

## MiniReview

## Detection of malachite green and leuco-malachite green in fishery industry

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**Abstract**

This article summarises the current methods for total malachite green (MG) detection which is known as a sum of MG and leuco-malachite green (LMG) that has been used extensively in aquaculture as fungicide, dye color in textile and other purposes in food industries. LMG is a reducing form of MG, where the MG is easily reduced due to the photo-oxidative demethylation process. Nevertheless, the use of MG had become an issue due to its toxicity effects. Many analytical instruments such as HPLC, LC-MS/MS, GC-MS, and spectrometry have been widely used for detection of MG. However, these methods require long time sample preparation and analysis, expensive, use hazardous reagents and indirect measurements. Hence, other analytical methods which are more sensitive, safe, rapid, inexpensive and portable are required. Alternatively, biosensors promise a more sensitive and rapid detection method for MG and LMG.

**Keywords**

Malachite green  
biosensor  
toxicity  
fishery products

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**Introduction**

Malachite green (MG) is a basic triphenylmethane dye with a molecular weight of 327. IUPAC name of MG is [4-[(4-dimethylaminophenyl)-phenylmethyidene]-1-cyclohexa-2, 5-dienylidene] dimethylazanium with chemical formula  $C_{23}H_{25}N_2^+$  (Liu *et al.*, 2009). MG has a high solubility in acidic organic solvent and lipid but less in water. MG is easily reduced into its reducing form, a leuco-malachite green (LMG). This dye is deactivated by light and may be reduced into LMG by photo-oxidative demethylation (Mitrowska *et al.*, 2007). The chemical structure of LG and LMG are shown in Figure 1. Ionization constant (pK) of MG is 6.90 in which being 0% ionized at pH 10.1, 50% at pH 6.9 and 100% at pH 4 (Srivastava *et al.*, 2004).

MG is commonly used as a dye in silk, jute, wool, cotton, leather, paper and acrylic industries since 1933. It is also used as food coloring agent and food additives (Liu *et al.*, 2009). In addition, MG is used as biological staining agent for microscopic analysis of tissue and cell samples, as well as direct endospores cells staining. The used of MG in

aquaculture industries is mainly caused by its easy availability, effectiveness, inexpensive and less restrictive to laws (Brandt *et al.*, 2004). MG has been found to be effective against white spot disease and ciliates (Wong and Cheung, 2009) and other disease in fish, fish eggs and crayfish (Sudova *et al.*, 2007). It is act as anti-parasitic, anti-fungal, anti-protozoan and plays a role in controlling skin and gill flukes (Liu *et al.*, 2009).

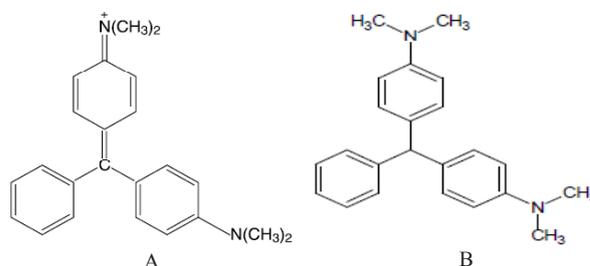


Figure 1. Chemical structure of Malachite Green (A) and Leuco-Malachite Green (B) (Liu *et al.*, 2009; Sudova *et al.*, 2007)

The use of MG, especially its reducing form (LMG) may pose potential hazard to human health because it is mutagenic and carcinogenic. LMG is also known as p,p'-benzylidenebis-N,N-dimethylaniline or 4,4'-Benzylidenebis (N,N-dimethylaniline),

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$C_{23}H_{26}N_2$  (Bergwerff *et al.*, 2004). LMG is very toxic to aquatic organisms as it is deposited in fatty tissue and remained for more than ten months after treatment (Jiang *et al.*, 2009). LMG is found in high concentration in liver and gall bladder (Sudova *et al.*, 2007). Furthermore, LMG will be slowly oxidized back to MG during storage or freezing of fish tissues (Stammati *et al.*, 2005).

The use of MG in food products has been prohibited in USA and European countries since 1983 (Jiang *et al.*, 2009). Committee on the Food and Animal Health of the European Commission stated that the minimum required performance limits (MPRLs) for total MG and LMG concentration is 2  $\mu\text{g}/\text{kg}$  (Sudova *et al.*, 2007). Due to this problem, detection and determination of total MG and LMG in aquaculture products are necessary. The current analytical methods for detection of total MG and LMG are HPLC, GC-MS, LC-MS/MS and spectrometry with a few type of detector (Wong and Cheung, 2009).

#### *The use of MG in aquaculture products*

High demand in fish, prawn and crab as protein rich food had aggravated the production of those commodity as well as other fisheries products. At the same time, the use of chemicals agents had also increase for preventing and controlling the disease in aquaculture products. MG is one of the most used chemicals agents to meet those purposes since 1993 (Rahman *et al.*, 2005). However, MG is classified as a Class II Health Hazard and show a significant health risk to humans through consumption of the fish that contain MG residues. In addition, MG is temperature stable and thus may not be degraded during routine fish processing (Mitrowska *et al.*, 2007).

The use of MG in fish farm is illegal and has been banned since May 1990 in Denmark and 1992 in Canada (Sudova *et al.*, 2007). Beside that, European United has banned the use of MG in food product in 2000. Although no allowable limit is determined, Czech Republic has stated that the fish withdrawal period is six month after treatment before sell at the market. In 2002, the largest numbers of positive tests of MG in aquaculture products were observed in Ireland followed by France, Austria and United Kingdom. However, in 2003, the number of positive results of MG decreased from 112 to 81 cases. Most of them are observed in United Kingdom, followed by France, Ireland and Austria (Sudova *et al.*, 2007).

In other cases, Hong Kong has imported fishes, crabs, eels and other aquaculture products from Taiwan and China in 2005, although their Health Department has found a trace of MG residues in

the products. Furthermore, United States Food and Drug Administration (FDA) have detected the MG residues in imported seafood from China in year 2006. Consequently, the Food and Drug Administration has blocked the importation of several type of seafood in June 2007 (Jiang *et al.*, 2009).

#### *Toxicological effects of MG*

MG and LMG are both toxic to aquatic organisms and human. Previous study demonstrated that these dye can be easily absorbed by fish tissues when it is entering water cycles and was reduced to LMG which is higher persistent than MG (Bauer *et al.*, 1988). They may influence the immune and reproductive system. It also carcinogenic, mutagenic, teratogenic, chromosomal fractures and also reduce fertility in fish such as rainbow trout. MG is sometime acts as a respiratory enzyme poison and may damage the cell ability to produce energy for metabolic processes in fish tissues (Srivastava *et al.*, 2004; Stammati *et al.*, 2005; Mitrowska *et al.*, 2005).

MG and LMG are high in fatty fish whereas the distribution of LMG is depends on the fat content in the fish tissue (Jiang *et al.*, 2009). Beside that, MG is highly cytotoxic to mammalian cells and act as liver tumor enhancing agent. In addition, fish treated with MG may have moderates regressive changes on gills and also moderates dystrophic changes in parenchymatous tissue. It also increases activation of macrophage (Sudova *et al.*, 2007), give abnormalities to head, spinal, fin and tail as well as delay the hatching time of rainbow trout (Srivastava *et al.*, 2004). The United States Food and Drug Administration stated that MG is carcinogenic chemical (Liu *et al.*, 2009) and cultural medium that contain 0.1 mg/L of MG might pose a lethal effect to fish (Baskaran *et al.*, 2011).

The absorption rate and side effects of MG may differ for different fish and fish eggs species. It may show a high mortality, anemia disease, lower weight gains and high possibility for tumors in rainbow trout. Meanwhile, it shows a cytostatic syndrome, a disruption of the chromosomal division process occurs in cyprinid fish. Beside that, it may slower the regeneration of damage gill epithelium but activate the reticulum endothelial system. Furthermore, it may cause inflammatory cells and high haemosiderosis in spleen and kidney (Sudova *et al.*, 2007). In rats and mice, they eat less, show decreasing in fertility and growth rates; may have some alterations in spleen, kidney, liver and heart; impose lesions on eyes, bones, and lungs and skin (Werth and Boiteaux, 1967; Culp *et al.*, 1999).

Other effects of the use of MG are restlessness

and uncoordinated movements of the fish in the tank followed by the loss of balance, apathy, agony and finally will die. Intoxification in fish was observed by a greenish tinge of fish skin, increased production of skin slime and oedematous gills with excessive amounts of mucous matter (Srivastava *et al.*, 2004). Toxicity of MG will increase with the decreasing of the pH and the increasing of temperature and exposure time (Theron *et al.*, 1991). MG also causes some critical modification in blood and increases the total levels of cholesterol in catfish (Yildiz and Pulatsu, 1999). Beside that, the presence of MG in fish may change metabolism of carbohydrate and osmoregulation, and also change the hepatic and muscle glycogenolysis (Tanck *et al.*, 1995; Srivastava *et al.*, 1996).

#### *Regulation for the use of LG and LMG in fishery industries*

The use of MG and LMG in aquaculture is more restricted in European countries, Canada and United States compared to that in Asian countries such as Malaysia, Thailand and Indonesia. Regulations and law that are commonly referred for MG and LMG residues are Commission of Codex Alimentarius, Commission of the European Communities (EU) and National Registration Authority (NRA) (Tang and Choi, 2005). The Codex regulation and law in food has specific criteria including toxicological information, analytical and intake data, technological consideration and also risk assessment and risk management consideration. Toxicological information of MG and LMG are needed including toxicokinetics and toxicodynamics, acute and long term toxicity and integrated toxicological information (acceptability and safety intake levels of contaminants) (Codex Alimentarius, 1995).

Maximum residue limit is the maximum concentration of MG and LMG residue which are legally permitted by the Community as acceptable in or on a food. For veterinary medicinal products include of MG and LMG residues, maximum residue limits (MRLs) are established according to the procedures laid down in Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009. Meanwhile maximum levels for contaminants are laid down in Commission Regulation (EC) 1881/2006. According to the Annex to Commission Decision 2002/657/EC, minimum required performance limits (MRPLs) is a minimum content of an analyte which is detected and confirmed in a sample. The MRPLs of MG and LMG residue in meat and seafood products that are established by Commission Decision 2004/25/EC

is 2.0 µg/kg (European Commission, 2007). Other regulation and law for use and detection of MG and LMG residues is by National Registration Authority (NRA). NRA Residue Guideline No. 26 is commonly used for Veterinary drug residue analytical methods. Nowadays, many countries are preferred to follow regulation and law of the Commission of the European Communities (EU) which is simpler but still meet the requirement of food safety. Furthermore, United States and European Union have been set the maximum residue limits for antimicrobial agents such as MG and LMG in foods by zero tolerance policy. Current US Food and Drug Administration detection levels of prohibited MG as antimicrobial agent (veterinary drug residue) in Seafood have been established as 1.0 µg/kg (Collette, 2006).

#### *Methods for the detection of MG and LMG*

MG residues had been found in many aquaculture products and it become a crucial problem when enter the human body through eating. Therefore, the detection of total MG and LMG are necessary to monitor the use of this chemical. To date, a several analytical methods that currently use are high performance liquid chromatography (HPLC), liquid chromatography with tandem mass spectrometry (LC-MS/MS), LC-TOF-Mass, capillary electrophoresis, electrochemistry, gas chromatography with mass spectrometry (GC-MS) and spectrophotometer (Lee *et al.*, 2007). Atmospheric pressure chemical ionization (APCI) or electro spray ionization and isotope dilution approaches (<sup>13</sup>C<sub>6</sub>-LMG and <sup>2</sup>H<sub>5</sub>-MG) are also used to determine MG residues (FAO/WHO Expert Committee, 2008).

The total MG and LMG residue in eel's plasma has been detected by Hajee and Haagsma (1995) using HPLC with post oxidation column that contain of PbO<sub>2</sub>. Tarbin *et al.* (1998) had detected MG and LMG in trout muscle using HPLC-Vis and ESP-LC-MS with Columbus C18 column. Visible detection probe contain lead (IV) oxide has been used as a detector for both instrumentation methods with a limit of detection of 5 µg/kg (Tarbin *et al.*, 1998). Beside that, Brandt and her group from Danish Institute had found MG and LMG in Danish and non-Danish fish through HPLC analysis with post oxidation column contains 20% PbO<sub>2</sub> (w/w) in Hyflo Super Cell and has detected by using UV-Vis detector at wavelength 618 nm. Lee *et al.* (2007) revealed that detection limit of MG and LMG using surface-enhanced raman microfluidic sensor is 0.6 and 0.7 µg/kg, respectively.

MG has been banned in many countries including the United States, Canada and European Union due

Table 1. Method used for detection of MG and LMG residues in aquaculture products

Detection method	Fish and its products	Reference
LC-MS/MS	Fresh water trout (caviar), shrimp	Tittlemier <i>et al.</i> (2007); Wu <i>et al.</i> (2007)
	Trout, pangasius	Scherpenisse and Bergweff (2005)
	Eel, Roasted eel meat	Ding <i>et al.</i> (2007); Wu <i>et al.</i> (2007)
	Salmon	Van de Riet <i>et al.</i> (2005); Dowling <i>et al.</i> (2007); Wu <i>et al.</i> (2007)
	Carp, trout	Tarbin <i>et al.</i> (1998); Effkemann (2007); Moller (2007)
	Edible gold fish	Lee <i>et al.</i> (2007)
	Edible fish	Zhu <i>et al.</i> (2007)
	Catfish, trout	Doerge <i>et al.</i> (1998)
LC-UV Vis	Salmon	Valle <i>et al.</i> (2005)
	Water	Allen <i>et al.</i> (1994); Meinertz <i>et al.</i> (1995); Safarik and Safarikova (2002)
	Trout and its organ	Fink and Auch (1993); Tarbin <i>et al.</i> (1998)
	Fresh flesh, egg, muscle and liver of rainbow trout	Bauer <i>et al.</i> (1988); Hormazabal <i>et al.</i> (1992); Meinertz <i>et al.</i> (1995); Swarbick <i>et al.</i> (1997)
	Eel plasma	Hajee and Haagsma (1995)
	Farming fish, river water	Pourreza and Elhami (2007)
	Fish plasma and muscle of channel fish	Plakas <i>et al.</i> (1995)
	catfish	Roybal <i>et al.</i> (1995)
	Carp and rainbow trout	Mitrowska <i>et al.</i> (2005)
HPLC-UV Vis	Eel, rainbow trout, fresh and smoked salmon	Bergweff and Scherpenisse (2003)
	Trout and catfish	Rushing and Hansen (1997)
	Fresh and deep frozen trout	Klein and Edelhäuser (1988)
LC-UV Vis or LC-MS	Chanel catfish, rainbow trout, tilapia, salmon, tiger shrimps	Andersen <i>et al.</i> (2005)
	Rainbow trout	Halme <i>et al.</i> (2007)
LC-DAD or LC-MS/MS	Edible fish	Stoev and Stoyanov (2007)
Spectrophotometer	MG standard	Barek <i>et al.</i> (1976)
	Rainbow trout	Fornier de Violet <i>et al.</i> (1995)
Partial Beam LC-MS and GC MS	catfish	Tumpseed <i>et al.</i> (2006)
LC-EC or LC-UV/VIS or LC-FD	catfish	Rushing and Hansen (1997)
ELISA	Edible fish	Yang <i>et al.</i> (2007)

to inappropriate use of MG residue as a veterinary drug to treat aquaculture fish and now routinely monitored by the Food and Drug Administrative and many other international agencies. Hence, Bergweff and Scherpenisse (2003) had successfully determined MG and LMG residues in aquatic organisms include rainbow trout, eel, prawn and canned salmon by using HPLC- reverse phase with pre-column oxidation reactor filled with lead (IV) oxide and celite. This analytical method has a limit detection of 1 µg/kg. Bergweff *et al.* (2004) has also used HPLC-reverse phase with Phenomenex LUNA phenyl-hexyl column for the detection of MG and LMG in prawn, finfish and eel. The limit of detection has been found to be 0.2 µg/kg (Bergweff *et al.*, 2004).

More analysis method has been done by researchers as their concern on the toxicity of MG and LMG. Mitrowska *et al.* (2005) has detected MG and LMG residues in carp muscle by using LC-VIS/FLD with visible and fluorescence detector. The limit detection of MG and LMG are 0.15 and 0.13 µg/kg, respectively. This analysis has done according to European United requirements and to fulfill the quality criteria of Commission Decision on 2002

which are less laboratories work and more convenient method for detection in matrix (Mitrowska *et al.*, 2005). Mitrowska *et al.* (2007) has also detected the MG and LMG residues in the same fish species sample using HPLC with a limit of detection of 0.15 µg/kg. Other MG and LMG analysis has done by Wong and Cheung (2009) by using LC-IDMS based on isotope dilution mass spectrometry. This analysis has done to swap eel sample (*monopterus albus*) by using C18 analytical column with a limit of detection of 0.4 µg/kg (Wong and Cheung, 2009). Furthermore, Jiang *et al.* (2009) has also detected MG and LMG using HPLC and LC-MS/MS. Table 1 shows the current methods for the detection and determination of MG and LMG in fish and aquaculture products.

LC-MS/MS and LC-UV/VIS are the most analytical methods use for the detection of MG and LMG. Most of researchers are used rainbow trout, salmon, eel, catfish, edible fish and carp as their sample meanwhile shrimp, pangasius, silver perch, basa, channel fish, tilapia, goldfish and shellfish are rarely use as a sample. Each analytical method has their own advantages and disadvantages based on sample type and condition for the detection of MG

Table 2. Advantages and disadvantages of analytical methods for detection of MG and LMG

Detection Methods	Advantages	Disadvantages	Reference
High Performance Liquid Chromatography (HPLC) include HPLC-Vis, HPLC-reverse phase	- As a screening and validation method which is specific and simultaneous analysis with a different detector.	- Expensive, time consuming and not adapted for in site and real time detection. - Requires highly trained personnel and unable to provide toxicity information of the sample. - Available only in sophisticated laboratories.	Scherpenisse <i>et al.</i> , 2003; Bergweff <i>et al.</i> , 2004; Anderssen <i>et al.</i> , 2005; Andreescu <i>et al.</i> , 2006; Mitrowska <i>et al.</i> , 2005, 2007, 2008
Liquid Chromatography with mass spectrometry (LC-MS/MS)	- Specific, highly selective and sensitive. - Fast analytical time, allow co-elution with a different detector. - Less laboratories and easily for the determination of MG and LMG from matrices. - Provide a highly accurate result of analysis and obey the quality criteria of Europe United Commission Decision 2002/657/EC.	- Expensive and require a long time for sample preparation. - Required experienced personnel for system maintenance and results interpretation.	Tarbin <i>et al.</i> , 1998; Bergweff <i>et al.</i> , 2004; Mitrowska <i>et al.</i> , 2005; Tang and Choi, 2005
Liquid Chromatography Ultra visible (LC-UV)	- Low cost. - Relatively sensitive at maxima wavelength	- Detect only at single wavelength - Not confirmative - Maximum wavelength of LMG at 266nm - Face interference problem	Tang and Choi, 2005.
Liquid Chromatography Diode Array Detector (LC-DAD)	- Multiple wavelength measurement. - Peak purity information.	- Relatively less sensitive. - Not confirmative compared to Tandem MS. - Need intense sample purification. - Prevent co-elution.	Tang and Choi, 2005.
Liquid Chromatography Fluorescence Detector (LC-FLD)	- High sensitivity than UV or DAD detectors. - Less background noise.	- Not confirmative. - Required intense sample clean-up. - Prevent co-elution.	Tang and Choi, 2005.
Gas Chromatography (GC) include GC-MS	- Earliest confirmatory method. - Most common MS in laboratories. - Relatively high sensitivity and selectivity than LC detector.	- Expensive and time consuming. - Not adapted for in site and real time detection. - Require highly trained personnel. - Available only in sophisticated laboratories. - MG is non volatile, thus less detectable in GC - High detection limit : 5 mg/g	Scherpenisse <i>et al.</i> , 2003; Bergweff <i>et al.</i> , 2004; Anderssen <i>et al.</i> , 2005; Tang and Choi, 2005; Andreescu <i>et al.</i> , 2006; Mitrowska <i>et al.</i> , 2005, 2007, 2008
Atmospheric Pressure Chemical Ionization (APCI)	- As a confirmation of MG and LMG analysis. - Very sensitive and selective technique. - The most efficient use of laboratory resources. - As an alternative for quantitative method with a lower limit of detection (LOD).	- Expensive and require a long time analysis. - Required highly trained personnel.	Tumipseed <i>et al.</i> , 2005 ; Valle <i>et al.</i> , 2005
Immunoassay -ELISA	- A common and near ideal rapid assay system. - It can be used in site and as a rapid test for screening large number of routine samples.	- Require a long time analysis and extensive sample handling. - Require expensive disposable plastic trays. - The detection limit, sensitivity, and reliability of the assay depend on the quality of the antibody used in a particular assay kit.	Mulchandini <i>et al.</i> , 1999; Yang <i>et al.</i> , 2007
Biosensor - include enzyme sensor, Surface Enhanced Raman micro-fluidic sensor	- A direct and real time measurement with a high specificity, sensitivity. - Provide a good stability, precision and accuracy. - Rapid, simple, user friendly operation, portable and economic. - Suitable for toxicity monitoring. - Are able to provide reliable information with a minimum sample preparation.	- Selective to certain analyte and cannot tolerate to high temperature.	Mulchandini <i>et al.</i> , 1999; Makower <i>et al.</i> , 2003; Andreescu <i>et al.</i> , 2006; Amine <i>et al.</i> , 2006; Lee <i>et al.</i> , 2007

and LMG residues (AOAC, 2008). Table 2 shows an advantages and disadvantages of current analytical methods for detection and determination of MG and LMG in fish and water.

Chromatography method of either liquid (LC) of gas (GC) combined with mass spectrophotometer is quite accurate, specific and reliable to determine MG or LMG. However, it have some limitation such as

Table 3. Biosensor method for detection of malachite green

Biosensor's Detector	Response time (min)	Limit of detection (LOD)	Reference
Multi-walled carbon nanotubes modified glassy carbon electrode (MWCNTs-GS)	5	0.006 ppb	Yi <i>et al.</i> , 2008
Multi-walled carbon nanotubes (MWCNTs)	3	2 ppb	Liu <i>et al.</i> , 2009
Fluorometric sensor with native double stranded DNA	<10	0.2 ng/mL	Cheng and Li, 2009

requires a long time sample preparation, measurement and analysis, expensive, use hazardous reagents, indirect measurements and needs highly trained person to perform the measurement. Hence, these analytical methods detection systems become less attractive but still needed (Lee *et al.*, 2007). To date, introduction of biosensor such an electrochemical method is an alternative and promise in food safety analysis which is simple, highly selective and sensitive, inexpensive and rapid response (Lee *et al.*, 2007). However, there is limited report regarding to electrochemical and biosensor determination of MG and LMG in fishery products which is needed more research. Table 3 shows biosensor with different detectors for the detection of MG and LMG in fishery products.

#### Biosensor for the detection of MG and LMG

Biosensor is an alternative method for simple, rapid, sensitive and economical measurement of contaminants such as MG and LMG residues in fishery products for on site monitoring purposes. This biosensor system comprise of transducer and bio-recognition elements such as enzymes, antibodies, nucleic acids and proteins. A transducer is responsible to convert the reactions between the bio-receptor and its target analyte into electronic signal as shown in Figure 2 (Chamber *et al.*, 2008).

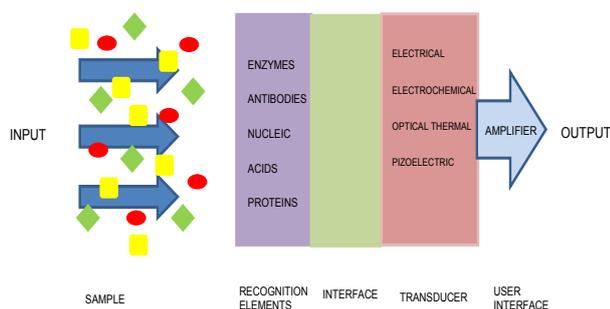


Figure 2. Configuration of a biosensor involves bio-recognition, interface and transduction elements

Biosensor based on enzyme inhibition is commonly used for detection of MG and LMG in aquaculture products where the butyrylcholinesterase enzyme (BuChE) is used as bio-recognition elements. Normally, BuChE enzyme hydrolyzes its substrate such a butyrylcholine (BC) and butyrylthiocholine (BTC) into butyric acid and choline or thiocholine. Instead of that, presence of MG and LMG inhibitor

(sample analyte) may cause an enzyme inhibition (enzyme inactivation). Therefore, the enzyme was no longer able to hydrolyse its substrate to butyric acid and choline or thiocholine as shown in Figure 3 below (Skladal *et al.*, 1992).

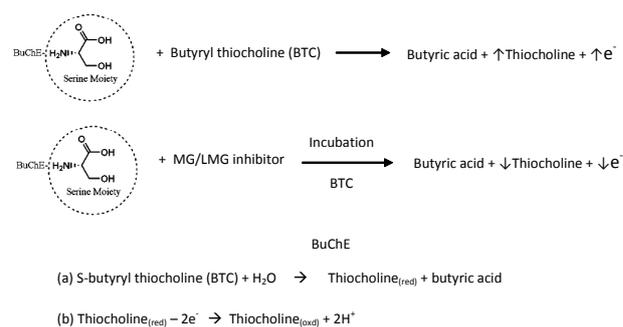


Figure 3. Principle of BuChE enzyme inhibition by MG and LMG inhibitor in the present of its substrate

Many studies have been focused to expand biosensor system for the detection of LG and LMG. Ngamukot *et al.* (2006) had developed MG and LMG biosensor system by using a flow cell of boron-doped diamond thin-film electrodes. Meanwhile, Yi *et al.* (2008) had worked on biosensor system by using multi-walled carbon nanotubes modified glassy carbon electrodes (MWNTs/GC). In addition, the voltammetry response of MG and LMG at the MWNTs/GC electrodes in the present of surfactant cetylpyridinium bromide has also been improved (Yi *et al.*, 2008; Liu *et al.*, 2009). However, most of the electrodes are measured the oxidation of MG only, which normally required oxidizing agents to oxidized back LMG (reducing form) to its parental, MG. Research reports on the development of biosensor system for the detection of MG and LMG is currently quite limited.

## Conclusion

MG and LMG residue remains for a long time in edible fish tissues and it may pose toxicity effect and harmful to human health through the food chain when consumers eat contaminated fish. The sum of MG and its metabolite LMG aggregate concentration was set at 2 µg/kg, stated as the minimum required performance limit (MPRL) that permitted in aquaculture industry either followed the EU or

CODEX limits. Previously, there has a lot of study on detection and determination of MG and LMG in fish through an analytical method such as HPLC, LC-MS/MS, GC-MS and spectrometry but it takes a long time analysis, expensive, use hazardous reagents and indirect measurements. To date, biosensors has offered more attractive detection method that are more sensitive, safe, simple, portable and rapid for analysis.

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