fish and its products such as mackerel (Shakila et al., 1996), carp roe (Krízek et al., 2011), and fermented anchovies (Mah and Hwang, 2009; Mah *et al.*, 2009). Shakila et al. (1996) reported that cinnamon and clove greatly inhibit histidine decarboxylase activity of *Morganella morganii* and concentration of 3% is quite effective to control histamine content in mackerel. The use of 5% (weight basis) of garlic extract can reduce the overall formation of biogenic amines by up to 8.7% during ripening of Myeolchi-jeot, a Korean salted and fermented anchovy (Mah et al., 2009). Formation of histamine, putrescine and cadaverine by *Bacillus licheniformis*, a strong amines producer from *Myeolchi-jeot* are significantly inhibited by glycine (Mah and Hwang, 2009). The authors revealed that addition of glycine (5% w/w) could reduced up to 63% overall biogenic amines formation during ripening of *Myeolchi-jeot*, as compared to control. Kang and Park (1984) reported that inhibition of histamine formation and histidine decarboxylase activity in mackerel stored at 25 °C for 25 days could be achieved by the addition of accidulants such as citric acid, malic acid and succinic acid as well as D-sorbitol. Abu Bakar et al., (2008a) had observed the effect of several preservatives including sucrose, sodium chloride, sodium metabisulphite, lactic acid and boric acids on biogenic amines level of freshwater prawn (*Macrobrachium renbergii*) during storage at icing condition for 25 days. The authors revealed that histamine was not detected in any of preservatives treated samples except for controls. In addition, cadaverine was also not detected in prawn body region samples

treated with sodium metabisulphite, boric acid and lactic acid throughout storage.

The use of food additives might incur adverse effects to certain food products. Bover-Cid et al. (2001) investigated the effect of sodium sulphite on biogenic amines accumulation during the ripening of slightly fermented sausages. They revealed that although sausage with sulphite showed lower microbial counts, only cadaverine production was inhibited, resulted in a lower level as compared to sausage without sulphite (control). In contrast, considerably higher level of tyramine and putrescine were observed in the treated sausage and their formation seems to be stimulated by the presence of sodium sulphite (Bover-Cid et al., 2001). Addition of sodium sorbate and sodium hexametaphosphate resulted in the formation of putrefactive odor in sardine stored for 2 days at chill temperature (Kang and Park, 1984). Other drawbacks of additives and preservatives used for controlling biogenic amines in foods are uncertain mechanisms of the inhibition and the lack of consumer acceptance. Component of spices such as capsaicin (red pepper) and thymol (thyme and oregano) have unpleasant pungent flavor which may not be accepted as food additives by consumers (Lee et al., 2008; Someya et al., 2003).

Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) combined with refrigeration has become a common packaging method in extending the shelf life of fish and its products (Del Nobile et al., 2009; Fagan et al., 2004; Genigeorgis, 1985; Torrieri et al., 2006). MAP is also a preferred method to control the formation of biogenic amines in fish products. Application of MAP is proven to be effective in controlling biogenic amines content during the storage of Atlantic herring (Ozogul et

al., 2002), tuna (Emborg et al., 2005), sardine (Ozogul and Ozogul, 2006) and garfish (Dalgaard et al., 2006).

Chong et al., (2012) had conducted a study to evaluate the effect of MAP on biogenic amines formation during chilled storage (5°C) of Indian mackerel. The beheaded and gutted samples were subjected to different conditions including air packaging as control (C), vacuum-packaging (VP) and modified atmosphere packaging (MAP) of CO_2:N_2:O_2 with ratio (in percentage) 30:65:5 (M30C), 60:35:5 (M60C), 80:15:5 (M80C) and 100:0:0 (M100C). Samples were then stored at chilled storage for 12 days. After storage, In general, MAP is effective in inhibiting biogenic amines concentration in Indian mackerel after chilled storage for 12 days. At the end of storage, histamine concentration was less 6.4%, 8.5%, 70.3%, 78.8% and 90.2% in samples treated with VP, M30C, M60C, M80C and M100C, respectively as compared to control samples that reached 430 ppm (Figure 10a). MAP exerts much greater effect in reducing putrescine content in mackerel. After 120 days of chill storage, putrescine concentration was less 71%, 79%, 81%, 90% and 94% in samples treated with VP, M30C, M60C, M80C and M100C, respectively as compared to control samples that reached 169 ppm (Figure 10b).

MAP also influences the accumulation of other amines content in different pattern. Although concentration of cadaverine is not significantly different among VP and MAP (all ratios) treated samples, the value was significantly lower than that in control samples that reached 182 ppm (Figure 10c). The effective way of MAP in inhibiting tyramine formation can only be achieved if CO2 concentration is above 60% (Figure 11a). Vacuum-packaging is not giving any inhibition effect on tyramine formation. At the end of storage, mackerel treated with M60C, M80C and M100C demonstrated lower tyramine levels of 30.54 ppm, 21.77 ppm

and 16.73 ppm, respectively. While, tyramine concentration in other treated samples are more 130 ppm. Chong et al., (2012) also observed the effect of MAP on spermine and spermidine in the samples. Spermidine content was lower in samples treated with VP and M30C than control samples (Figure 11b). Stronger inhibition of spermidine formation was observed in samples treated with CO2 at above 60%. Nevertheless, neither of VP nor MAP treatments were exhibited inhibition effect of spermine (Figure 11c). In addition to inhibit biogenic amines formation, MAP also effective to prolong the shelf life of Indian mackerel stored at chill temperature and samples treated with that method shows better appearance compared to other samples (Figure 12).

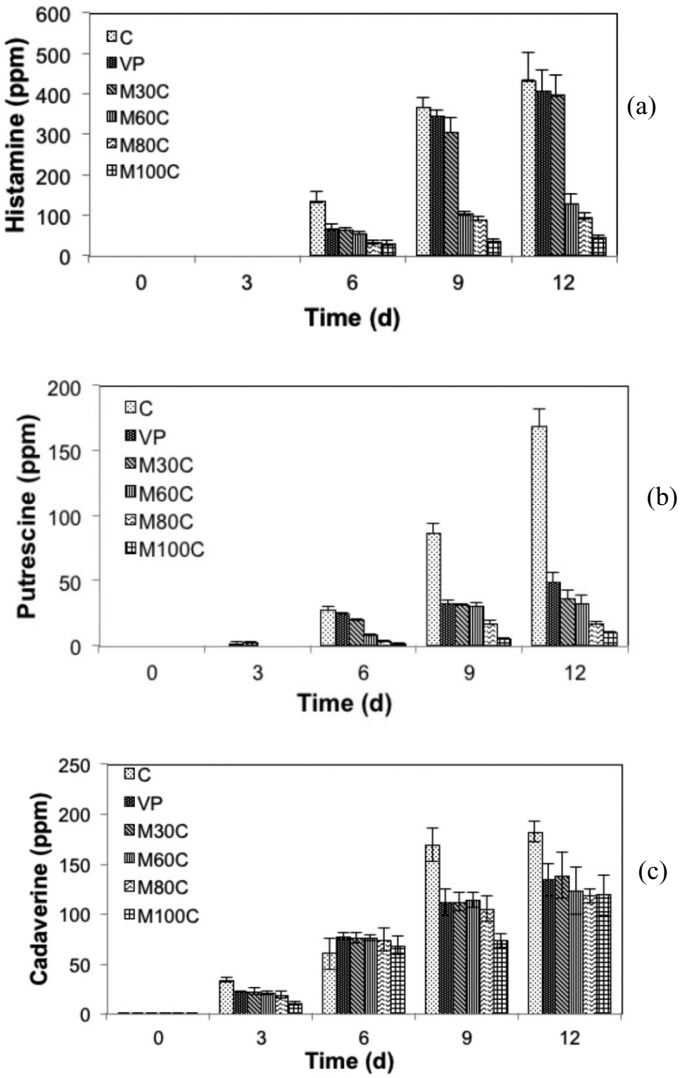


Figure 10 Changes in histamine (a), putrescine (b) and cadaverine (c) levels in Indian mackerel packed with air (C), vacuum packaging (VP) and modified atmosphere packaging (MAP) throughout 12 d of chilling storage (5°C)

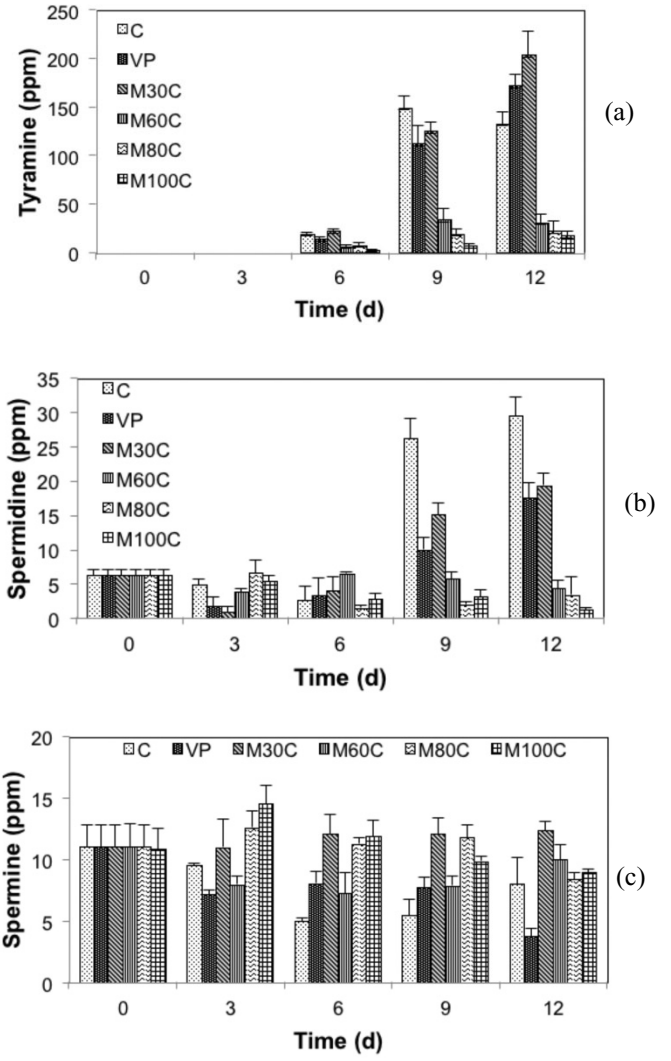


Figure 11 Changes in tyramine (a), spermidine (b) and spermine (c) levels in Indian mackerel packed with air (C), vacuum packaging (VP) and modified atmosphere packaging (MAP) throughout 12 d of chilling storage (5°C)

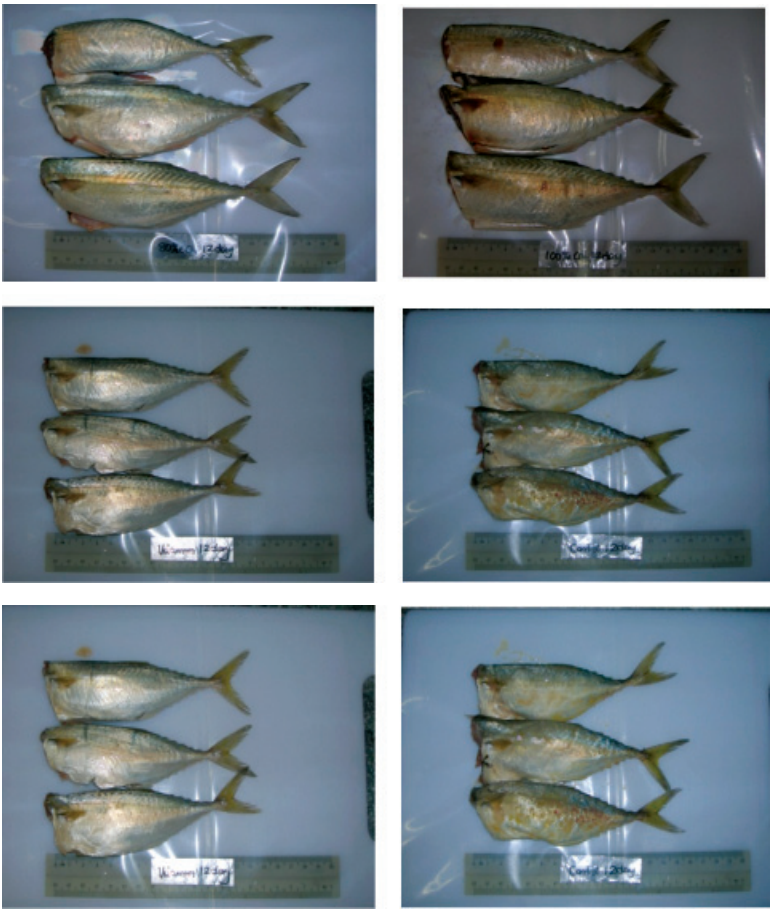


Figure 12 Appearance of beheaded and gutted Indian mackerel treated with vacuum packaging and modified packaging after stored at 5°C for 12 days

Inhibition of histamine formation was achieved when MAP with a gas composition of 40% CO_2/60% O_2 was applied to tuna stored for 28 days at 2 °C (Velu et al., 2013; Emborg et al., 2005). Dalgaard et al. (2006) also reported that MAP with a gas composition of 40% CO_2/60% N_2) exhibits a synergistic effect with

freezing and thawing in controlling histamine formation in garfish. When the garfish was frozen, thawed and stored at 5 °C, the shelf life was 70% longer under the MAP gas mixture than storage in air. The histamine formation was also reduced with that particular condition (Velu et al., 2013). The authors stated that the reduction of histamine in MAP treated samples was due to the growth inhibition of *Photobacterium phosphoreum* and *Morganella morganii*, which are identified as strong histamine producers. Active packaging using oxygen scavengers are also used to reduce biogenic amines formation in seer fish (Mohan et al., 2009). Mohan et al. (2009) found that biogenic amines formation in seer fish steak increase with the presence of oxygen, but biogenic amines in the fish were lower when oxygen (99%) was removed by O_2 scavenger. The shelf life of seer fish was also extended from 12 (in air) to 20 days. Many biogenic amines producing enzymes are more active under aerobic condition, although some of them are capable to decarboxylaze amino acids in the absence of oxygen (Halasz et al., 1994). Several anaerobic bacteria are known as biogenic amines producers. Thus, the effectiveness of MAP in inhibiting biogenic amines formation is principally dependent on the type of microbial flora and their environmental condition such as temperature and the composition of gas used in the packaging.

Non-producing Amines Starter Culture

Biogenic amines content in fermented foods is usually higher than other foods since most of starter cultures used for fermentation possess amino acid decarboxylase. Thus, the use of non-producing amines starter cultures is thought to be effective to inhibit or delay the formation of biogenic amines in fermented foods. This technique has been applied in the fermentation of meat sausage (Bover-Cid et al., 1999; Gençcelep et al., 2007; Komprda et al., 2001; Lu et al.,

2010; Sara et al., 2000), fish sausage (Hu et al., 2007), sauerkraut (Kalac et al., 2000; Spicka et al., 2002) and cheese (Fernandes-Garcia et al., 2000; Nieto-Arribas et al., 2009). Sara et al. (2000) found that sausage treated with *Lactobacillus sakei* CTC494 (a non decarboxylating strain) as starter culture exhibit remarkably lower tyramine content as compared to the control. The culture also notably limits the formation of putrescine and cadaverine. Formation of tyramine, putrescine and cadaverine in sauerkraut can also be suppressed by the inoculation of non producing amines *Lactobacillus plantarum* during fermentation (Kalac et al., 2000). Histamine degrading *Staphylococcus carnosus* FS19 was also found as non histamine, putrescine and cadaverine producers (Zaman et al., 2010).

Non-producing amines bacteria were also applied as combined cultures and their synergistic effect to inhibit amines formation was observed. Genccelep et al. (2007) investigated the effect of starter culture A (*Lactobacillus sakei* and *Staphylococcus carnosus*) and B (*Pediococcus acidilactici*, *Staphylococcus xylosus* and *Lactobacillus curvatus*) on biogenic amines formation in *sucuk*, Turkish dry fermented sausage. They found that *sucuk* produced through fermentation with either starter culture A or B has significantly lower histamine, putrescine and cadaverine and tyramine content as compared to the natural fermentation (without starter culture). Various combination of *Staphylococcus xylosus* with some lactic acid bacteria include *Lactobacillus plantarum*, *Lactobacillus casei* subsp. casei and *Pediococcus acidilactici* were also found to suppressed accumulation of histamine, putrescine, cadaverine, tyramine and tryptamine content in silver carp sausages (Hu et al., 2007). The use of bacteriocin producing bacteria can also decrease biogenic amines content in foods. Histamine was not found in cheese with bacteriocin producing starter compared with

200 ppm in control cheese (without bacteriocin) after 4 months of ripening (Joosten and Nunez, 1996). Moreover, Halasz et al. (1994) reported that inoculation of *Lactobacillus curvatus* as a starter culture in cabbage at the range of 5×10^6 to 2×10^7 cfu/g resulted in notably lower concentration of tyramine as compared to natural fermentation of sauerkraut.

Application of negative amines producer starter culture may be effective only when optimal growth condition of cultures are achieved, and thus they can dominate over biogenic amines producing and contaminant bacteria (Hu et al., 2007; Lu et al., 2010; Xu et al., 2010). Lu et al. (2010) reported that mixed starter culture *Lactobacillus farciminis* and *Staphylococcus saprophyticus* were dominate microbial flora and inhibited indigenous biogenic amines producing bacteria during sausage fermentation, and thus reduced the level of histamine, putrescine, cadaverine, phenylethylamine, and tyramine. The use of starter cultures decreased the pH quickly; enable the inhibiting effect to the growth of amines producing bacteria (Hu et al., 2007).

Application of Biogenic Amines Degrading Bacteria

Biogenic Amines Degrading Bacteria

Biogenic amines can be degraded through the oxidative deamination process catalyzed by amine oxidases with production of aldehydes, ammonia and hydrogen peroxide. A typical reaction of amines degradation (Ishizuka et al., 1993; Murooka et al., 1979; Yamashita et al., 1993) is following to the equivalence below:

$$R\text{-}CH_2\text{-}NH\text{-}R' + O_2 + H_2O \rightarrow R\text{-}CHO + H_2N\text{-}R' + H_2O_2$$

The formed aldehydes are then oxidized to form corresponding acid, catalyzed by aldehyde dehydrogenase (Teti et al., 2002). Medina et al. (2003) revealed that based on the cofactor attached,

amine oxidases can be differentiated into two main groups. One group has flavin adenine dinucleotide (FAD) and includes both monoamine oxidase (MAO), and polyamine oxidase (PAO). The other group has one or more carbonyl groups, which appears to be topaquinone (TPQ), usually called copper containing semicarbazide sensitive amine oxidases (SSAO) and include diamine oxidases (DAO), cell surface and soluble SSAO and extracellular lysyloxidase (Medina et al., 2003).

Amines oxidase is ubiquitous and plays an important role in the metabolism of biogenic amines in human, plant and animal cells. Many bacterial strains have been recognized to exhibit amines oxidase activities (Cuskey and Olsen, 1988; Dapkevicius et al., 2000; Ishizuka et al., 1993; Leuschner and Hammes, 1998; Leuschner et al., 1998; Martuscelli et al., 2000; Murooka et al., 1979; Parrot et al., 1987; Yamashita et al., 1993; Zaman et al., 2010). Table 2 show biogenic amines degrading activity by bacteria isolated from foods. Amine dehydrogenase activity was also found in extremely halophilic archaea *Natrinema gari* isolated from fish sauce (Tapingkae et al., 2010a; Tapingkae et al., 2010b). While a thermostable histamine oxidase was observed in cells of *Arthrobacter crystallopoietes* KAIT-B-007 isolated from soil (Sekiguchi et al., 2004). Instead of amine oxidase, some bacterial strains of *Pseudomonas aeruginosa*, *Pseudomonas putida* and the methylotroph *Paracoccus versutus* used amine dehydrogenase to oxidaze amines (Hacisalihoglu et al., 1997). Biogenic amines degradation was influenced by environmental condition such as pH, temperature and salt concentration. Zaman (2011) revealed that although histamine degradation by *Staphylococcus carnosus* FS19 was occurred at a wide range of condition, its optimal degradation was observed pH 6, salt 9% and temperature 40 °C. Parrott et al. (1987) revealed that the growth of bacteria can be supported by

acids and ammonia which are produced during the sequential action of amine oxidase and aldehyde dehydrogenase in the presence of electron acceptor such as oxygen. Therefore, amine degradation was thought to be restricted only to aerobic microorganisms (Leuschner et al., 1998). Nevertheless, fermentative degradation of putrescine was observed at strictly anaerobic isolates from various anoxic sediment samples, which resembles to *Acetobacterium woodii*, *Clostridium barkeri* and *Eubacterium limosum* (Matthies et al., 1989). The utilization rate of primary amines as a carbon and energy source was varied among microorganisms (Hacisalihoglu et al., 1997).

Murookaa et al. (1979) found membrane bound monoamines oxidase in some strains of the family *Enterobacteriaceae* such as *Klebsiella*, *Enterobacter*, *Escherichia*, *Proteus*, *Salmonella*, and *Serratia*, as well as *Pseudomonas aeruginosa* IFO 3901, *Micrococcus luteus* IFO 12708 and *Brevibacterium ammoniagenes* IAM 1641. They revealed that the amine oxidases of those bacteria are highly specific for tyramine, octopamine, dopamine, and norephinephrine. Parrot et al. (1987) reported that the catabolism of 2-phenylethylamine by *Escherichia coli* K12 is occurred by conversion of 2-phenylethylamine via pehnylacetaldehyde to phenylacetic acid. They revealed that phenylacetaldehyde was formed by the action of inducible amine oxidase. While the flavin adenine dinucleotide (FAD) containing putrescine oxidase was found in *Micrococcus rubens* (Ishizuka et al., 1993).

Table 2 Biogenic amines degradation in buffer medium by bacteria isolated from foods

Bacterial Strains	His (%)	Tym (%)	Put (%)	Cad (%)	Incubation	Reference
Lactobacillus sakei 15.05	56.2	-	nd	nd	30 h 30 °C	1
Staphylococcus xylosus S206	92.6	30.1	nd	nd	48 h 30 °C	2
Staphylococcus xylosus S79	68.1	19.9	nd	nd	48 h 30 °C	2
Micrococcus varians LTH 1540	-	99.2	nd	nd	24 h 30 °C	3
Micrococcus varians LTH 1534	36.8	36.9	nd	nd	24 h 30 °C	3
Brevibacterium linens LTH 3809	98.7	47.4	nd	nd	24 h 30 °C	3
Staphylococcus xylosus 0538	38.1	4.4	nd	nd	24 h 30 °C	4
Bacillus coagulans 1051	34.2	-	nd	nd	24 h 30 °C	4
Bacillus amyloliquefaciens	59.9	nd	7.5	26.4	24 h 37 °C	5

Staphylococcus carnosus	29.1	nd	7.8	8.4	24 h 37 °C	5
Staphylococcus condiment FS22	27.4	nd	4.4	6.8	24 h 37 °C	5
Bacillus humi FS13	32.8	nd	14.8	23.9	24 h 37 °C	5
Staphylococcus intermedius FS20	10.8	nd	30.4	28.0	24 h 37 °C	5

[1]Dapkevicius et al. (2000), [2]Martuscelli et al. (2000), [3]Leuschner et al. (1998), [4]Mah and Hwang (2009). [5]Zaman et al. (2010)

Application of Amines Degrading Bacteria in Seafood

Leuschner et al. (1998) have investigated the potential role of microorganisms involved in food fermentation to degrade histamine and tyramine. They include bacteria that belong to the genera *Lactobacillus*, *Micrococcus* and *Arthrobacter* as well as to the species *Pediococcus acidilactici*, *Brevibacterium linens*, *Geotrichum candidum* and *Staphylococcus carnosus*. Some tested strains were reported to have histamine and tyramine oxidase at different activity level. They found that *Micrococcus varians* LTH 1540 exhibited the highest tyramine acitivy, at which degraded 100% of tyramine in a buffer system within 24 h at 30 °C. Optimal tyramine degradation activity of resting cells *Micrococcus varians* LTH 1540 was observed at pH 7 at 37-40 °C. The presence of salt as well as glucose and hydralazine inhibited the enzyme activity. On the contrary, histamine degradation activity of *Natrinema gari* BCC 24369 was observed at salt concentration of 4.0-5.0M, pH of 6.5-7.5 and temperature of 40-55 °C (Tapingkae et al., 2010a).

Dapkevicius et al. (2000) also found that the degradation of histamine by diamine oxidase (DAO) was temperature dependent. Although the degradation rate of this amine was still considerable at 15 and 22 °C, but the highest rate was observed at 37°C. They also reported that five strains of *Lactobacillus sakei* could degrade histamine as much as 20-56% in a model system within 30 hours. Several strains of *Staphylococcus xylosus* isolated from sausages were also exhibited the ability to degrade histamine and tyramine in 0.05 M phosphate buffer at pH 7 (Martuscelli et al., 2000). *Staphylococcus xylosus* S81 was observed to degrade 100% of histamine, although it could only degrade 11.0% of tyramine under the same condition. However they also found that *Staphylococcus xylosus* S142 could degrade tyramine by 63% and histamine by

47% in the same buffer medium from their initial concentration after incubation for 48 h at 37°C (Martuscelli et al., 2000).

Zaman et al. (2011) have conducted as study to investigate effect of amines degrading bacteria on biogenic amines accumulation during fish sauce fermentation. In their study, they used *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05 as starter culture. Both bacteria were isolated from fish sauce and found to be histamine degrader (Zaman et al., 2010). Fermentation was held at controlled temperature (35 °C), which is almost similar to the average temperature in most manufacturing site in northeastern region of Malaysia (Figure 13). The authors found that concentration of histamine, putrescine, cadaverine and tyramine increased throughout fermentation (Figure 14, 15, 16, 17). The increase was progressive during the first 20 days of fermentation. However, after 120 days fermentation, histamine concentration was 27.7% and 15.4% less in samples inoculated with *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05, respectively, as compared to control samples. This has proven that both cultures could reduce histamine accumulation. Presumably, no other factors might be taken into consideration as factors responsible for histamine degradation during fermentation. It should be noted that histamine is quite resistant to degradation once it is formed in food products. Heat treatment such as autoclaving was found to be not effective to degrade histamine and other amines (Luten et al., 1992).

Figure 13 Fermentation of fish sauce with amines degrading starter culture in laboratory of microbiological food safety, UPM

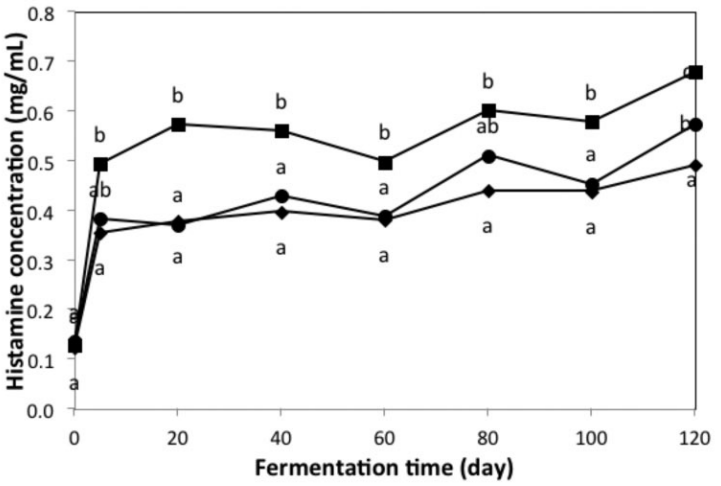


Figure 14 Histamine concentration of fish sauce during fermentation at 35°C for 120 days. ♦: treated with *Staphylococcus carnosus* (FS19), ●: treated with *Bacillus amyloliquefaciens* (FS05), ■: without treatment (control). Points at the same fermentation time marked with different letters are significantly different ($p<0.05$)

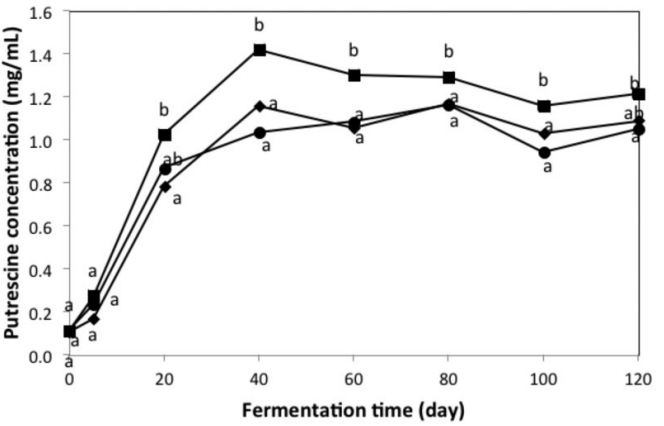


Figure 15 Putrescine concentration of fish sauce during fermentation at 35°C for 120 days. ♦: treated with *Staphylococcus carnosus* (FS19), ●: treated with *Bacillus amyloliquefaciens* (FS05), ■: without treatment (control). Points at the same fermentation time marked with different letters are significantly different ($p<0.05$)

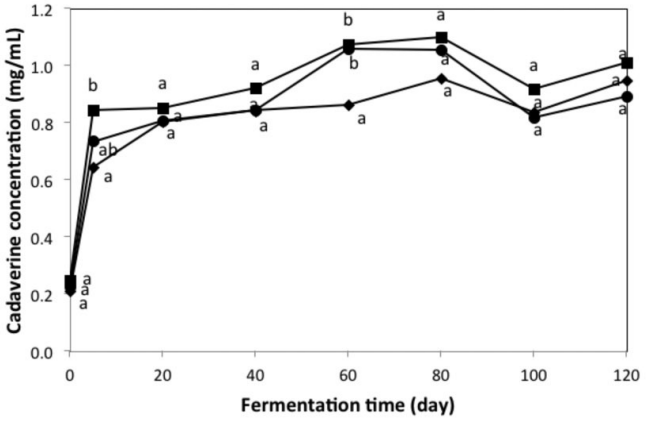


Figure 16 Cadaverine concentration of fish sauce during fermentation at 35°C for 120 days. ♦: treated with *Staphylococcus carnosus* (FS19), ●: treated with *Bacillus amyloliquefaciens* (FS05), ■: without treatment (control). Points at the same fermentation time marked with different letters are significantly different ($p<0.05$)

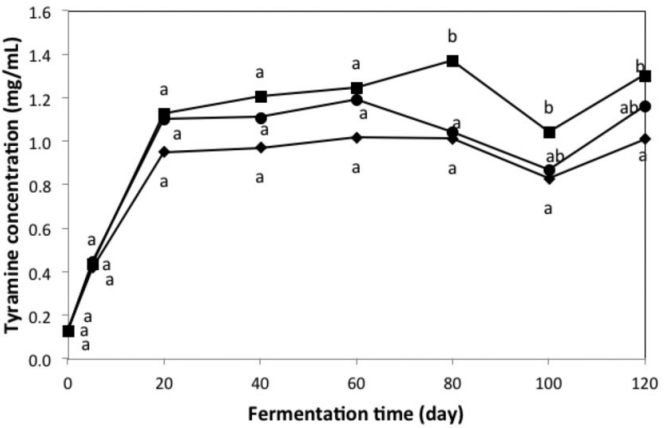


Figure 17 Tyramine concentration of fish sauce during fermentation at 35°C for 120 days. ♦: treated with *Staphylococcus carnosus* (FS19), ●: treated with *Bacillus amyloliquefaciens* (FS05), ■: without treatment (control). Points at the same fermentation time marked with different letters are significantly different ($p<0.05$)

The authors had also observed that degrading ability of both cultures are different in buffer system and food complex system. In their previous study, Zaman et al. (2010) observed that *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05 exhibited the ability to degrade 29.1% and 59.9% of histamine in a buffer system, respectively. However, when applied to fish sauce, *Bacillus amyloliquefaciens* FS05 showed lower histamine degradation activity. The authors suggested that it could be due to the fact that the histamine degrading enzyme of *Bacillus amyloliquefaciens* FS05 less adapted to complex substances in fish sauce; also the bacteria has to compete for survival with indigenous bacterial flora during fermentation. Some species of bacteria (possibly the starter culture) could stop growing when other bacterial species or group reaches it maximum population density

in a particular time (Ross, 2008). Nevertheless, *Staphylococcus carnosus* FS19 showed a better tolerance to fish sauce fermentation system as evidenced by its ability to retain the degradation activity close to that in a buffered system.

Table 3 Percentage of biogenic amine degradation by *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05 in buffer system and fish sauce sample

Biogenic amines	***Staphylococcus carnosus* FS19**		***Bacillus amyloliquefaciens* FS05**	
	Buffer system[a]	**Fish sauce[b]**	**Buffer system**	**Fish sauce**
Histamine	29.1 %	27.7 %	59.9%	15.4%
Putrescine	7.8 %	10.2 %	7.5%	13.5%
Cadaverine	8.4 %	6.2 %	26.4%	11.6%
Tyramine	nd	22.4 %	nd	10.9%
Overall[c]	nd	15.9%	nd	12.5%

nd: not determined. [a]: phosphate buffer system at pH 7.0, [b]: percentage of degradation was calculated from the data presented in Figure 14-17, [c]: total amines (histamine, putrescine, cadaverine and tyramine).

Futhermore, the use of starter culture reduced the overall amines concentration in fish sauce (Table 3). After 120 days of fermentation, the overall biogenic amines concentration was 15.9% and 12.5% less in samples inoculated with *Staphylococcus carnosus* FS19 and *Bacillus amyloliquefaciens* FS05, respectively, as compared to control samples. Mah and Hwang (2009) reported in their study that *Staphylococcus xylosus* which was applied as a protective culture in a salted and fermented anchovy could reduce overall amines by 16.0% as compared to the control. Other researchers also reported a reduction of biogenic amines concentration in other

food products (Kalac et al., 2000; Leuschner and Hammes, 1998). The study emphasized that application of starter cultures which possess amines oxidase enzyme in food fermentation is effective in inhibiting biogenic amines accumulation.

RAPID METHODS OF DETERMINATION FOR CONTAMINANTS IN SEAFOOD

Health issues regarding these chemicals prompted the Malaysian Authority under the Ministry of Agriculture, Ministry of Health and other relevant agencies to monitor the presence of these contaminants in our processed muscle foods throughout the country for this decade. However, the inconsistency in the final results and methodology used posed the greatest problem thus far.

The rapid detection of pathogens and other chemical contaminants in food is critical for ensuring the safety of consumers. Traditional methods to detect foodborne contaminants often rely on time-consuming procedures, followed by tedious biochemical protocols, and sometimes serological techniques. Recent advances in technology make detection and quantification faster, more convenient, more sensitive, and more specific than conventional assays (Shafiquzzaman et al., 2012).

The development of rapid methods is probably one of the promising ways to solve some problems concerning sensitive, fast and inexpensive measurements for medicine, biotechnology and environmental monitoring purposes. The scope of this study is focused on processed muscle foods. Through this study, it is hoped that the analytical reports on fast and reliable detection of the presence of microbes, contaminants and prohibited chemicals in muscle foods commodity analyzed can draw the attention of the authorities regarding the related issues for the sake of public's health safety (Sabah et al., 2011).

Advent of biotechnology has greatly altered food testing methods. Improvements in the field of immunology, molecular biology, automation and computer technology continue to have a positive effect on the development of faster, more sensitive and more convenient methods in food safety. Rapid detection techniques continuing to advance at a great pace, the next generation of assays currently being developed potentially has the capability for near real time and online monitoring of multiple pathogens (Shafiquzzaman et al., 2011). Modern methods are based on molecular biology techniques like PCR, RFLP, DNA microarray assay, immunological techniques like ELISA, biophysical and biochemical principles with the application of biosensors like bioluminescence sensor, bio-analytical sensors utilizing enzymes, electrical impedometry and flow cytometry (Hisham et al., 2011).

Development of Enzyme-based Biosensor Technology for Detection of Contaminants in Seafoods

Histamine

The *in vitro* determination of histamine level in seafood products is normally by chromatography analysis, which sometimes requires toxic reagents, expensive instrumentation and not practical for *in situ* analysis due to complex sample treatment and the requirement of trained personnel. Amperometric sensors based on screen-printed electrodes allow the production of simple, inexpensive and portable devices for rapid determination of freshness and spoilage in seafood products in the field (Ching et al., 2007). In this study, an amperometric histamine biosensor was developed based on immobilizing the enzyme diamine oxidase (DAO) in a photocured poly(2-hydroxyl ethyl methacrylate)-film directly deposited on a screen-printed carbon paste electrode. The use of direct photocuring

enable a simple fabrication procedure for constructing a histamine biosensor to be used in rapid analysis of histamine, a substance related to the freshness and spoilage in seafood products. The study also involved in the evaluation of the enzyme immobilization characteristics and the electrochemical behaviour of the screen-printed histamine biosensors (Ching et al., 2012). Two types of screen-printed histamine biosensors were evaluated, i.e. the screen-printed working electrode alone and a biosensor consisted of screen-printed working, counter and reference electrodes (all-screen-printed biosensor). The use of potassium hexacyanoferrate (III) as a possible mediator to improve the response of the all-screen-printed histamine biosensor was also examined.

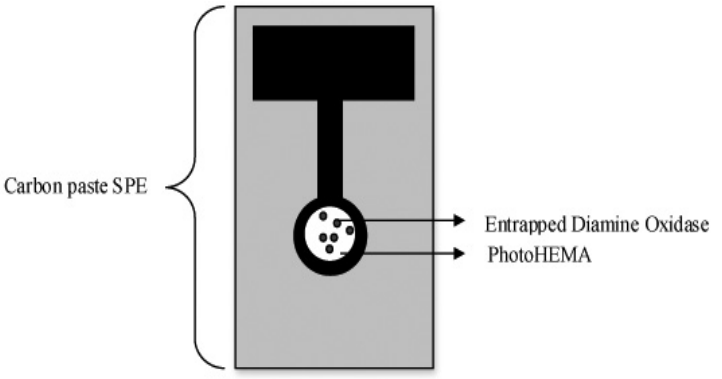


Figure 18 Immobilization of DAO on top of carbon paste screen-printed electrode (SPE) with active area of 4 mm^2

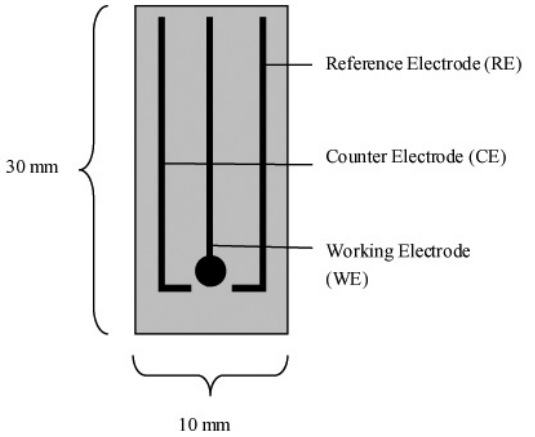


Figure 19 Modified SPE from SPE Electro deposited of potassium hexacyanoferatte on the surface of working electrode. With silver basal track for 3 electrodes:

- RE = Ag/AgCI
- CE = Carbon paste
- WE = Working Electrode

The histamine sensitive membrane with immobilized DAO, after this device used for determination histamine in tiger prawns. The histamine biosensor showed a good reproducibility and repeatability. Due to the simplicity in preparation of these ferrocene-containing films, photolithography technique can thus be applied to the fabrication of various reagentless biosensors (Ching et al, 2012). Thus, a rapid progress in biosensor technology is needed to contribute to the availability of inexpensive, accurate and simple techniques for the monitoring of toxic compounds in food and the environment (Helmi et al., 2012).

Formaldehyde

Formaldehyde is widely used in food processing for its bleaching effect and also as preservative in order to prevent the product from spoilage by microbial contamination. Formaldehyde was used as a preservative in dried foods, fish and certain oils and fats, disinfections for containers as well as modifying starch for cold swelling. It is sometimes added inappropriately in food processing for its preserving and bleaching effects such as dried foods, vermicelli, tripe and chicken paws. It is also found in cheese as bacteriostatic agent. In the sugar industry, it is added while producing juices as infection inhibitor. Nowadays, the safety and quality of seafood has arose much public attention. Before putting on shelf, seafood is firstly dipped in formaldehyde-water solution for a period of time by dishonest mongers, in order to prevent from spoiling and to increase the storage time (Nur Indang et al., 2009).

The seafood dipped with formaldehyde is a big danger to the physical health of consumer. Besides, it also used as such as herring and caviar. Due to this issue, voices have to protest to the practice of such chemical in foods. Even though chemical treatment is helpful in controlling spoilage on fish, these chemicals brought about the negative adverse effects upon human consumption, yet such issue is still under controversy. Since formaldehyde can cause adverse effect to human health, they are prohibited under the Food Regulation 1985. Although regulation has been enforced but still there are reports that revealed the practice of such prohibited chemicals in food. Formaldehyde is classified as a mutagen and possible human carcinogen being proved by experiments on microorganisms (mutagenic effect), mice and rats (induction of cancer). Recently, formaldehyde has been described as one of the chemical mediators of apoptosis (Norliana et al., 2009).

Formaldehyde is classified as a potential human carcinogen. It has often been abusely used as a preservative in food industries but it is also produced naturally in fish by postmortem enzymatic reaction (Noordiana et al., 2011). The level of formaldehyde was studied in 20 species of seafood bought from local wet markets. Several species of fish were selected to evaluate the inherent level of formaldehyde produced during storage at frozen (-20 ^{o}C), iced and ambient storage using Nash method. Among the 20 species of fish bought from the local wet markets, only lizardfish (*Saurida micropectoralis*) contained highest level of formaldehyde. Therefore, only lizardfish (*Saurida micropectoralis*) and Indian mackerel *(Rastrelliger kanagurta)* were selected for storage study. There was no significant changes ($p<0.05$) of formaldehyde levels observed for Indian Mackerel. However, for lizardfish, the formaldehyde level was decreased during ambient temperature and iced storage but it increased during frozen storage ($p<0.05$) (Norliana et al., 2009).

The determination of formaldehyde in foods especially fish and seafood required a fast, simple and sensitive method than the conventional methods which have many disadvantages such as time consuming, suffered of toxic reagent (acetylacetone, trichloroacetic acid, 2,4-dinitrophenylhydrazine) as well as easily interrupted by various interferences (methanol, aldehyde, ethanol) (Nur Indang et al., 2009). Methods such as Nash, HPLC, gas chromatography and fluorescence have been used before. In these methods have many disadvantages which involved the usages of toxic reagent in the procedure, suffer a lot of interference, time-consuming, expensive and required well-train operator (Nur Indang et al., 2012a). In this article, the developed formaldehyde biosensor was used to monitor the formaldehyde redox behaviour by performing cyclic voltammetry and differential pulse voltammetry (DPV) method.

Formaldehyde dehydrogenase NAD+ was used as the biorecogniton receptor in the system. The determination of formaldehyde based on some parameters such as the effect of pH, scan rate, multiple cycling, response ranges using immobilized enzyme, repeatability, reproducibility, storage stability, interferences and validity.

The developed formaldehyde biosensor used with free form enzyme and immobilized in Nafion polymer due to improve its stability and then deposited on the gold electrode. Both condition yielded current changes which is correlated with the concentration of substrate. Using free enzyme, the peak's potential reduction of NAD+ has found at -0.2 V. A limit of detection is 0.016 ppm of formaldehyde in aqueous solution and a response time less than 1 min. It also showed a high reproducibility, repeatability, storage stability and interferences for the determination of formaldehyde in fish sample. Product of the innovation is Rapid method for the determination of formaldehyde in fish and fish products at levels of 0.1 to 20 ppm using an enzyme formaldehyde dehydrogenase-based biosensor (Nur Indang et al., 2012b).

The newly formaldehyde biosensor developed by Nur Indang et al., (2012b) was based on coupling the enzyme (*nicotinamide adenine dinucleotide,* NAD+) and formaldehyde dehydrogenase (FDH) for the detection of formaldehyde in fish samples. To maximize the reaction rate, the enzyme acts as biorecognition immobilized with Nafion membrane which chemically modified on gold electrode. The enzyme required nicotinamide adenide dinucleotide (NAD+) as a cofactor which then reduced to NADH during enzymatic reaction. In the system, 0.1 M of potassium phosphate was used as the supporting electrolyte and 0.5 mM of NAD+ was added as the coenzyme. The optimum scan rate was found at 0.1 V/s while the optimum pH was at 8 via cyclic voltammetry. A linear response was ranged from 1 to 10 ppm of

formaldehyde, with correlation coefficient (R2) equals to 0.9865 (RSD < 3.05%). The response time was found less than 1 min. Formaldehyde biosensor showed reproducibility with no significant different ($p > 0.05$) at 1, 5 and 10 ppm of formaldehyde (n = 10). For interferences study, it was showed that the biosensor response retained its specificity for formaldehyde and did not respond to equivalent additions of methanol and also ethanol and gave the percentage of formaldehyde recovered ranging from 99.0% to 99.8%. Thus, formaldehyde biosensor is a promising tool and has a potential application for simple, fast, reusability, reproducibility, sensitivity, storage stability, validity, interferences and convenient method for the determination of formaldehyde in fish samples.

Figure 20 The laboratory instrumentation used for the development of enzyme-based biosensor for the determination of formaldehyde in fish

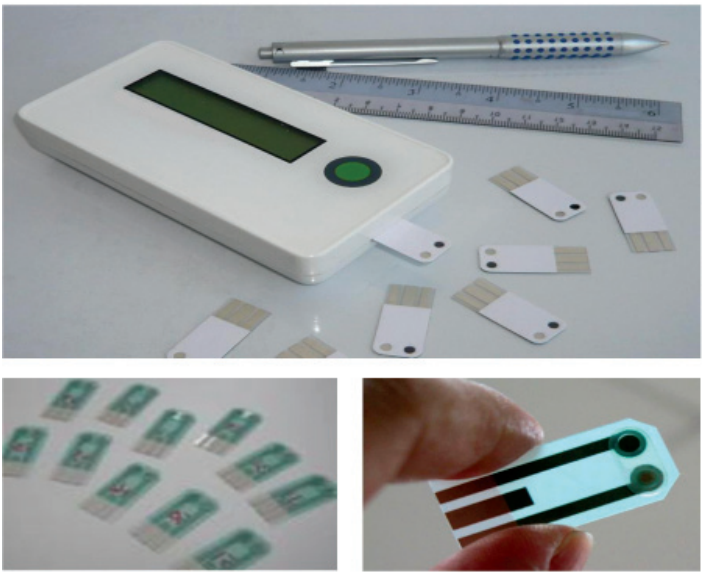


Figure 21 The development of pre-commercialised enzyme-based biosensor for the detection of formaldehyde in fish

THE WAY FORWARD

Considering national food production systems, it is recommended that food safety should be considered using a food safety management systems approach, for example hazard analysis and critical control point (HACCP) and as part of this Good Agricultural Practice and Good Manufacturing Practice. It is considered important to identify the main food safety hazards, where they occur in the food production and marketing chain and their potential risk to consumer health. A multi-disciplinary approach to hazard identification, based on risk assessment, has always been proposed. This should include collection of data on occurrence of food hazards (for example, pathogens), and the conditions and handling practices that lead to their presence in food systems. To support the case for

further investment in food safety interventions it is suggested that there is a need to calculate the potential economic value resulting from improved food safety and consumer health.

It was always considered important that education in food safety should be addressed throughout society, for example in schools, households, work place and in food processing and catering businesses.

Urbanisation can lead to environmental changes which can in their turn affect food systems which can rapidly evolve in an unregulated situation. Food quality and safety in particular may be affected. Food consumption habits and food demand may also change as a result of urbanisation. However, little is known or documented about this in many nations. It is useful to determine consumer perceptions, barriers and responses to emerging new foods. This information can help with the development of food supply and catering businesses that can adapt in response to changing pattern of urbanisation. Needs assessment must be used to inform research and consumer reaction must be taken into consideration before products are promoted.

It is suggested that there is a need to define appropriate food safety legislation, which respond to both global and domestic challenges. Access to information on international food standards was considered difficult for many individuals and organisations in this region. Enforcement of food standards was an issue as there may be limited resources for inspection, enforcement, and access to accredited laboratories that provide reliable food safety information. Sensitisation of consumers and food handlers about food safety is important. The informal food processing sector (for example, street food vendors) should receive specific attention with respect to legislation since it provides a significant proportion of food consumed in many places. Coordination between local authorities

and food standards and regulatory bodies was considered necessary. In support of the required regulatory framework, it is important to ensure that staffs are trained and that their working conditions are sufficient to reduce the risk of corruption.

- The importance of food safety issues on livelihoods and consumer health needs to be higher on the political agenda of the country.
- Food safety management systems in this country need to be able to respond to both global and domestic challenges
- There is a need to identify food safety hazards of main concern to consumer health and livelihoods and where they occur on the food chain. It was recommended that a multidisciplinary approach based on the HACCP system be used.
- There is a need to quantify potential economic benefits resulting from improvements in food safety and consumer health.
- Appropriate food safety legislation needs to be introduced in consultation with all players in the food production, processing, processing and catering sectors.
- There is a need for national food safety control systems which can be supported by appropriate food laws, enforcement and support (for example accredited laboratories).
- Consumers, food handlers and processors need to be educated in food safety issues. Food inspectors need appropriate training so that they can contribute effectively.
- It is necessary to form and/or strengthen consumers' associations and integrate them at both local and international levels
- There is need to develop appropriate evaluation procedures for food safety hazards through provision of accredited analytical laboratories.

- The rapid detection of pathogens and other chemical contaminants in food is critical for ensuring the safety of consumers. Due to their widespread use and potential health hazards, it is important for us to be able to monitor their presence, even at very low level (ng/mL level) in the agricultural products.
- Lastly, technology continues to advance at a great pace and next generation assays, such as biosensors and DNA chips already are being developed that potentially have the capability for near real-time and on-line monitoring of multiple pathogens and contaminants in processed foods.

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BIOGRAPHY

Fatimah Abu Bakar was born in Teluk Intan, Perak. She received her secondary education at Sekolah Tun Fatimah Johor Bahru, Johor and did her Matriculation at the Norwood High School, Adelaide South Australia. She then obtained her Bachelor of Science Degree with a First Class Honours in Microbiology from Universiti Kebangsaan Malaysia (UKM) in 1985 of which she won the UKM Gold Medal and also the Tan Sri Hj. Mohd Noah Award for being the best student at the Faculty of Life Sciences.

She started her career as a tutor in January 1986 at the Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia (now Universiti Putra Malaysia, UPM). Later in the same year, she went on to do my Master of Science in Microbial Physiology Related to Biotechnology at King's College, University of London under the UPM SLAB ("*Sistem Latihan Akademik Bumiputera*") Program. In October 1987, she became a lecturer at the Department of Food Science, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia and received her Ph.D in Food Microbiology from the Faculty of Science and Environmental Studies, Universiti Putra Malaysia in March 2001.

As a lecturer, she had taught courses in Food Microbiology, Biochemistry and Food Safety including Basic Food Microbiology, Food Microbiology, Industrial Microbiology, Food Fermentation Technology, Food Biochemistry, Introduction to Food Science, Food Hygiene and Sanitation, Food Toxicology and Nutritional Changes in Food. Fatimah had also taught the post-graduate courses in the field of Food Safety and Quality such as Microbiology of Food Safety, Microbial Ecology of Food Commodities and the Post-harvest Physiology and Biochemistry of Food Commodities. She has been the Coordinator for Industrial Training for the Bachelor

of Food Science and Technology (BSTM) and Bachelor of Food Studies (BSPMK) Programs since the year 2002.

She was the prime mover in the curriculum development for the Master of Food Safety and Quality Assurance (non-thesis) Course work program, given the task to lead, comprehend, develop and prepare new courses synopsis for the curriculum mainly the field of Food Quality Control and Food Safety, Food Toxicology and Advanced Food Microbiology. With her expertise in the field of Food Microbiology and Food Safety, she was invited by the Universiti Darul Iman (UDM), Terengganu now known as UniZA in sharing with them by teaching their Bachelor of Food Technology program students in Basic Food Microbiology courses for the year 2007 and 2008. Fatimah has been a trainer and often invited as a resource person to lecture or give academic talk at various training workshops such as the *Basic Food Microbiology Training Workshop* and *Microbiological Risk Assessment Workshops* organized not only by the Centre of Excellence for Food Safety, UPM but also by other agencies in Malaysia for such workshops in the *Good Manufacturing Practice (GMP) Workshop* organized by the Malaysian Institute of Food Technology (MIFT) throughout Malaysia and those organized by the Ministry of Health and private agencies. She was also involved in setting and evaluating examination questions for the *Penilaian Tahap Kecekapan; PTK 5* and *6* and even *TK 1, 2 and 3* and answers for the laboratory staff (science officers, assistant science officers and the laboratory technicians) at the faculty mainly in the field of food microbiology and safety. She has been a referee for promotion exercise for those candidates applying for the post of associate professors in UPM and also from other universities.

Being in the academic world, she cannot run away from supervising students. Thus up to December 2012, she has been

the main supervisor for eight (8) Ph.D, twenty eight (28) M.Sc, and another more than one hundred twenty (120) undergraduate students. Out of those numbers, seven (7) Ph.D and ten (10) M. Sc. had already graduated. At the same time, she is also actively involved with co-supervising another ten (10) Ph.D students, including five (5) had already graduated, and another 12 M.Sc students with six (6) of them had already graduated. These students supervision may run across the different faculties and institutes in the University as well as those from other universities mainly in the field of Food Biotechnology, Food Safety and Biosensor Technology. Apart being surrounded by all these students, and through her successful students' supervision, she had been entrusted to lead an international project under the Arab Science and Technology Foundation (ASTF) grant with a post-doctoral candidate from the year 2007 till 2011. She is an external examiner for M.Sc and Ph.D students from USM and UTM.

As an academician, all of us believe that research and teaching are equally important. She had been actively involved in research covering subjects on food microbiology and food safety, biotechnology, biochemistry and quality mainly of seafood and seafood products. In this capacity, as a project leader, she is responsible for managing more than twenty research projects from various government and private agencies with a total funding of more than RM 3,000,000.00. Then with her international standing and reputation in the field of food safety and food microbiology she had been entrusted to lead five important oversea/international projects from Japan, Singapore, Australia and the United Arab Emirates. She started with the Japanese International Corporation Agency (JICA) way back in the year 1988-1990 on the project *Freshness and Quality of Freshly landed Fish in Malaysia,* then work on *Effect of 4-hexylresorsinol on freshness and quality of*

prawns with Pfizer Limited, Singapore in the year 1989-1991 had brought her to the ASEAN region in promoting the preservative compound. Another project sponsored by the Australian Wheat Board (AWB) on the *Spoilage of Yellow Wet Noodles* from the year 2004-2006 while the Arab Science and Technology Foundation (ASTF) awarded her grants for the two projects on *Development of Rapid Diagnostic kits for multivariant drug resistance* for the year 2007-2011.

Fatimah is also a member of National Biosensor Technology Research Group that conducted research to develop biosensor apparatus in the form of enzyme-based electrodes to determine the quality and safety of fish. This Top-Down Project engendered in 2003 until 2010. As a co-researcher, she had been actively involved in the studies of *Development of Biosensor Technology for determination of contaminants in agricultural products and the environment* funded for more than RM 3,000,000 over a period of 6 years by the Ministry of Science, Technology and Innovations (MOSTI) under the 8th and 9th Malaysia Plan top down IRPA projects, working in close collaboration with other institutions such as Universiti Kebangsaan Malaysia (UKM), Universiti Teknologi Malaysia (UTM), SIRIM, Malaysian Agriculture Research and Development Institute (MARDI), Universiti Teknologi Mara (UiTM) and Universiti Sains Malaysia (USM). As this is an important national and public agenda, the research on fish and fishery products has made the Lembaga Kemajuan Ikan Malaysia (LKIM) appoint her to be their consultant under the Post harvest Losses of Fish Project working closely with the Ministry of Agriculture (MOA) and Ministry of Health, Malaysia. In 2003, she had been given some grant for a project monitoring prohibited chemicals namely formaldehyde and boric acid contamination at various fish entry and landing ports throughout the country, which

is very much of national and public interests. Closely related to that, for the year 2004, she was immediately entrusted by the LKIM to train their Fish Inspectors and help set up a National Quality Control and Fish Safety Laboratory for the authority at the Kompleks Sultan Abu Bakar, Johor Second Link, Pasir Gudang, Johor. A few other private companies (such as Gold Carbon Sdn Bhd and Atlantic Three) had also engaged her as consultant for the project on monitoring post-harvest losses and quality and safety of fish and aquaculture products. Later in the year 2005 and 2006, she was involved with the DUMEX Laboratory Quality staff-training program in managing, teaching and training the laboratory technical staff in the field of Food Microbiology and Food Safety at DUMEX Quality Control Laboratory in Nilai, Negri Sembilan for thirty of its laboratory technicians. The purpose was to upgrade their hands-on knowledge and keeping abreast with the latest technology and issues in food safety.

Apart from those projects mentioned above, recently in 2009, she also secured quite a number of research projects funded by other government agencies such as the Ministry of Health (MOH) and Jabatan Kemajuan Islam Malaysia (JAKIM) under the project of *Determination of Alcohol Contents in Foods and Drinks in Malaysia,* from the Ministry of Agriculture (MOA) on *Modelling Of The Intoxication Effect Of Selected Fermented Alcoholic Foods In Malaysia* and from the Ministry of Higher Education on the project entitled *Development of Rapid biotechnological techniques for determination of Formaldehyde in Fish and Seafood Products* and *Novel Starter Culture for Cheese Manufacturing.* The other prominent research projects and grants that she shared with the other researchers entitled *Production of fermented foods high in gamma-butyric acid as designer food* from MOSTI for more than RM 1 million and *Thermostable lipase from local strains of lipase*

producing thermophilic bacteria for RM 300,000 also from MOSTI. Apart from these projects, there are other grants for projects related to food safety and food biotechnology which are also carried out by our group funded either by the Ministry of Agriculture (MOA), private agencies, or even UPM under the Research University Grant Scheme (RUGS).

From all these projects carried out successfully, as the main or co-author, she managed to receive more than 30 local and international awards, published over 121 publications in refereed citation index international journals, 1 textbook translation, 4 chapters in books and another 150 over in either international or national proceedings and paper presentations during conferences and seminars. Of these awards, she had won gold, silver and bronze medals for various research projects on food safety and food biotechnology at the university and international levels of Invention, Innovation and Technology Exhibition. The project on *Halotolerant Staphylococcus carnosus FS19 as potential histamine degrader* had won gold medal at the 23rd International Invention Innovation, Industrial Design & Technology Exhibition (ITEX 2012) while *Thermostable lipase from local strains of lipase producing thermophilic bacteria* had won gold medal at the 17th International Invention Innovation, Industrial Design & Technology Exhibition (ITEX 2006), had 2 silver and 1 bronze medals during Malaysia Technology Exhibition (MTE2011) for the projects on *Use of enzyme-based biosensor for determination of formaldehyde in fish and fish product, A DNA-based Biosensor for detection of bacterial and fungal pathogens* and *Use of Capacitance-based Biosensor for histamine determination* respectively. She also obtained 1 Intellectual property for the project *Thermostable food-grade lipase producing bacteria isolated from Malaysia hot springs* and gene partial sequences deposited in Genbank Data

Library. She was also awarded the best posters presentation at the *Malaysian Science and Technology Congress (MSTC) 2000*, *Regional Biosensors and Biodiagnostics Conference, 2008, Kuala Lumpur and 30th Malaysian Microbiology Symposium, 2008* in Kuantan. Thus far, she had won numerous medals during the annual research and innovation exhibitions (PRPI) held at UPM. Apart from all these publications and awards, within her research groups, she had filed 12 patents, of which 4 as the project leader. One patent published with WIPO from the *Use of Amperometric Biosensor for histamine determination in fish*, 1 patent pending on the *Use of Capacitance-based Biosensor for histamine determination* projects, another 2 pending on the *Use of enzyme-based biosensor for determination of formaldehyde in fish and fish products,* and on *A DNA-based Biosensor for detection of bacterial and fungal pathogens.* 2 European patents published in 2010 for the *Methods for Virus Design* and in 2011 for *Phage-Based Limulus Amoebocyte Lysate Assay For The Rapid Detection Of Bacteria* from the ASTF project, 1 patent shared from the *Reduction of Mercury levels in Fish* and 6 others filed from the other projects. The work on *Use of enzyme-based biosensor for determination of formaldehyde in fish* has brought her one step further and at the moment, in the process of pre-commercialization/licensing stage.

As a scientist in the field of Food Safety and Food Microbiology she was entrusted to be a reviewer for the various local and international citation-indexed journals such as the *Food Control, International Journal of Food Microbiology, International Journal of Applied Microbiology, Letters of Applied Microbiology, Journal of Food Science and Agriculture, Sensors Letters, Biotechnology, Analytica Chimista Acta*, *Journal of Agricultural Sciences and Technology (JAST), Pertanika International Journal of Tropical Agriculture, ASEAN Food Journal, International Food Research*

Journal, Mardi Bulletin and some others mainly in the field of food science and Technology.

Currently, she sits in the technical committee as panel evaluator for UPM research proposals and MOSTI in evaluating e-Science and Technofund research grant proposals in the fields of Food Science, Food Safety and Food Biotechnology.

She began involving in the administrative work at the Faculty as a Food Microbiology Teaching and Food Safety Research Laboratory Manager from 2001-2005, looking after the day-to-day running and maintenance of the teaching and research laboratories. In 2004, she was the Chairman for the Staff Training Division at the Faculty, coordinating the training needs of the faculty academic and supporting staff.

She was the Deputy Dean of Academic and Student Affairs at the Faculty of Food Science and Technology, handling the academic, students' mobility and student affairs' division of the Faculty from June 2010 till February 2012 before changing portfolio to Resource Management Division until today. Prior to that, she was appointed as the Head of Department of Food Science for 5 years from June 2005 till May 2010. Being entrusted as the Head and Chairman of UPM Food Science and Technology degree (BSTM) program, she was also active as a committee member of the ISO 9001-2000 and now the ISO 9001-2008 team, Industrial Training, Asset Management, Food Analysis Unit, Human Resource Development, Trustee Account, Curriculum Revision, and Post-graduate Studies. Fatimah had also played an active role in reviewing and setting up previously a three-year program of the Bachelor of Food Science and Technology in the 1990s and later reverted to the four-year program since 2005. Currently, she is also the Head of Industrial Unit for the Faculty.

Since the year 2001, Fatimah had been appointed as panel evaluator for the Lembaga Akreditasi Negara (LAN), now known as the Malaysia Qualifications Agency (MQA) either as the chairperson or member of the panel evaluators. Being an evaluator, she is actively involved with the evaluation and accreditation of new academic programs across the levels of diplomas, Bachelor, Master and Doctor of Philosophy, mainly in the field of food science, food technology, health sciences and biotechnology related programs throughout the country. Her responsibility is to help the board in evaluating and accrediting courses and new programs for several private universities and colleges throughout Malaysia especially with the improvement of Academic Performance Audit (APA), Code of Practice for Program Accreditation(COPPA) and Code of Practice for Institutional Accreditation (COPPIA) very much in-line with the aspiration of the Ministry of Higher Education (KPT). In 2011, she was appointed by KPT as the resource person/ advisor to the Department of Polytechnic, KPT for the development of the Advanced Diploma in Food Technology program. Besides, Universiti Malaysia Terengganu (UMT) has appointed her as their external assessor for three years (2012-2015) evaluating the Bachelor of Food Science (Food Technology) academic program. Since the year 2008, *Dewan Bahasa dan Pustaka* (DBP) appointed her to sit as the expert panel for English-Malay translation in the field of Food Science and Technology.

She is a member of several professional affiliations mainly the Malaysian Institute of Food Technologists, an Executive committee (EXCO) member and Life member of the Malaysian Society for Microbiology and a member of the Malaysian Nutrition Society. Through these experiences, she was the prime mover/the chairperson for the International Conference on Food Research in November 2010 (ICFR2010) at Putrajaya Marriot, in collaboration

with ILSI and ICSMF. Apart from that, to mention a few, she has been the Chairperson of the organizing committee for auspicious occasions such as *the Faculty Alumni Nights* consecutively for the year 2011 and 2012 and as advisor to the students *International Food Festival* event last November 2012.

She was awarded the Excellence Service Award for the 2002, 2003, 2005, 2006, 2007, 2008, 2009, 2010 and 2011 by the faculty. She is an associate researcher at the Institute of Bioscience, IBS, since April 2002 and since 2008 as an associate fellow researcher at the Institute of Halal Products, UPM.

On the community front, she is often called by the magazines, media etc to give advice and opinion in the various fields of food science, food safety and quality. Among them, in 2008, she was invited by *TV3* to appear *live-on air* for the programme on *Wanita Hari Ini* to talk about the *Effect of Fungi in Food.* While in 2010, she helped UPM to promote the use of enzyme-based biosensor for the determination of formaldehyde in fish and seafood products, appearing in almost all national mainstream media (TV and newspapers). For 2011, she had been interviewed by the New Sunday Times for the article on "Expired Foods" while last June 2012 she appeared on *TV Al-Hijrah* to speak on the *Halal* and safety of fermented fruits and vegetables and in October 2012 she appeared in *Berita Harian* to talk about her research on antiviral activities of red cabbage. Being an all rounder, she had represented the Faculty in the Inter-Faculty Badminton tournament, playing in the Women Doubles for the year 2004-2006.

Alhamdulillah, having working very hard towards achieving her goals in life, she is indeed very happy to help UPM in achieving its mission of being a leading institution of higher learning and research, contributing not only to human advancement and discovery of knowledge, but also towards the creation of wealth and

nation building. At the same time, she is proud to have contributions in the different field of activities throughout her stay at UPM thus far, not only towards the advancement of the students and university but also the community, public, related industries, Malaysia and the world as a whole. Finally but most importantly, she is happily married to Raja Ramle bin Raja Mansor and blessed with two lovely daughters Siti Noor Fathilah and Nur Fara Asyiqin.

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I am forever grateful to all the previous deans at the Faculty: Prof Dato' Dr Mohamed Mahyuddin Mohamed Dahan who took me to the post of a tutor in 1986, Prof Dr Gulam Rusul, Rahmat Ali made me a lecturer at the Department of Food Science in late 1987, Prof Dr Jinap Selamat, not only as a good friend, colleague but also for giving me support and encouragement all the way through and to the present dean, Prof Dr Mohd Yazid Abdul Manap for his support and trust.

I would also like to acknowledge all my co-researchers for sharing their knowledge and experiences with me. My deepest appreciation to all my undergraduate and postgraduate students, and to my two post-doctoral candidates (Dr Ahmed and Dr Zukhruf); whom without them I would not be able to be where I am today. At the same time, I am indebted to UPM, JPA, MOHE, MOA, MOSTI, JICA, AWB, PFIZER and ASTF for giving me the grants to pursue my research.

I have too many other individuals to thank; to all my colleagues and staff at the Faculty of Food Science and Technology, those who have contributed directly or indirectly to my success today, please forgive me because it is impossible to list them all here, I wish to say that I am grateful to have known and work with all of you. Last but not least, my deepest gratitude goes to those who matters the most to me, none other than my beloved family members; my mother, Arbaayah, my husband Raja Ramle Raja Mansor, my wonderful daughters Siti Noor Fathilah and Nur Fara Asyiqin, my son-in law, Faredi Abdul Razak and my little grandson Ahmad Faris Danial for their constant prayers, endless love, encouragement, patience, sacrifices and understanding. I am also forever indebted to my late father Allahyarham Haji Abu Bakar Haji Mohamad for his guidance and *May Allah Sentiasa Mencucuri RahmatNya ke atas rohmu. Ameen.* To all my siblings, in- laws, cousins, nephews, nieces and children, I appreciate the love and invaluable support.

To All of You Many Thanks Again
MAY ALLAH BLESS YOU

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LIST OF INAUGURAL LECTURES

1. Prof. Dr. Sulaiman M. Yassin
   The Challenge to Communication Research in Extension
   22 July 1989

2. Prof. Ir. Abang Abdullah Abang Ali
   Indigenous Materials and Technology for Low Cost Housing
   30 August 1990

3. Prof. Dr. Abdul Rahman Abdul Razak
   Plant Parasitic Nematodes, Lesser Known Pests of Agricultural Crops
   30 January 1993

4. Prof. Dr. Mohamed Suleiman
   Numerical Solution of Ordinary Differential Equations: A Historical Perspective
   11 December 1993

5. Prof. Dr. Mohd. Ariff Hussein
   Changing Roles of Agricultural Economics
   5 March 1994

6. Prof. Dr. Mohd. Ismail Ahmad
   Marketing Management: Prospects and Challenges for Agriculture
   6 April 1994

7. Prof. Dr. Mohamed Mahyuddin Mohd. Dahan
   The Changing Demand for Livestock Products
   20 April 1994

8. Prof. Dr. Ruth Kiew
   Plant Taxonomy, Biodiversity and Conservation
   11 May 1994

9. Prof. Ir. Dr. Mohd. Zohadie Bardaie
   Engineering Technological Developments Propelling Agriculture into the 21st Century
   28 May 1994

10. Prof. Dr. Shamsuddin Jusop
    Rock, Mineral and Soil
    18 June 1994

11. Prof. Dr. Abdul Salam Abdullah
Natural Toxicants Affecting Animal Health and Production
29 June 1994

12. Prof. Dr. Mohd. Yusof Hussein
Pest Control: A Challenge in Applied Ecology
9 July 1994

13. Prof. Dr. Kapt. Mohd. Ibrahim Haji Mohamed
Managing Challenges in Fisheries Development through Science and Technology
23 July 1994

14. Prof. Dr. Hj. Amat Juhari Moain
Sejarah Keagungan Bahasa Melayu
6 Ogos 1994

15. Prof. Dr. Law Ah Theem
Oil Pollution in the Malaysian Seas
24 September 1994

16. Prof. Dr. Md. Nordin Hj. Lajis
Fine Chemicals from Biological Resources: The Wealth from Nature
21 January 1995

17. Prof. Dr. Sheikh Omar Abdul Rahman
Health, Disease and Death in Creatures Great and Small
25 February 1995

18. Prof. Dr. Mohamed Shariff Mohamed Din
Fish Health: An Odyssey through the Asia - Pacific Region
25 March 1995

19. Prof. Dr. Tengku Azmi Tengku Ibrahim
Chromosome Distribution and Production Performance of Water Buffaloes
6 May 1995

20. Prof. Dr. Abdul Hamid Mahmood
Bahasa Melayu sebagai Bahasa Ilmu- Cabaran dan Harapan
10 Jun 1995

21. Prof. Dr. Rahim Md. Sail
Extension Education for Industrialising Malaysia: Trends, Priorities and Emerging Issues
22 July 1995

22. Prof. Dr. Nik Muhammad Nik Abd. Majid
The Diminishing Tropical Rain Forest: Causes, Symptoms and Cure
19 August 1995

23. Prof. Dr. Ang Kok Jee
The Evolution of an Environmentally Friendly Hatchery Technology for Udang Galah, the King of Freshwater Prawns and a Glimpse into the Future of Aquaculture in the 21st Century
14 October 1995

24. Prof. Dr. Sharifuddin Haji Abdul Hamid
Management of Highly Weathered Acid Soils for Sustainable Crop Production
28 October 1995

25. Prof. Dr. Yu Swee Yean
Fish Processing and Preservation: Recent Advances and Future Directions
9 December 1995

26. Prof. Dr. Rosli Mohamad
Pesticide Usage: Concern and Options
10 February 1996

27. Prof. Dr. Mohamed Ismail Abdul Karim
Microbial Fermentation and Utilization of Agricultural Bioresources and Wastes in Malaysia
2 March 1996

28. Prof. Dr. Wan Sulaiman Wan Harun
Soil Physics: From Glass Beads to Precision Agriculture
16 March 1996

29. Prof. Dr. Abdul Aziz Abdul Rahman
Sustained Growth and Sustainable Development: Is there a Trade-Off 1 or Malaysia
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