MEDWELL PUBLISHING
Research Journal of Pharmacology 3 (2): 31-47, 2009
ISSN: 1815-9362

A Review: Methods of Determination of Health-Endangering Formaldehyde in Diet

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Abstract: The sea food immersed with formaldehyde to prevent spoilage by dishonest fish mongers is a big risk to the health of consumers especially risk of cancer development. Formaldehyde is a toxin for viable cell. With increasing awareness of food safety and authenticity issues, new and sophisticated techniques for the authentication of seafood are developed. Therefore, many methods are proposed to assure the quality of seafood by determining the formaldehyde content. Various ranges of methods have been proposed such as colorimetric method to high technology device methods and E-nose has been applied in seafood. Specificity, simplicity and quick response of the method is most considered to choose for application *in situ*. This review demonstrates the potential of technological applications have so far been used in determining formaldehyde in seafood and their setbacks, disadvantages and advantages. This review is aimed to be as a reference of formaldehyde detection techniques in seafood.

Key words: Formaldehyde, seafood, review, toxicology, determination, E-nose

INTRODUCTION

Recent trends in global food production, processing, distribution and preparation create an increasing demand for food safety research to ensure a safer global food supply. The World Health Organization (WHO) works mutually with the Food and Agriculture Organization of the United Nations (FAO) to address food safety issues along the entire food production chain from production to consumption using new methods of risk analysis, taking into account the potential risks of susceptible populations as well as of combined low-level exposure to several chemicals (WHO, 2002). Microbiological and chemical hazards result in the most significant sources of foodborne diseases to consumer. Among them, great attention has been paid toward volatile toxic aldehydes especially formaldehyde, recently classified by the International Agency for Research on Cancer (IARC) in the Group 1 'as carcinogenic to humans (IARC, 2004). It has been described as one of the chemical mediators to cause apoptosis, i.e. programmed cell death. Moreover, the formaldehyde generated *in situ* from some N-methylated compounds can induce apoptosis or retardation of cell proliferation of tumorous cells or known as cancer (Korpan *et al*., 2000).

Formaldehyde is easily flammable, colorless and readily polymerized gas at ambient temperature. The most common commercially available form is a 30-50% in aqueous solution (WHO, 1989). Formaldehyde is the most widespread carbonyl compound and it is widely used in consumer goods to protect the products from spoilage by microbial contamination. Formaldehyde is often added to keep food pleasing to the consumers, but this chemical poses a hazard threat to human health (Cui *et al*., 2007).

In food industry, it is widely used in food processing as for its bleaching effect and also as preservative in order to prevent the product from spoilage by microbial contamination especially seafood (Wang *et al*., 2007). Formalin which contains 37% formaldehyde has been used as a therapeutant to control ectoparasites and aquatic fungi disease events occurring at fish culture facilities (Schnick, 1991; Rach *et al*., 1997). Commonly, the fish are dipped in a formaldehyde bath for this purpose (Greg *et al*., 2003). Its residues in the food for human consumption are proscribed because of possible carcinogenicity (Jung *et al*., 2001).

There are 3 commercial formaldehyde products approved by US Food and Drug Administration (FDA), which have similar formulations of about 37% formaldehyde, for use in US aquaculture as parasiticides:

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Parasite-S (for use on all finfish and penaeid shrimp; Western Chemical), Paracide-F (for use on bluegill, catfish, largemouth bass, salmon and trout; Argent Chemical Laboratories) and Formalin-F (for use on bluegill, catfish, largemouth bass, salmon and trout; Natchez Animal Supply (FDA, 1998). According to the label recommendations, routine treatment concentrations of formalin range from 15-250 mg L^{-1} for control of protozoan and monogenetic trematodes on fish and shrimp and up to 2000 mg L^{-1} for control of fungi on fish eggs (Jung *et al*., 2001).

This compound is also produced from the enzymatic reduction of trimethyamine-N-oxide to formaldehyde and dimethylamine during frozen storage and it causes protein denaturation and muscle toughness (Bianchi *et al*., 2007; Sotelo *et al*., 1995). Moreover, it result in the loss of food quality because of unacceptable texture, undesirable flavour, odour, colour and its harm for consumers. Formaldehyde accumulates during the frozen storage of some fish species, including cod, pollack and haddock (Sotelo *et al*., 1995). Deterioration in quality due to micro-organisms and various biochemical processes is nearly eliminated, but some enzymatic activities cause changes in the products of frozen fish. These changes are of great commercial importance, because they are limiting factor for the shelf-life of frozen seafood (Benchman, 1996).

From previous studies, formaldehyde has been detected as a result of postmortem change in the tissues of cod (*Gadus macrocephalus*), Alaska pollock (*Theragra chalcogramma*), blue shimp (*Penaeus stylirostris*) and pacific shrimp (*Pandalus jordani*) (Amano and Yamada, 1964; Flores and Crawford, 1973; Hose and Lightner, 1980). Endogenous formaldehyde residues ranging from 0.1-31.8 μg g^{-1} were detected in several species including eel (*Anguilla japonica*) (Ueno *et al*., 1984), striped bass (*Morone saxatilis*) (Xu and Rogers, 1995), banana shimp (*Penaeus merguiensis*) (Yamagata and Low, 1995) and Nile tilapia (*Tilapia niloticus*) (Xu and Rogers, 1995). The highest level of formaldehyde (e.g. 10-20 mg kg^{-1}) in fish may not be considered as palatable as human food source (Yasuhara and Shibamoto, 1995). An acceptable daily intake of 0.2 mg kg^{-1} body weight has been set by the United States Environment Protection Agency, whereas, values of 60 mg kg^{-1} for *Gadidae* and crustaceans, respectively were proposed in 1985 by the Italian Ministry of Health (Bianchi *et al*., 2007).

Taking into account that fish and fish products play an important role in human nutrition as a source of biologically-valuable proteins, fats and fat-soluble vitamins and frozen and fresh fish are the most commercialized products, this study aims to highlight the methods used to determine formaldehyde content of seafood maintained under different conditions. This study was done to look for the most advanced detection methods for formaldehyde in seafood and a comparison of these methods was done based on their advantages and disadvantages.

SPECTROPHOTOMETRY

Spectrophotometer method

Nash mehod: The very early basis of a spectrophotometric procedure proposed by Nash (1953) is the Hantzsch reaction between acetylacetone, ammonia and formaldehyde to form 3,5-diacetyl-1,4-dihydrolutidine (λ_{max} = 412 nm). To complete the reaction, the procedure requires pH adjustment with ammonium acetate and heating. But under strongly acidic conditions, it may be used in the presence of trioxan and other compounds, which degrade to formaldehyde. The sensitivity may be increased by measuring the fluorescence spectrum. These methods are relatively sensitive and selective for formaldehyde and it is useful on account of the mild conditions employed. However, the procedure needs long reaction times and cannot be simply adopted for an automatic analysis. In order to develop a simple and automated method of analysis for formaldehyde, a Flow Injection Analysis (FIA) system with an incorporated gel-filtration chromatography column is proposed and have been applied to determine formaldehyde in fish (Benchman, 1996).

4-Amino-3-hydrazino-5-mercapto-1, 2,4-triazol (AHMT) method: AHMT method procedure determines formaldehyde in fish, olive flounder (*Paralichthys olivaceus*) and black rockfish (*Sebastes schlegeli*). Two milliliters of deionized water as control and 2 mL of the extract are pipetted into separate tubes and 2 mL of 5 N KOH are added to each tube. Then 2 mL of 0.5% AHMT dissolved in 0.5 N HCl and the solutions are gently mixed. A stopper is put on each test tube, which is allowed to stand for 20 min at room temperature. Afterwards, 2 mL of 0.75% KIO solution prepared in 0.2 N KOH 4 are added. The mixture is shaken gently and by spectrophotometry (DMS 80 UV-visible, Varian, England). The absorbance of the violet color is read at 550 nm. The amount of formaldehyde in fish (μg g^{-1} or μg mL^{-1}) is calculated from standard curves (Jung *et al*., 2001). However, the AHMT method needs a very strong base as the reaction medium, which is not desirable especially as carbonate formation will occur.

Kinetic spectrophotometric method: Spectrophotometric catalytic kinetic methods are based on the catalytic effect of the element upon the reactions whether in colored (Vis) or colorless (Dirksen *et al*., 2001) solutions (Rancic *et al*., 2005). The application of these methods offers some advantages such as improved selectivity and high sensitivity. Rhodamine B (RhB) compounds are a group of xanthene dyes that have been widely used in analytical chemistry and the photophysical properties of rhodamines in solution have been extensively studied. Formaldehyde could act as an effective catalyst for the catalysis of formaldehyde in the developed system. The reaction rate was spectrophotometrically monitored by measuring the decrease colour from purple to colorless in the absorbance at 515 nm (Lazrus *et al*., 1988). The catalytic effect of formaldehyde on the indicator reaction between RhB and potassium bromate is very sensitive. The method has a detection limit as low as 2.90 μg L^{-1} and an analytical working range of 10-100 μg L^{-1}. The method is simple, rapid, precise and it is successfully applied to the analysis of formaldehyde in foods especially seafood (Cui *et al*., 2007).

CHROMATOGRAPHY

High performance liquid chromatography: This method is based on steam distillation and 2,4-dinitrophenylhydrazine derivatization (2,4-DNPH). Formaldehyde is analyzed by HPLC using ODS-C18 column at UV detector (355 nm) and have been applied to squid (Li *et al*., 2007a). By this method, the formaldehyde content of squid muscle and viscera, dried squid thread and boiled squid were determined. Detection limit was 8.92 μg L^{-1} in standard solution and 0.18 mg kg^{-1} in sample and recovery was 83.09-103.20%. The results showed that variable formaldehyde levels were observed among 4 species squid, which was generally far higher in viscera than in muscle of frozen squid. And cooking accelerated the formaldehyde production of squids. The study indicated that the HPLC method, with a better selectivity, precision and accuracy, was available to determine the formaldehyde in squid products with satisfactory results (Li *et al*., 2007a). However, the disadvantage of this method where 2,4-DNPH can react with many aldehydes and ketones and the 2,4-DNPH derivatization reaction takes 1 h for a complete reaction.

Solid Phase Microextraction (SPME)-GC-MS method: The formaldehyde content in different fish products is evaluated using a solid phase microextraction (SPME)-GC-MS method based on fiber derivatisation with pentafluorobenzyl-hydroxyl-amine hydrochloride (Bianchi *et al*., 2007). As formaldehyde is a very volatile compound, solvent-free techniques like Solid Phase Microextraction (SPME) can be easily applied for the analysis of organic compounds especially formaldehyde, thus combining sampling and preconcentration in a single step (Pawliszyn, 1997). An innovative method based on SPME with *in situ* derivatisation with Pentafluorobenzyl-hydroxylamine Hydrochloride (PFBHA) have been developed and validated for the determination of formaldehyde at ultratrace levels in frozen fish samples (Bianchi *et al*., 2005). Higher formaldehyde levels were founds in species belonging to the Gadidae family, whereas, fresh-water fish as well as crustaceans were generally characterised by lower values. Using this method, LOD and LOQ values of 17 and 28 μg kg^{-1}, respectively were calculated (Bianchi *et al*., 2007).

Electronic nose: Electronic noses have been applied in lots of applications (Ampuero and Bosset, 2003; Zhang *et al*., 2005, 2006), especially in the quality control of food industry (Haugen *et al*., 2006; Marilley and Casey, 2004; Marti *et al*., 2005) and food safety detection (Magan and Evans, 2000; Needham *et al*., 2005; Rajamäki *et al*., 2006).

Electronic nose technology, which is a rapid means of analysis and can be easily used in conjunction with chemometrics, has had a good degree of success in the authentication of a wide range of food types (Reid *et al*., 2006).

The developed solid-state-based prototype gas-sensor system, FishNose, has been applied to determination of quality smoked Atlantic salmon showed a good performance with regard to sampling, repeatability and sensitivity. This was partly due to the development of an application specific sampling unit interfaced with the sensor module, which had been optimized for direct analysis of smoked salmon. This allowed sequential sampling without purging the sampling system between the measurements, thus shortening the analysis time and maintaining a good repeatability of the system within 5% on real samples (Haugen *et al*., 2006).

An E-nose with 6 TGS gas sensors was used for spoiling and formaldehyde-containing detection of seafood in octopus. Two static features R_0 (resistance in the air), S (sensor response) and one dynamic feature DR (desorption rate) were extracted by Zhang *et al*. (2009). Fresh octopus samples dipped in water solutions with different formaldehyde concentrations were measured. In these measurements, the stability of sensors and features was evaluated and compared. The mean relative errors of

Table 1: Some conventional methods to determine formaldehyde

Methods	Detection regaent	Remarks	Reference
Colorimetric methods	Pararosaniline	Although, pararosaniline-based Schiff reaction has been used widely for formaldehyde determination, color development is relatively slow and sensitivity is limited and sulfur dioxide residue in food will influence the result.	(Pedrero Muñoz *et al*., 1989)
	Chromotropic acid (1,8-dihydroxy naphthalene-3,6-disulphonic acid	Chromotropic acid method usually quite rapid, however, its not only need oil of vitriol as the medium but also was interfered by acetaldehyde.	(Altshuller *et al*., 1961)
	3-methyl-2-benzothiazolonehy drazone (MBTH)	MBTH is expensive and color development is relatively slow and sensitivity is limited.	(WHO, 1989)
Fluorometric determination	Hantzsch reaction		
	2,4-pentanedione (acetylacetone)	The techniques is specific, non-destructive and quantitative and allow the continuous detection, however, the requirement of large, complex and expensive instrumentation makes the method not suitable for routine applications.	(Reche *et al*., 2000)
	2,4-pentanedione (acetylacetone) with flow injection system	Require high reaction temperatures, so that high backpressure, a postcooling device or a debubbling diffusion cell are necessary to prevent the bubble generation and the increase in consequent noise.	(Sritharathikhun *et al*., 2005)
	5,5-dimethyl-1,3-cyclohexa-nedione (dimedone)	Time consuming n tedious work.	(Sawicki and Carnes, 1968)
	5,5-dimethyl-1,3-cyclohexa-nedione (dimedone) with flow injection system	Highly sensitive fluorometric FIA system with dimedone and measured gaseous formaldehyde after absorbing in aqueous solution.	(Sakai *et al*., 2002)
	1,3-cyclohexanedione (CHD)	The procedure by a batchwise method needs long reaction times and cannot be simply adopted for an automatic analysis.	(Sawicki and Carnes, 1968)
	1,3-cyclohexanedione (CHD) with flow injection	The sensitivity of CHD system is very good; LOD is 10-15 nM.	(Li *et al*., 2001)
	4-amino-3-pentene-2-1 (Fluoral-P)	Time consuming n tedious work.	(Compton and Purdy, 1980)
	Acetoacetanilide (AAA) with flow injection	An expensive instrument as a detector is needed. Moreover, organic solutions such as acetonitrile, acetone or ethanol are necessary.	(Li *et al*., 2007b)
	Methyl Acetoacetate (MA)	Need high reaction temperature	(Li *et al*., 2008)
Gas chromatography	2, 4- DNPH	2,4-DNPH can react with many aldehydes and ketones and the 2,4-DNPH derivatization reaction takes 1 h for a complete reaction.	(Dalena *et al*., 1992)

these 3 features were 23.6, 19.7 and 4.1%, respectively. The results showed that the dynamic feature was more stable. With principal component analysis, the spoilage of seafood could be easily detected. And the correct recognition rate of different octopus samples was 93.1%. The results showed that electronic nose analysis could be an efficient method for seafood quality assessment (Zhang *et al*., 2009).

Other methods of determination of formaldehyde: There are many other methods have been developed to determine formaldehyde.

The methods are colorimetric, fluorometric, gas chromatography and biosensors. Most of them were not tested on seafood but using standard formaldehyde for academic studies purpose and applied to air, textile, furniture, cosmetics and others. Some of the conventional methods to determine formaldehyde are summarized in Table 1.

ENZYMATIC METHODS

Biosensor: Biosensors can be classified in agreement to the type of involving active biological component in the mechanism or the mode of signal transduction or combination of these 2 aspects. Figure 1 shows some analytes (substrate) possible to be analyzed immobilizing the biological components, separately, in several transducers. Besides that the choice of the biological material and the adjusted transducer depends on the properties of each sample of interest and the type of physical magnitude to be measured. The degree of selectivity or specificity of the biosensor are determined by the type of the biocomponent. Thus, the biological recognizers are divided into 3 groups: biocatalytic, bioaffinity and hybrid receptors (Mello and Kubota, 2002).

Biological transducers can be immobilized on a solid support in many ways. Methods for immobilization of the biological component such as adsorption, cross linking, covalent bonding, entrapment, encapsulation and others

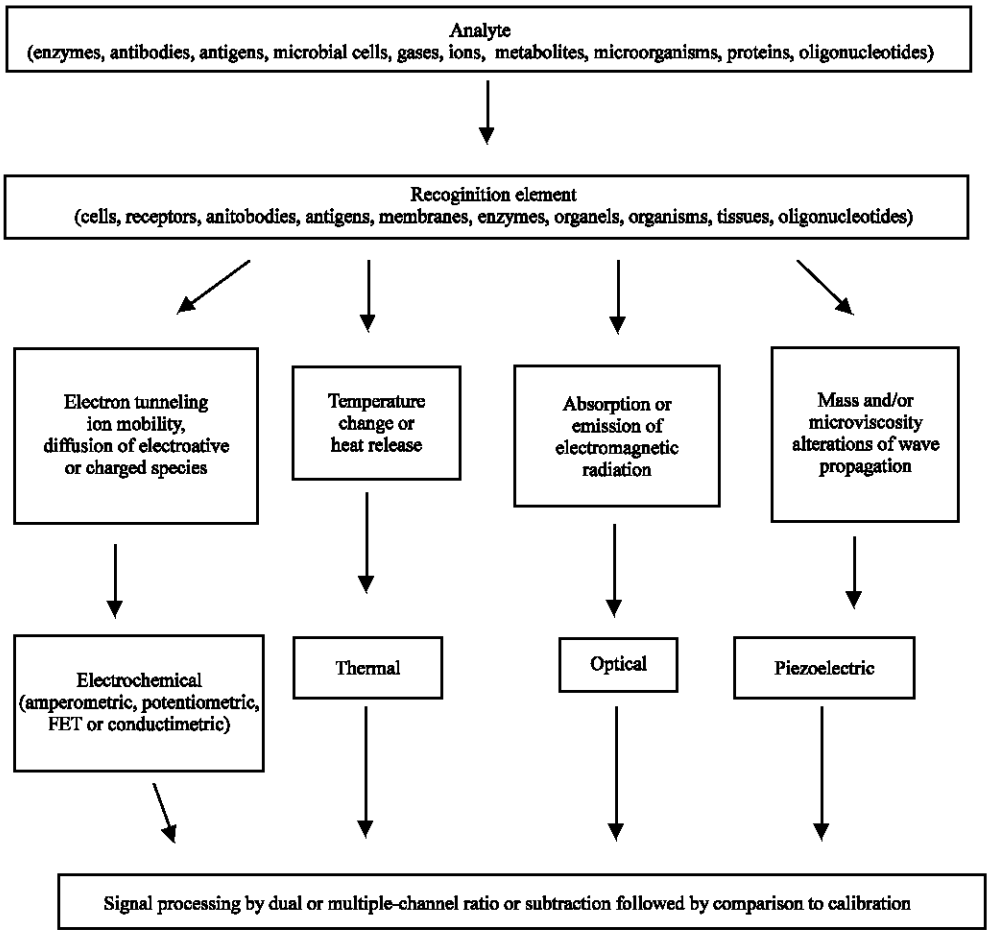

Fig. 1: A scheme shows the biorecognition and transducer employed in the construction of formaldehyde. Source: Mello and Kubota (2002)

as use of solid binding matrices. The immobilization matrix may function as a support or may also be concerned with mediation of the signal transduction mechanism.

The aim of any immobilization method is to retain maximum activity of the biological component on the surface of the transducer (Mello and Kubota, 2002).

The selection of an appropriate immobilization method depends on the nature of the biological element, type of the transducer used, physicochemical properties of the analyte and operating conditions for the biosensor system (Luong *et al*., 1988). The most common methods used for the immobilization of biocomponents are adsorption and covalent bonding. In this system, the activity of the biological component for a substrate can be monitored by the oxygen consumption, hydrogen peroxide formation and changes in NADH concentration, fluorescence, absorption, pH change, conductivity, temperature or mass. Thus, the biosensor can be categorized in several types according to the transducer (Fig. 1): potentiometric (Ion-Selective Electrodes (ISEs), Ion-Sensitive Field Effect Transistors (ISFETs)), amperometric, impedimetry, calorimetric, optical and piezoelectric transducers. In fact, many biosensors used for food analysis are based on oxidase systems like an aerobic microorganism in combination with electrochemical transducers, in particular, amperometry devices (Mello and Kubota, 2002).

According to the IUPAC definition, an electrochemical biosensor is a self-contained integrated device, which is able to provide specific quantitative or semiquantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct and spatial contact with the transduction element (Thévenot *et al*., 2001). Biosensors based on electrochemical transducer have the advantage of being economic and present fast response, thus, the possibility of automation allows application in a wide number of samples (Luong *et al*., 1988).

The determination of formaldehyde using biosensors have been developed such as biosensors based on cells (Korpan *et al*., 1993; 2000) or enzymes, either Alcohol Oxidase (AOX) (Korpan *et al*., 1997; 2000;

Dzyadevych *et al*., 2001) or Formaldehyde Dehydrogenases (FDH) (Herschkovitz *et al*., 2000; Kataky *et al*., 2002). The measurement of formaldehyde is based on enzymatic oxidation of formaldehyde by means of formaldehyde dehydrogenase to formic acid with ensuing reduction of NAD^+ as electron transfer to NADH. Then reduced NADH is oxidized to NAD + by electrochemical reaction, thus, obtaining the oxidizing current of its reduction form at the electrode.

Enzymatic reaction:

$$HCHO + NAD^+ + H_2O \xrightarrow{FDH} HCOOH + NADH + H^+$$

Electrochemical reaction:

$$NADH \rightarrow NAD^+ + H^+ + 2e^-$$

A number of sensor approaches for the detection of formaldehyde concentration have been published including systems operating in gas (Dennison *et al*., 1996; Hämmerle *et al*., 1996; Vianello *et al*., 1996) and organic phases. Voltammetric devices (Kettrup, 1993) and chemosensors based on graphite paste electrode modified by palladium, have been proposed and also optical biosensor (Rindt and Scholtissek, 1989). Recently, a new polarographic method has been developed for the determination of formaldehyde traces by direct *in situ* analyte derivatization with (carboxymethyl) trimethyl ammonium chloride hydrazide (Girard T-reagent) (Chan and Xie, 1997). The drawback of this method is the requirement for expensive apparatus as well as the necessity to remove oxygen traces by sparging with pure nitrogen.

Potentiometric: In biosensors based on potentiometry, a membrane or sensitive surface to a desired species generates a proportional potential to the logarithms of the concentration of the active species, measured in relation to a reference electrode. The potentiometric devices measure changes in pH and ion concentration and it is possible to use transistors as electric signal amplifiers coupled to ISE, called ISFET. These biosensors are based on the immobilization of a biological active material, enzymes, on a membrane, on the surface of a transducer as ISE that answers for the species formed in the enzymatic reaction. The strength of the electrical field at the membrane surface controlled by the conductivity of the n-channel region in the p-type silicon and is measured by application of a voltage between the source and drain electrodes. The solution-membrane interface should remain ideally polarized and impermeable to the passage of charge for proper functioning. If failure to meet this criterion, it results in poor sensitivity (Deshpande and Roco, 1994).

For formaldehyde detection, consisting of pH sensitive field effect transistor as a transducer and either the enzyme alcohol oxidase (in a highly or partially purified form), or permeabilised yeast cells (containing AOX), as a biorecognition element have been described Korpan *et al*. (2000).

This biosensor has demonstrated a high selectivity to formaldehyde with no interference response to methanol, ethanol, glucose and glycerol. All the results obtained with methanol look rather unusual because it is the preferred substrate for most Alcohol Oxidase (AOX), being directly oxidized to formaldehyde, as shown in the first stage of reaction in Fig. 2 (Korpan *et al*., 2000).

According to the second step of the reaction, it would be expected that the formaldehyde produced from methanol (first step of reaction would be oxidised giving a signal from the biosensor. The absence of a measurable response to methanol may be due to:

- The rate of methanol oxidation in AOX reaction is about 10-fold higher than formaldehyde (Kato *et al*., 1976; Van Der Klei *et al*., 1990). So methanol may cause competitive inhibition of formaldehyde oxidation. Indeed, the results obtained with the mixture of methanol (or ethanol) and formaldehyde in equimolar (10 mM) concentrations show the evidence of the competitive inhibition-real time response of all biosensors developed appeared to be negligible
- As in trigliceride assay system, effective oxidation of methanol is likely to result in the local oxygen depletion in the bioactive zone limiting the oxygen available for formaldehyde oxidation (Shephard and Whiting, 1990) using glycerol-3-phosphate oxidase (these 2 explanations may be also attributed to ethanol in equimolar mixture with, which formaldehyde cannot be detected as well)
- Formaldehyde produced from methanol can diffuse without oxidation from the bioactive zone back into the bulk solution (Korpan *et al*., 2000)
- Formaldehyde, being very reactive, thus reducing its amount for further oxidation. It is likely to bind covalently with NH_2-groups of AOX (all sensors) or cell constituents (the cell sensor). The spontaneous reaction of formaldehyde with amino groups of proteins is well known (Tome *et al*., 1985) and has been discussed above for other amines. All these factors may result in a decrease of the concentration of formic acid produced from formaldehyde in bioactive membranes to a level less than the sensitivity of the biosensors and therefore, no response to methanol is apparent (Korpan *et al*., 2000)

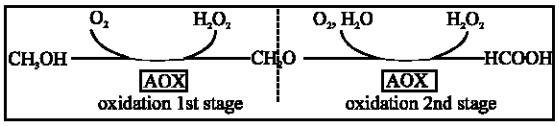

Fig. 2: Formaldehyde reaction catalyze by Alcohol Oxidase (AOX). Source: Korpan *et al.* (2000)

Amperometric: In the amperometric biosensors, the current produced is measure for the chemical reaction of an electroative species to an applied potential, which is related to the concentration of the species in solution. The amperometric biosensor is fast, more sensitive, precise and accurate than the potentiometric ones because it is not necessary to wait until the thermodynamic equilibrium is obtained and the response is a linear function of the concentration of the analyte. However, the disadvantages where selectivity of the amperometric devices is only governed by the redox potential of the electroative species present. As the result, the current measured by the instrument can include the contributions of several chemical species (Mello and Kubota, 2002).

On food analysis, the majority of the electrochemical biosensors are based on the amperometric in combination with oxidases enzyme. Amperometric electrodes and enzymes have shown good results because the enzymatic react with their substrates and the facility to measure, associated with high sensitivity. Among the amperometric, transducers that are based on the measuring of hydrogen peroxide present a higher sensitivity than those with detection of the oxygen consumption (Mello and Kubota, 2002).

The amperometric (Winter and Cammann, 1989; Hall *et al*., 1998) biosensors have been suggested for the determination of formaldehyde level. Intact and permeabilized yeast cells were tested as the biorecognition elements for amperometric assay. The mutant C-105 (gcr1 catX) of the methylotrophic yeast *Hansenula polymorpha* with a high activity of AOX was chosen as the biorecognition. Different approaches were used for monitoring FA-dependent cell response including analysis of their oxygen consumption rate by using a Clark electrode and assay of oxidation of redox mediator at a screen-printed platinum electrode covered by cells entrapped in Ca-alginate gel (Khlupova *et al*., 2007).

Bi-enzyme biosensor using diaphorase from *Bacillus stearothermophilus*, nicotinamide adenine dinucleotide (NAD^+)- and glutathione (GSH)-dependent formaldehyde dehydrogenase (FDH) from the genetically-engineered methylotrophic yeast *Hansenula polymorpha* as bio-recognition elements have been developed for determination of formaldehyde (Nikitina *et al*., 2007). NAD (P)-dependent dehydrogenase-based biosensors have possess several advantages, which make them an obvious candidate for formaldehyde monitoring. One advantage is that molecular oxygen is not involved in the enzymatic reaction, thus it does not interfere with the result of the detection. However, they display one main drawback, namely, the enzymatically generated reduced cofactor NAD (P)H must be efficiently reoxidized (Lobo *et al*., 1997). As early as in 1975, it was shown that the electrocatalytic oxidation of NADH could be measured electrochemically (Blaedel and Jenkins, 1975). However, this electrocatalytic oxidation needs a high over-potential, where unwanted side reactions in combination with electrode fouling can occur and therefore was not highly suitable for biosensing applications (Gorton and Domínguez, 2002).

Therefore, in order to improve NAD (P)H-electrooxidation, 2 types of osmium redox polymers were tested. Both, poly (vinylpyridine)-[osmium- (N, N'-methylated-2, 2'-bimidalzole)$_3$]$^{3+/2+}$ complex (PVP-Os) and poly (1-vinylimidazole) complexed with [Os (4, 4' dimethylbipyridine)$_2$ $Cl^{3+/2+}$ (PVI-Os) have been successfully used to wire a series of different redox enzymes and were applied for the construction of many biosensors (Nikitina *et al*., 2007). However, it has disadvantages where it is tedious and time consuming work especially in the preparation during enzyme immobilization.

Reagentless formaldehyde-selective amperometric biosensors were also developed based on NAD^+- and glutathione-dependent formaldehyde dehydrogenase isolated from a gene-engineered strain of the methylotrophic yeast Hansenula polymorpha. Electron transfer between the immobilized enzyme and a platinized graphite electrode was constructed using a number of different low-molecular free-diffusing redox mediators or positively charged cathodic electrodeposition paints modified with Os-bis-N, N-(2, 2'-bipyridil)-chloride ((Os (bpy)$_2$Cl)) complexes (Demkiv *et al*., 2008).

Besides that combinations of enzyme and nanoparticles have been also suggested to determine formaldehyde. In this research, quantum-sized CdS nanocrystals were synthesized using a quaternary water-in-oil microemulsion and immobilized onto gold working electrode by self-assembled monolayers techniques. Formaldehyde dehydrogenase, the enzyme was covalently immobilized onto a protecting membrane, which was stratified on part of the semiconductor

nanoparticles modified electrode. The role of covalent enzyme immobilization is to improve the stability of the catalytic oxidation of formaldehyde, which occurs after light stimulation of the semiconductor through the electron/hole recombination. A study about the best electrochemical oxidation potentials under different flow conditions was performed and preliminary sensor stability and interferences tests were also carried out, for a sensitive and selective detection of formaldehyde. A detection limit of 41 ppb of formaldehyde was calculated and an operational stability of 6 h was achieved under flow conditions. This novel amperometric biosensor based on FDH-semiconductor hybrid systems, not requiring NAD^+/NADH as charge transfer in the enzymatic reaction (Vastarella and Nicastri, 2005).

However, there are several serious problems restricting wide commercial application of these biosensors. In the case of formaldehyde dehydrogenase-based biosensors such difficulties arise from the necessary addition of a cofactor enabling formaldehyde conversion and of electrochemical mediators (Hall *et al*., 1998).

For methylotrophic yeast-based biosensors, the difficulties are caused by the dramatic dependence of the biosensor response on buffer capacity and insufficient storage stability (Korpan *et al*., 1993). Moreover, chemo-as well as biosensor systems often are not quite selective.

Capacitance-based: A new formaldehyde-selective biosensor by capacitance versus voltage and impedance measurements using bi-layer bio-recognition membrane have also been developed. As transducers, gold electrodes SiO2/Si/SiO2/Ti/Au and Electrolyte-Insulator-Semiconductor Si/SiO2 (EIS) structures have been used (Ben Ali *et al*., 2006).

As a bio-selective element, recombinant FDH isolated from the gene-engineered strain of the thermotolerant methylotrophic yeast *Hansenula polymorpha* Tf 11-6 was used as biorecognition (Van Dijk *et al*., 2000). On the contrary to bacterial FDH produced from *Pseudomonas putida* (Tanaka *et al*., 2003; Fujii *et al*., 2004), yeast FDH is dependent on 2 low molecular negatively charged compounds-NAD and glutathione (Schütte *et al*., 1976).

Therefore, to solve the cofactors problem, NAD^+ and reduced Glutathione (GSH) were non-covalently incorporated into a bio-selective sensor membrane in high concentrations (Ben Ali *et al*., 2006), which supply the sufficient levels of the cofactors for a great number of assays. An additional negatively charged Nafion membrane covering the enzyme- and cofactors-containing one supported the high levels of the cofactors in biomembrane, which creates a diffusion barrier preventing a leakage of the cofactor from the bioactive zone of biosensors. However, the detection limit using yeast is lower than bacteria with comparison of 10 μM (Ben Ali *et al*., 2007) and 1-100 μM (Ben Ali *et al*., 2006) It also has been shown that formaldehyde can be detected within a concentration range from 1 μM to 20 mM (bacteria) 10 μM to 20 mM (yeast).

Besides that the key problems which seriously hamper successful commercialization of the NAD^+-based biosensors developed earlier and their wide usage are necessity to add exogenous cofactor (NAD^+) into the samples to be analyzed, to incorporate into biologically active membrane of sensors covalently bounded NAD^+ and to supply the analytical systems by NAD^+-regeneration system (Ben Ali *et al*., 2007).

Conductometric or impedimetry: Conductimetric biosensors are based on the principle of change of conductivity of the medium when microorganisms metabolize uncharged substrates, such as carbohydrates, to intermediates, such as lactic acid. The changing was measured to detect small changes in the conductivity of the medium between 2 electrodes. The amount of charged metabolites is directly proportional to the growth rate of the organism and it is easily quantifiable. Moreover, many biological membrane receptors may be monitored by ion conductometric or impedimetric devices using inter digitated microelectrodes. However, conductimetric biosensors are usually non specific and have a poor signal/noise ratio and therefore have been little used (Mello and Kubota, 2002).

Meanwhile, a conductometric enzyme biosensor for determination of formaldehyde in aqueous solutions using interdigitated thin-film planar electrodes and immobilised alcohol oxidase from *Hansenula polymorpha* has been developed. The operational stability was not <20 h (Dzyadevych *et al*., 2001) and under continuous flow conditions by an on-line system using formaldehyde dehydrogenase, the stability was increased to at least 3 month (Vianello *et al*., 2007). In the reaction, formaldehyde dehydrogenase in the presence of a cofactor, NAD^+ (oxidized nicotinamide adenine dinucleotide), catalyzes the oxidation of the stripped formaldehyde to formic acid with the consequent increase of the conductivity of the reaction medium, were NADH is the reduced form of nicotinamide adenine dinucleotide and $HCOO^-$ represents formate ion. The change of conductivity of the stripping solution due to this reaction is meauredd by a flow conductivity cell with a sensitivity of 20 μS ppm^{-1} formaldehyde and with a detection limit of 50 ppb (Vianello *et al*., 2007). An analytical characteristic for formaldehyde assay is summarized in Table 2.

Table 2: Formaldehyde assay analytical characteristics

Sensoring system	Linear dynamic range	Detection limit	Remarks	References
Bio-Sniffer	40-3000 ppb	410 ppb	Have good selectivity based on substrate specificity of enzyme	(Mitsubayashi *et al.*, 2008)
Capacitance EIS/FDH	1-25 mM	1 mM		(Ben Ali *et al.*, 2007)
Impedance Au/FDH	1 μM to 1 mM	1 μM (30 ppb)		(Ben Ali *et al.*, 2007)
Potentiometric, ISFET			The sensitivity strongly	(Korpan *et al.*, 1993;)
yeast cells-based	1-300 mM	1 mM	depends on buffer capacity	(Gonchar *et al.*, 2002)
Chemoluminescence chemosensor		1 ppm	Non selective	(Nakagawa, 1995)
Amperometric FDH	1-6 ppm	0.3 ppm	Stable functioning during 7 h	(Hämmerle *et al.*, 1996)
Potentiometric, ISFET FDH	10-200 μM	10 μM (after enrichment)	Enzyme and cofactor are added to the bulk solution	(Vianello *et al.*, 1996)
Potentiometric, ISFEt alcohol oxidase	5-100 mM	5 mM		(Korpan *et al.*, 2000)
Thin-film resistive chemosensor	100-600 ppm	122 ppm	Non selective	(Dirksen *et al.*, 2001)
Conductometric alcohol oxidase	0.1-100 mM	0.1 mM	The sensitivity strongly depends on buffer capacity and ionic strength	(Dzyadevych *et al.*, 2001)
Amperometric, FDH-mesoporous silica		1.2 μM	Selective, reusable and stable for 80 days.	(Shimomura *et al.*, 2008)
Amperometric, FDH-PVP	50-500 μM	32 μM		(Nikitina *et al.*, 2007)
Amperometric, screen-printed electrode FDH	0.1-1 mM	0.1 mM		(Kataky *et al.*, 2002)
Amperometric, FDH, fluorescence	0.5-10 μM	0.1 μM		(Kiba *et al.*, 1999)
Amperometric FDH-Pos-EA	1-500 μM	1 μM		(Herschkovitz *et al.*, 2000)
Photochemosensor	1-100 mM	0.3 mM		(Mohr, 2003)
Fluorometric algal cells-based	0.05-1 ppm	10 ppb		(Podola *et al.*, 2004)
Photoelectrochemical FDH/CdS	0.05-1 ppm	41 ppb	Authors do not use NAD in the system, so the mechanism of the response is unclear	(Vastarella and Nicastri, 2005; Curri *et al.*, 2002)
Photodiode gas chemosensor	0.04-1 ppm	0.04 ppm		(Kawamura *et al.*, 2005)
Piezoelectric chemosensor molecularly imprinted polymer	1.25-14.25 μM	2.05 μM		(Feng *et al.*, 2005)

CONCLUSION

Increasing concern over the effects of prolonged exposure to formaldehyde, coupled with the widespread use of this substance especially in seafood, drives likely the trend towards lower threshold limit values. The International Agency for Research on Cancer has concluded that formaldehyde is a potential carcinogen for animals and evidence for the carcinogenicity of formaldehyde in human beings has been reported and discussed. Consequently, the development of simple and sensitive methods for monitoring formaldehyde is of great interest from the analytical and toxicological viewpoints.

Accurate and increasingly sensitive analytical techniques are therefore, required and developed, which should be simple and suitable for on-site testing. Of the techniques reviewed, the Nash method is dangerous due to the usage of perchloric acid for extraction of the seafood and need high temperature for the system. More studies should be done to develop new extraction with low reaction temperature. AHMT method have also been also proposed but needs a very strong base as the reaction medium is not desirable especially because of the as carbonate formation. Solid Phase Microextraction (SPME)-GC-MS method based on fiber derivatisation with pentafluorobenzyl-hydroxyl-amine hydrochloride have a good advantage to be applied to seafood for determining of formaldehyde due to its ability to overcome the problem where formaldehyde is easily to volatile and be applied on site. Besides that chromotropic acid method is widely tested and therefore remains popular. However, it color development is relatively slow and sensitivity is limited. Formaldehyde kit based on color detection is now marketed but it is not really sensitive. Rhodamine B as a detection reagent offers some advantages as alternative using kinetic spectrophotometer method.

High-pressure liquid chromatography is rapidly becoming a standard laboratory technique and the sensitivity and short analysis time for the determination of formaldehyde suggests that it will become more widely used for this purpose. However, the problem associated with this method is the interference of many carbonyls substances, including acetaldehyde and acetone. If the difficulties of derivative preparation and solvent handling can be overcome, this technique is most likely to be applied on-site.

Development of electrochemical technology especially biosensor such as potentiometric, amperometric, conductormetric and capacitance widen the research world for determination of formaldehyde. However, none of them have been applied in seafood. The quantitative determination of formaldehyde in seafood as consumer rich protein source is developed and commercializes and is a trend for the future. Factors such

as stability, storage and sensitivity are still not equated in its totality, but they can be modified in the materials of electrodes, techniques of immobilization of the biocomponent, use of different mediators, addition of stabilizers and a pre-treatment of the sample, when necessary. Combining with nanoparticles such as Cadmium Sulfide (CdS) will give more advantage to increase the sensitivity and limit of detection of biosensor techniques.

Electronic nose is a newly efficient method for detection of formaldehyde and have been applied to smoked Atlantic salmon and octopus. With principal component analysis, the spoilage of the seafood could be easily detected. It is rapid and easily used, thus, it is most chosen device for the determination of formaldehyde in seafood quality. In conclusion, the electronic nose appears to be promising for the quality assessment of seafood in future.

ACKNOWLEDGEMENTS

The authors thank the Top Down Sub-Project from MOSTI of Malaysia for financial support. The authors are grateful to Associate Professor Fatimah Abu Bakar and to the anonymous reviewers for their valuable corrections and suggestions.

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