ENHANCEMENT OF MERCURY DEPURATION FROM COCKLES (*ANADARA GRANOSA)* UTILIZING CATALYTIC CHELATION TECHNIQUE

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ABSTRACT

Cockles, *Anadara Granosa* is one of the common shellfish consumed as a source of protein by the Indo-Pacific Asians including Malaysia. This bivalve is a filter-feeding mollusk and can be easily contaminated by different types of pollutants. Since the cockle's habitat is in the estuarine sediment, its soft tissue usually accumulated with a high concentration of toxic and heavy metals such as mercury. Cockles themselves have the ability to remove toxic metals out from their soft tissues, which is called a depuration process. However, the natural depuration process is very slow and needs to be enhanced. Developing a method that can safely enhance the depuration process and remove mercury from cockles is the main concern, thus the depuration of mercury from cockles was enhanced through this study utilizing the sodium acetate (CH_3COONa) as the chelating agent, and catalyzed by the heterogeneous alumina supported calcium oxide catalyst. The treatment optimization was performed by applying the response surface methodology (RSM) combining the pH ranging from 7 to 10, the dosage of the chelating agent from 400 ppm to 600 ppm, temperature from 29.5 to 37.5 ^{0} C, and time of reaction between 1, 3 and 5 hours. The cockle's soft tissues were prepared using the nitric acid digestion method adopted from AOAC 999.10 for the analysis of mercury content using Flow Injection Mercury System (FIMS) based on cold vapor atomic absorption spectroscopy (CVAAS). The LOD and LOQ of the method were 0.028 mg/kg and 0.090 mg/kg respectively. The results obtained as statistically analyzed and it has been found that the maximum depuration achieved at ±99% of mercury removal and the mercury content in the cockle's soft tissue could comply with the standard regulations for mercury content in food.

ABSTRAK

Kerang daripada spesis *Anadara Granosa* adalah salah satu kerang yang kerap digunakan sebagai sumber protein oleh orang-orang Asia Indo-Pasifik termasuk Malaysia. Bivalvia adalah moluska penapis yang mudah dicemari oleh pelbagai jenis bahan cemaran dan kotoran. Oleh kerana habitat kerang yang berada di sedimen estuari, tisu lembutnya cenderung untuk mengumpul kepekatan logam toksik yang berat dan tinggi seperti merkuri (Hg). Kerang sendiri mempunyai keupayaan untuk mengeluarkan logam beracun daripada tisu lembut mereka, yang juga dipanggil proses pembersihan. Walau bagaimanapun, proses pembersihan semulajadi sangat perlahan dan perlu dipertingkat. Justeru, usaha untuk membangunkan satu kaedah yang selamat bagi meningkatkan proses pembersihan dan mengeluarkan merkuri daripada kerang adalah amat diperlukan, oleh itu pembersihan merkuri daripada kerang dipertingkat melalui kajian ini dengan menggunakan natrium asetat (CH_3COONa) sebagai agen pengkelat, dan dipangkin oleh kalsium oksida yang dipadukan di atas pepejal alumina (CaO / Al2O3). Bagi mendapatkan rawatan yang optimum, kaedah permukaan tindak balas (RSM) digunakan dengan cara menggabungkan pH antara 7 hingga 10, dos ejen pengkelat dari 400 ppm hingga 600 ppm, suhu 29.5 hingga 37.5 ^{0} C, dan masa tindak balas antara 1, 3 dan 5 jam. Tisu lembut kerang disediakan mengikut kaedah pencernaan asid nitrik daripada piawaian AOAC 999.10 bagi menganalisa kandungan merkuri menggunakan Sistem Mercury Injection Mercury (FIMS) di mana fungsinya berasaskan spektroskopi penyerapan atom sejuk (CVAAS). Hasil kajian daripada kaedah LOD dan LOQ menunjukkan keputusan 0.028 mg/kg dan 0.090 mg/kg bagi setiap kaedah. Hasil kajian yang diperoleh secara statistik menunjukkan bahawa pembersihan maksimum yang dicapai pada ± 99% penyingkiran mercury dan kandungan merkuri dalam tisu lembut kerang mematuhi peraturan standard untuk kandungan logam berat dalam makanan.

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LIST OF SYMBOLS/ ABBREVIATIONS

Hg	-	mercury
RSM	-	Response Surface Methodology
pH	-	pH value
ppm	-	parts per million
ppb	-	parts per billion
FIMS	-	Flow Injection Mercury System
CVAAS	-	Cold Vapor Atomic Absorption Spectroscopy
MT	-	Metric Tons
ppt	-	parts per thousand
Cd	-	cadmium
Pb	-	lead
As	-	arsenic
Ha	-	Hectare
BBD RSM	-	Box-Behnken Design Response Surface Methodology
kg	-	kilogram
mg	-	milligram
µg	-	microgram
g	-	gram
S	-	Sulphur
N	-	nitrogen
O	-	oxygen
L	-	liter
AES	-	Atomic Emission Spectroscopy
AAS	-	Atomic Absorption Spectroscopy
FIAS	-	Flow Injection Analysis System
IDC	-	Initial Demonstration of Capability
IDL	-	Instrument Detection level

LLD	-	Lower level of Detection
MDL	-	Method Detection Level
LFB	-	Laboratory Fortified Blank
LFM	-	Laboratory Fortified Matrix
LFMD	-	Laboratory Fortified Matrix Duplicate
CCD	-	Central Composite Design
QC	-	Quality Control
LOD	-	Level of Detection
LOQ	-	Level of Quantitation
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemists
QA	-	Quality Assurance
ICV	-	Initial Calibration Verification
CCV	-	Continue Calibration Verification
CB	-	Calibration Blank
µL	-	microliter
mL	-	milliliter
h	-	hours
LOF	-	Lack of Fit
3-D	-	Three dimensional
A	-	Absorbance
RSD	-	Relative Standard Deviation
SD	-	Standard Deviation
p-value	-	Probability Value
DF	-	Degree of Freedom
C.V%	-	Variation Coefficient
Pred R^2	-	Predicted R^2
Adj R^2	-	Adjusted R^2
%	-	Percentage
℃	-	Degree Celsius

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CHAPTER 1

INTRODUCTION

The cockles, *Anadara granosa* which is locally known as 'Kerang' is one of the most favored seafood, consumed by people of coastal areas such as Malaysia. This bivalve mollusk has been cultured in Malaysia since 1948 in Perak and then distributed to the western coast of Peninsular Malaysia including from Kedah to Johor. According to Ibrahim (1995), almost 5500 hectares of grounds produced about 55500 tons of matured cockles in 1991, which indicates a high demand for cockles. Cockles are naturally cultivated in coastal areas such as Kedah, Penang, Perak, Selangor and the Johor States, furthermore, they have been found to be cultivated in the sheltered coastal areas such as Pahang as well as along the west coast of Peninsular Malaysia. The production of cockles in 1980 was about 123,862 MT but decreased to 45,664 MT in 1986, due to overfishing and environmental problems (FAO, 2019).

1.1 Background of Study

Countries with a large population living in coastal areas, are the major consumers of seafood as a high source of protein. Furthermore, animal sources of protein are not sufficient as the population is increasing day by day. So turning to seafood is a need for humans to ensure the completion of their diet (Abbas Alkarkhi *et al.*, 2008). Since Malaysia is one of the countries with lots of coastal areas, aquaculture has been started here in the early 1920s. Marine shrimp trapping first developed in Johor in the 1930s followed by culture of blood cockles (*Anadara granosa*) at the 1940s in Perak and has been developed into significant aquaculture industry in Malaysia.

Culture of cockles is mostly limited to the coastal areas of Kedah, Penang, Perak, Selangor and Johore (FAO, 2019). The level of annual production of these bivalves in Malaysia is listed in Table 1.1 from the year 1980 to 1986 which indicated a reduction in the production of shellfish during that period of time due to the number of obstacles such as overfishing and environmental problems.

Table 1.1 Annual production of commercially important shellfish in Malaysia, 1980–1986 (FAO, 2019)

SPECIES	YEAR						
	1980	**1981**	**1982**	**1983**	**1984**	**1985**	**1986**
Anadara granosa	123,862	72,743	57,530	44,789	68,231	44,761	45,664
Paphia undulata	3,597 A	3,597 A	3,597	3,828	3,704	2,016	357
Perna viridis	2,121	2,211	3,755	2,005	629	604	709
Crassostrea sp.	3	3	4	2	10	10 B	10 B

Cockles, *Anadara granosa* is a marine bivalve mollusk which can live in a water depth of 20 m but mostly found in the littoral areas with relatively low salinity, they are filter feeders and mostly feed on organic detritus, phytoplankton and algae (Andayani and Sumartono, 2012). Cockles are seasonal organisms which mostly available from August to February and they can adapt in an environment with a salinity of 14-300 ppt and temperature range from 20-30 ℃(Broom, 1982). The intertidal zone of the sea with a salinity of around 10-32 ppt is a suitable place for the cultivation of cockles and normally harvesting of cockles is started when it reaches a size of 24-30 mm (FAO, 2019).

Pollution of water and contamination of marines are of a major concern since Malaysia is going for industrial development rather than agriculture and mining, thus a large number of industrial pollutions which contains poisonous chemicals especially heavy metals is going to water streams and is the main reason for seafood contamination by heavy metals (Abbas Alkarkhi *et al.*, 2008). The dosage of trace heavy metals in the marine ecosystem is damn low. However, according to Kahle and Zauke (2002), the marine bivalves including cockles are capable of concentrating

heavy metals in their tissues to a million times compared to their concentration in the habitat.

Cockles are one of the marine mollusks which is widely consumed in Malaysia. These bivalves are easy to trap, a high biological value source of protein, vitamins, minerals, and are ample. They can be easily polluted by effluents coming from anthropogenic activities, sewage discharge, shipping activities, agricultural activities, and other contamination sources. As long as heavy metals coming from industrial effluents or by natural means is harmful and even can be carcinogenic, thus it is a big concern for humans as the consumers of seafood (Kahle and Zauke, 2002).

Mercury is one of the most toxic heavy metals which is a shiny silver-white odorless liquid and is very poisonous and cumulative to the living organism cells especially the marine environment. Because mercury is a highly toxic metal, it harms most of the organs such as the brain, kidneys, and muscles, furthermore, it disrupts the membrane potential and intracellular calcium homeostasis. Mercury becomes a colorless gas when heated and the vapors can cause bronchitis, asthma and brief respiratory problems (Jaishankar *et al.*, 2014). Humans' exposure to mercury is normally through dental amalgam and the diet, mostly from marine products which are the main sources of methylmercury in the diet. Several metabolic pathways can transform mercury into other types by oxidation of mercury to divalent mercury, reduction of divalent mercury to metallic mercury, methylation of mercury, which all are toxic and harmful for human organs (Rahman *et al.*, 2000).

1.2 Statement of Problem

Cockles are one of the most extensively used seafood in Malaysia and are popular in coastal areas, also they are cheap sources of protein and can be a proper alternative for animal proteins in tropical areas, furthermore, cockles can be exported to the European countries which are a big consumer of seafood. Therefore because of the excessive use of cockles and the increase of population in cities, the demand for

cockles had been raised and almost 5000 ha of mudflats along the west coast of Malaysia has been used for semi-culture of cockles (Abbas Alkarkhi *et al.*, 2008).

Since Malaysia is more to industrial development so there are a significant amounts of wastes from these industrial activities and even by natural means which contains toxic heavy metals and discharge of these byproducts especially heavy metals such as mercury to water stream produce a considerable environmental contamination, by which the seafood including cockles will be polluted by mercury, furthermore cockles are lower feeding organisms and have the ability of absorbing mercury from sediment and accumulating on their tissues which can be harmful for health (Ibrahim, 1995).

Although contamination of cockles by heavy metals especially mercury which is a potentially toxic heavy metal, is a big concern among environmental researchers, but still no commercially applicable research has been concentrated on a method for removal of mercury from cockles, thus this research utilizes sodium acetate trihydrate, $CH_3COONa.3H_2O$ as a chelating agent catalyzed by heterogeneous catalyst to enhance depuration of mercury from cockles to meet the EU standards and Malaysian Food Regulations (1985). Moreover, the optimization for the factors affecting mercury removal was done by using the BBD RSM method.

1.3 Objectives of the Study

i. To validate FIMS (Flow Injection Mercury System) for determination of mercury in cockles.
ii. To enhance mercury depuration from cockles utilizing chelation agent.
iii. To optimize the removal of mercury from cockles using response surface methodology (RSM).

1.4 Scope of the Study

This study was conducted to investigate the enhancement of mercury depuration from cockles, *Anadara granosa* by catalytic chelation technique. Sodium acetate trihydrate, $CH_3COONa.3H_2O$ was used as a chelating agent and calcium oxide on an alumina support (CaO/Al_2O_3) was used as a catalyst (Azelee *et al.*, 2014). Optimization of the parameters for higher removal efficiency of mercury was done by the Box-Behnken design of response surface methodology.

The sample was provided from the wet market and stored at 4℃ prior to treatment. Sample preparation was done using the wet digestion method by nitric acid, HNO_3 and hydrogen peroxide, H_2O_2 and flow Injection Mercury System (FIMS) which works based on cold vapor technique is validated for the determination of mercury in cockles through this study.

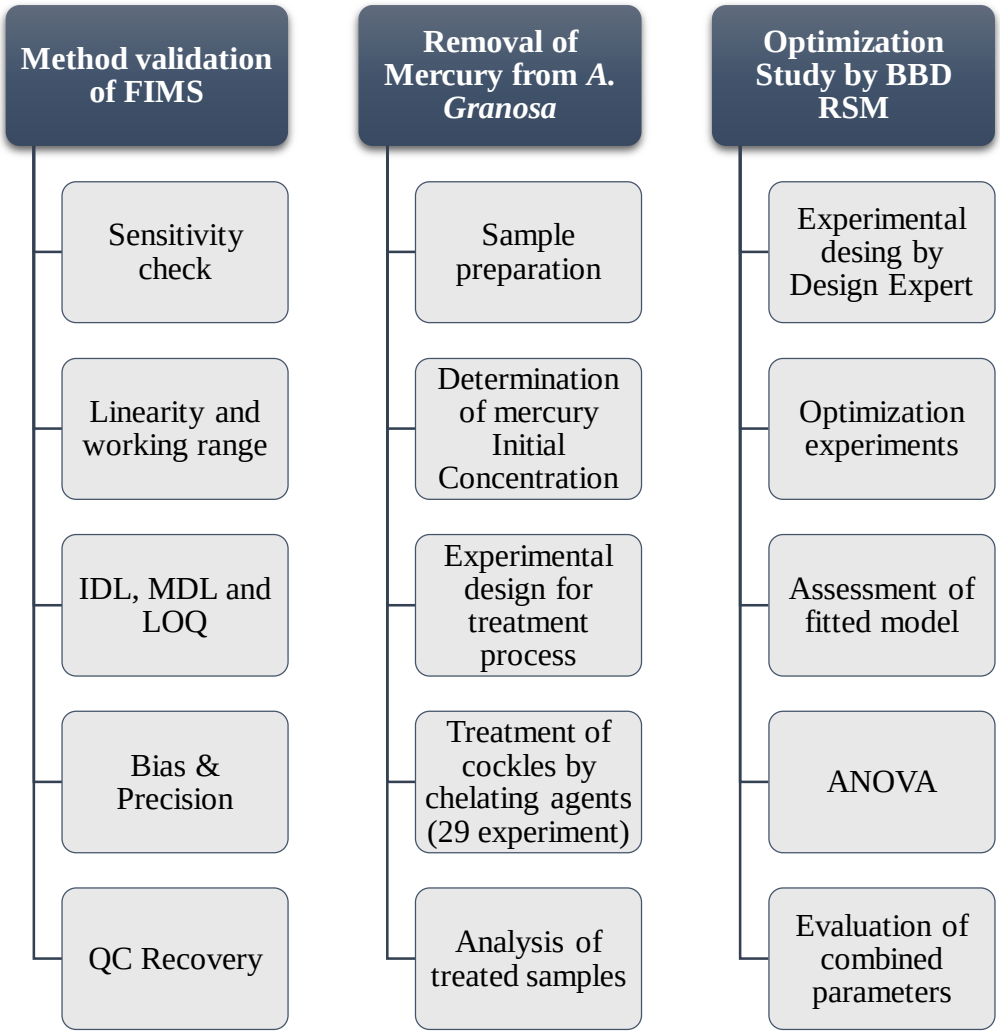

Figure 1.1 Conceptual design of the experiment

1.5 Significance of the Study

Because of the rapid industrial improvements in Malaysia, there is a high risk of loading toxic heavy metals into water stream and removal of these toxic and heavy metals such as mercury is of a high concern, because it diminishes the quality of marine bivalves which can be exported to other countries, moreover cockles (*Anadara granosa*) has the ability to absorb toxic heavy metals especially mercury, and accumulate them on its tissues, which is hazardous and even carcinogenic for humans,

thus it is highly needed to have a method for treatment of cockles from mercury to increase the cockles' quality and ensure the mercury content satisfying to the international health standards.

Utilizing chelating agents for the removal of mercury is a time and cost-effective method to enhance the depuration of mercury from cockles by forming a ring-like complex with mercury which later can be easily excreted out of the cockles.

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