



UNIVERSITI PUTRA MALAYSIA

**TOTAL MERCURY AND METHYLMERCURY CONCENTRATION IN
FISH AND THEIR REDUCTION THROUGH PROCESSING**

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**TOTAL MERCURY AND METHYLMERCURY CONCENTRATION IN
FISH AND THEIR REDUCTION THROUGH PROCESSING**

By

PARVANEH HAJEB

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

June 2009



DEDICTED TO MY BELOVED FAMILY



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

**TOTAL MERCURY AND METHYLMERCURY CONCENTRATION IN
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PARVANEH HAJEB

June 2008

Chairman : Jinap Selamat, PhD

Faculty : Food Science and Technology

This research has been conducted to study the levels of total mercury and methylmercury, and their correlation in different marine fish species available for consumption in Peninsular Malaysia. Artificial methods have been used to remove mercury from fish. Method for methylmercury determination in fish samples was optimized using response surface methodology (RSM). Total mercury and methylmercury levels were determined using Cold vapor atomic absorption spectrophotometry (CV-AAS) and Gas chromatography-micro electron capture detector (GC- μ ECD), respectively. Samples of twelve species of common marine fish consumed by Malaysians were collected from local wholesale market in Malaysia. On the basis of total mercury and methylmercury levels measured in commonly



consumed fish, two species, long tail tuna and short-bodied mackerel identified with high mercury contents were sampled from east and west coast of Peninsular Malaysia. Methods for elimination of mercury in raw fish fillet has been developed using acidic solutions containing mercury chelating agents. The optimum conditions for methylmercury extraction were found by using acid concentration of 12.118 M, cysteine concentration of 2.375%, solvent volume of 1.5 ml, and extraction time of 35 min. Total mercury and methylmercury levels in fish samples studied were in the range of not detected to 1.010 and not detected to 0.914 $\mu\text{g/g}$ wet wt, respectively. The methylmercury to total mercury ratio ranged from 49.1% to 87.5%, with the highest ratio was in predatory fishes. All of the fish species showed strong positive correlation between methylmercury and total mercury levels ($R^2 > 0.86$). High levels of total mercury and methylmercury were detected in short-bodied mackerel and long tail tuna. Samples of these two species from east coast of Peninsular Malaysia showed higher levels of mercury compared to those from west coast. In all of the locations, significant positive correlations were found between fish body weight and mercury content. The industrial optimized method produced a solution which can remove mercury from raw fish fillet up to 91%. The optimum conditions for mercury reduction was achieved using cysteine concentration of 1.25 %, EDTA of 275 (mg/L), NaCl of 0.5 (%), pH of 3.75 and exposure time of 18 min. The home-used optimized protocol produced a solution which can remove mercury from raw fish fillet up to 81%. The overall optimal condition resulting to the maximum mercury removal in fish fillet was obtained at combined level of pH of 2.79, 0.5% NaCl, and 13.5 (min) of exposure time.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCEMARAN JUMLAH MERKURI DAN METILMERKURI DI DALAM IKAN DAN PENGURANGANNYA SEWAKTU PEMROSESAN

Oleh

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Penyelidikan ini telah dijalankan bagi mengkaji tahap jumlah merkuri dan metilmerkuri serta kaitannya dengan pengambilan pelbagai spesis ikan laut yang didapati di Semenanjung Malaysia. Satu kaedah yang diubahsuai telah digunakan bagi membuang merkuri dari ikan. Pemoptimuman kaedah bagi pengesanan metilmerkuri di dalam ikan telah dijalankan dengan menggunakan kaedah 'Response surface methodology' (RSM). Manakala tahap jumlah merkuri dan metilmerkuri masing-masing dikesan dengan menggunakan spektrofotometri serapan atom wap sejuk dan kuromatografi gas-penangkapan elektron. Dalam kajian ini sebanyak 12 spesis ikan laut yang biasa dimakan oleh penduduk Malaysia telah diambil dari pasar borong tempatan. Tahap jumlah merkuri dan metilmerkuri, nisbah metilmerkuri



dengan total merkuri (%MeHg) dan kaitannya dengan isi ikan serta jantungnya telah dikesan. Berdasarkan kepada keputusan yang diperolehi, didapati 2 spesis ikan yang biasa dimakan iaitu tongkol dan kembong telah dikenalpasti mengandungi kandungan merkuri yang tertinggi, dimana sampel tersebut telah diambil dari pantai timur dan barat Semenanjung Malaysia. Kaedah bagi menyingkirkan merkuri dari filet ikan mentah telah dibangunkan dengan menggunakan larutan berasid yang mengandungi agen pengkelat merkuri. Keadaan optimum bagi pengekstrakan metilmerkuri adalah kepekatan asid 12.118M, kepekatan cysteine 2.375, isipadu pelarut 1.5 ml dan masa pengekstrakan 35 min. Tahap jumlah merkuri dan metilmerkuri dalam ikan yang masing-masing dikaji adalah di antara julat tidak dikesan-1.010 µg/g dan tidak dikesan-0.914 µg/g berat basah. Manakala julat nisbah metilmerkuri dengan jumlah merkuri (%MeHg) adalah di antara 49.1% dan 87.5%, yang mana nisbah yang tertinggi adalah dari ikan-ikan pemangsa. Semua spesis ikan menunjukkan korelasi yang kuat secara positif di antara metilmerkuri dan tahap jumlah merkuri ($R^2 > 0.86$). Tahap jumlah merkuri dan metilmerkuri dikesan paling tinggi di dalam ikan tongkol dan ikan kembong. Berdasarkan kepada kedua-dua spesis ikan tersebut didapati spesis ikan dari pantai timur Semenanjung Malaysia mempunyai merkuri lebih tinggi berbanding dari pantai barat. Data dari semua lokasi pensampelan menunjukkan terdapat korelasi positif di antara berat badan ikan dan kandungan merkuri. Untuk penggunaan peringkat industri, larutan yang digarakan pada keadaan optimum bagi kepadatan menyingkirkan merkuri dari filet ikan mentah sehingga 91%. Keadaan optimum bagi penyingkiran merkuri adalah kepekatan cysteine (1.25%), kepekatan EDTA (275 (mg/L)), kepekatan NaCl (0.5

%), pH (3.75) dan tempuh pendedahan (18 min). Manakala untuk penggunaan di rumah, lamtan yang digunakan pada keadaan optimum dapat mengeluarkan merkuri dari filet ikan mentah sebanyak 81%. Keadaan optimum bagi penyingkiran merkuri secara maksima dari fillet ikan diperolehi dengan menggabungkan tahap pH (2.79), kepekatan NaCl (0.5%) dan masa pendedahan (13.5min).

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I certify that an Examination Committee met on 19 / 06 / 2009 to conduct the final examination of Parvaneh Hajeb on his PhD degree of Food Science thesis entitled “Concentration of mercury in fish muscle and its reduction through washing treatment” in accordance with Universiti Pertanian Malaysia (higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at UPM or at any other institutions.

PARVANEH HAJEB

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LIST OF ABBREVIATIONS

Ag	Silver
AES	Atomic Emission Spectrometry
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Bi	Bismuth
Br⁻	Bromide
CaNa₂EDTA	Calcium disodium ethylenediaminetetraacetate
CH₃Hg⁺	Methylmercury
CH₃HgCl	Methylmercury chloride
(CH₃)₂Hg	Dimethylmercury
C₆H₅Hg⁺	Phenylmercury
CCD	Central composite design
CCFAC	Codex Committee on Foods Additives and Contaminants
Cd	Cadmium
CE	Capillary electrophoresis
CP	Center point
Cl	Calcium
Cr	Chromium
CRM	Certified reference materials
CV-AAS	Cold vapor atomic absorption spectrophotometry
CV-AFS	Cold vapor atomic Fluorescence spectrophotometry



DMPS	2,3 dimercaptopropane-1-sulfonate
DMSA	Dimercaptosuccinic acid
DPA	D-penicillamine
ECD	Electron capture detector
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
Eq	Equation
EWI	Estimated weekly intake
F⁻	Fluoride
FAO	Food and Agricultural Organization
FDA	Food and drug analysis
g	Gram
GC	Gas chromatography
GC-AFS	Gas chromatography-atomic fluorescence spectrometry
GC-ICP-MS	Gas chromatography-inductively coupled plasma-mass spectrometry
GC-MS	gas chromatography-mass spectrometry
g/s	Gram/ second
h	Hour
HCl	Hydrochloric acid
Hg	Mercury
HNO₃	Nitric acid
H₂SO₄	Sulfuric acid
HPLC	High performance liquid chromatography



HPLC-MS	High performance liquid chromatography-mass spectrometry
I	Iodide
ICP-MS	Inductively coupled plasma-mass spectrometry
IUPAC	International Union for Pure and Applied Chemistry
JECFA	Joint Expert Committee on Food Additives
KBr	Potassium bromide
Kg	Kilogram
L	Liter
LC	liquid chromatography
LKIM	Lembaga Kemajuan Ikan Malaysia
LOD	Limit of detection
LOQ	limit of quantification
M	Molar
mg	Milligram
MeHg	methylmercury
MeOH	Methanol
min	Minute
MIP-AES	Microwave induced plasma-atomic emission spectrometry
mL	Milliliter
MOH	Ministry of Health Malaysia
MS	Mass spectrometry
MT	Metallothionein

