



UNIVERSITI PUTRA MALAYSIA

**EAVAILABILITY AND ACTIVITY OF COENZYME Q10 IN SELECTED
MALAYSIAN FRESHWATER FISH**

NUR SUMIRAH MOHD DOM

FBSB 2009 5





*Your complimentary
use period has ended.
Thank you for using
PDF Complete.*

[Click Here to upgrade to
Unlimited Pages and Expanded Features](#)

AVAILABILITY AND ACTIVITY OF COENZYME Q₁₀ IN SELECTED MALAYSIAN FRESHWATER FISH

By

NUR SUMIRAH MOHD DOM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirement for the Degree of Master of
Science**

May 2009



AVAILABILITY AND ACTIVITY OF COENZYME Q₁₀ IN SELECTED MALAYSIAN FRESHWATER FISH

By

NUR SUMIRAH MOHD DOM

May 2009

Chairman: Associate Professor Muhajir Hamid, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

The present study was done to determine the availability of Coenzyme Q₁₀ and to screen the antioxidant activity of the liver, intestine, muscle and egg of selected Malaysian freshwater fish extracts using DPPH assay. Besides, the study was also done to see the interactions of CoQ₁₀ and α -tocopherol in *in vitro* assay. The presence of Coenzyme Q₁₀ (CoQ₁₀) in the muscle, liver, intestine and eggs of nine selected Malaysian Freshwater Fish consisting of Keli (*Clarias batrachus*), Puyu (*Anabas testudineus*), Tilapia Merah (*Oreochromis niloticus*), Tilapia Hitam (*Oreochromis mossambicus*), Rohu (*Labeo rohita*), Patin (*Pangasius polyuranodon*), Lampam Jawa (*Puntius gonionotus*), Lampam Sungai (*Puntius schwanenfeldii*) and Lee Koh (*Cyprinus carpio*) were determined using High Performance Liquid Chromatography (HPLC).

The results demonstrated the presence of CoQ₁₀ at variable amounts in different tissues and species of the Malaysian freshwater fish studied. The total amount of CoQ₁₀ present in all the tissues were found in the order of Tilapia Hitam

) (8.251 g/g), Tilapia Merah (*Oreochromis niloticus*) (8.255 g/g), Keli (*Clarias batrachus*) (3.263 g/g), Puyu (*Anabas testudineus*) (2.446 g/g), Lampam Jawa (*Puntius gonionotus*) (1.776 g/g), Patin (*Pangasius polyuranodon*) (1.436 g/g), Rohu (*Labeo rohita*) (0.519 g/g), Lee Koh (*Cyprinus carpio*) (0.362 g/g) and Lampam Sungai (*Puntius schwanenfeldii*) (0.237 g/g) wet weight.

In this study, n-hexane/ ethanol method was used to extract the Coenzyme Q₁₀. According to the previous study, the Bligh and Dyer method extracted mainly tocopherol, retinol and small amounts of CoQ. However, in this study, -tocopherol was also detected in the n-hexane/ ethanol extract. Four fish species identified to have the highest amount of Coenzyme Q₁₀ namely Tilapia Hitam (*Oreochromis mossambicus*) Puyu (*Anabas testudineus*), Tilapia Merah (*Oreochromis niloticus*) and Keli (*Clarias batrachus*) were selected for quantitation of the -tocopherol and their antioxidant activities using 2,2-diphenyl-1-picrylhydrazil (DPPH) assay. The total body contents of -tocopherol were found to be 12.635 g/g, 12.631 g/g, 7.152 g/g and 4.482 g/g wet weight tissues in the respective fish.

From the findings, antioxidant that contributes the most to the antioxidative activity of the various tissues of fish was found to be -tocopherol. As the fish shows low levels of Coenzyme Q₁₀ instead of -tocopherol and with the inability of the TBA assay to quantify the antioxidative effects in the tissues studied, further studies were carried out to assess the effectiveness of synthetic

and combination of both *in vitro* as a comparison to previous studies on other fish.

In this study, mixtures of reduced CoQ₁₀ and α -tocopherol standards were used. It was shown that the percentage of inhibition increased in the presence of both CoQ₁₀ and α -tocopherol suggesting synergistic effect. Meanwhile, α -tocopherol exhibited a higher percentage with 10.99 - 60.60 % on its own compared to reduced CoQ₁₀ with percentage inhibition ranging from 7.81 - 49.76 %. This would explain the variation of DPPH activities in the various tissues of fish and it can be concluded that α -tocopherol contributed more to the antioxidant activity compared to CoQ₁₀ in the freshwater fish examined.

In conclusion, as the Coenzyme Q₁₀ and α -tocopherol plays vital roles in protecting human body from free radicals and retard the growth of many chronic diseases, the Malaysian freshwater fish species examined are recommended as part of the diet as both the lipophilic antioxidants are present in their tissues. The fish extracts can also be used as an alternative source of natural antioxidants to replace synthetic antioxidants in pharmaceutical aspects, as food supplement as well as in cosmetic and medical applications.

KEHADIRAN DAN AKTIVITI KOENZIM Q₁₀ DALAM IKAN AIR TAWAR TERPILIH DI MALAYSIA

Oleh

NUR SUMIRAH BINTI MOHD DOM

MEI 2009

Pengerusi : Prof. Madya Muhajir Hamid, PhD

Fakulti : Fakulti Bioteknologi dan Sains Biomolekul

Kajian ini dijalankan untuk menentukan kehadiran koenzim Q₁₀(CoQ₁₀) dan juga melihat aktiviti antioksidan pada hati, usus, otot dan telur pada ekstrak spesies tertentu ikan air tawar di Malaysia menggunakan kaedah 2,2-diphenyl-1-picrylhydrazil (DPPH). Di samping itu, kajian ini juga dijalankan untuk melihat interaksi antara CoQ₁₀ dan α -tokoferol secara *in vitro*. Kehadiran koenzim Q₁₀ dalam otot, hati, usus dan telur sembilan spesies ikan air tawar di Malaysia yang terdiri daripada Keli (*Clarias batrachus*), Puyu (*Anabas testudineus*), Tilapia Merah (*Oreochromis niloticus*), Tilapia Hitam (*Oreochromis mossambicus*), Rohu (*Labeo rohita*), Patin (*Pangasius polyuranodon*), Lampam Jawa (*Puntius gonionotus*), Lampam Sungai (*Puntius schwanenfeldii*) and Lee Koh (*Cyprinus carpio*) ditentukan menggunakan teknik Kromatografi Cecair Prestasi Tinggi (HPLC).

Keputusan yang diperolehi menunjukkan kehadiran CoQ₁₀ pada jumlah berbeza pada beberapa jenis tisu dan spesies ikan air tawar di Malaysia yang digunakan.

o yang terkandung di dalam semua tisu adalah mengikat taratan lemak, di mana Tilapia Hitam (*Oreochromis mossambicus*) (8.251 g/g), Tilapia Merah (*Oreochromis niloticus*) (6.259 g/g), Keli (*Clarias batrachus*) (3.263 g/g), Puyu (*Anabas testudineus*) (2.446 g/g), Lampam Jawa (*Puntius gonionotus*) (1.776 g/g), Patin (*Pangasius polyuranodon*) (1.436 g/g), Rohu (*Labeo rohita*) (0.519 g/g), Lee Koh (*Cyprinus carpio*) (0.362 g/g) dan Lampam Sungai (*Puntius schwanenfeldii*) (0.237 g/g) berat basah.

Dalam kajian ini, kaedah n-heksana/ etanol digunakan untuk mengekstrak koenzim Q₁₀. Menurut kajian lepas, kaedah Bligh and Dyer hanya mengekstrak tocopherol, retinol dan hanya sedikit kuantiti CoQ. Namun, dalam kajian ini, kaedah n-heksana/ etanol dapat mengesan kehadiran α -tokoferol. Empat spesis ikan yang dikenalpasti mempunyai jumlah koenzim Q₁₀ tertinggi iaitu Tilapia Hitam (*Oreochromis mossambicus*) Puyu (*Anabas testudineus*), Tilapia Merah (*Oreochromis niloticus*) and Keli (*Clarias batrachus*) dipilih untuk mengesan jumlah α -tokoferol yang terkandung dan juga aktiviti antioksidan yang terdapat menggunakan kaedah DPPH. Jumlah keseluruhan α -tokoferol adalah sebanyak 12.635 g/g, 12.631 g/g, 7.152 g/g and 4.482 g/g berat basah pada ikan tersebut di atas.

Daripada penemuan tersebut, diketahui bahawa antioksidan yang paling banyak menyumbang kepada aktiviti antioksidan pada pelbagai tisu ikan adalah α -tokoferol. Oleh kerana ikan menunjukkan kuantiti koenzim Q₁₀ yang rendah berbanding α -tokoferol dan juga disebabkan oleh kegagalan kaedah asid

melihat kesan antioksidatif pada otot, hati, usus dan
telur ikan air tawar di Malaysia, kajian lanjut telah dijalankan untuk melihat
keberkesanan sintetik koenzim Q₁₀, -tokoferol and kombinasi keduanya secara
in vitro sebagai perbandingan kepada kajian yang dijalankan pada ikan.

Bagi kajian ini, campuran piawai CoQ₁₀ terturun dan -tokoferol telah
digunakan. Ia telah menunjukkan bahawa peratusan perencatan telah
meningkat dengan kehadiran CoQ₁₀ dan -tokoferol, mencadangkan kesan
sinergistik antara keduanya. Sementara itu, -tokoferol telah menunjukkan
peratusan perencatan yang lebih tinggi iaitu 10.99 . 60.60 % berbanding CoQ₁₀
terturun dengan peratusan perencatan terdiri dari 7.81 . 49.76 %. Ini dapat
menjelaskan kepelbagaian aktiviti DPPH yang diperolehi pada beberapa jenis
tisu ikan dan dapat disimpulkan bahawa -tokoferol lebih banyak menyumbang
kepada aktiviti antioksida berbanding CoQ₁₀ pada ikan air tawar yang
dikajiselidik.

Pada kesimpulannya, disebabkan koenzim Q₁₀ dan -tokoferol memainkan
peranan penting dalam melindungi badan manusia dari radikal bebas dan
merencat pembentukan penyakit-penyakit kronik, spesis ikan air tawar di
Malaysia yang dikajiselidik disyorkan sebagai sebahagian daripada diet
disebabkan kehadiran kedua-dua antioksida tersebut. Ekstrak ikan juga boleh
digunakan sebagai sumber alternatif kepada antioksida semulajadi bagi
menggantikan antioksida sintetik dalam aspek farmaseutikal, sebagai makanan
tambahan serta dalam aplikasi kosmetik dan perubatan.

ACKNOWLEDGEMENTS

First and foremost, the thesis would never be completed without the Almighty Allah blessings and power. *Alhamdulillah.*

I would like to give my sincere appreciation to my previous supervisor, Associate Professor Dr. Juzu Hayati Arshad for her guidance, helpful hands and never ending supports during my entire research time. Hope she is doing great in her retirement time now. I am also very thankful to Associate Professor Dr. Muhajir Hamid as my current supervisor and Associate Professor Dr. Mohd. Yunus Abd. Shukor (co-supervisor) for giving me help, support and positive suggestions in completing the thesis.

I would like to extend my heartfelt gratitude to my colleagues, Ezarul Faradianna Lokman, Rafidah Saat, Farida Haryani Abd. Aziz, Hasliza Hassan, Nor Aishah Abu Shah and for those who indirectly contributes for making this thesis a success, thank you for the help and encouragement. My thanks also goes to En. Jasni and all the staff at the hatchery unit of Universiti Putra Malaysia for providing me the Malaysian freshwater fish needed in this project and all the staffs in the Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences for providing me lab facilities.

Most importantly, none of this would have been possible without the love and patience of my family. I would like to thank my dear beloved parents, Haji Mohd



*Your complimentary
use period has ended.
Thank you for using
PDF Complete.*

[Click Here to upgrade to
Unlimited Pages and Expanded Features](#)

ah Mek Rakiah Latif, my dear husband, Muhammad
Faisal Hamid and all my siblings, Nur Shukriah, Mohd Suffian, Mohd Sharizal,
Mohd Shazwan and Mohd Shazrin for supporting and never ending love. To my
precious sweethearts; Aisyah Humaira and Khadijah Nur Syifaaq Muhammad
Faisal, both of you are my truly inspiration.





*Your complimentary
use period has ended.
Thank you for using
PDF Complete.*

[*Click Here to upgrade to
Unlimited Pages and Expanded Features*](#)





*Your complimentary
use period has ended.
Thank you for using
PDF Complete.*

[Click Here to upgrade to
Unlimited Pages and Expanded Features](#)

The Senate of Universiti Putra Malaysia has been approved the requirements for the degree of master of Science. The members of the Supervisory committee are as follows:

Muhajir Hamid, PhD

Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Yunus Abd. Shukor, PhD

Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOHD. GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 May 2009



*Your complimentary
use period has ended.
Thank you for using
PDF Complete.*

[Click Here to upgrade to
Unlimited Pages and Expanded Features](#)

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 Universiti Putra Malaysia or at any other institutions.

NUR SUMIRAH MOHD DOM

Date: 1 April 2009

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xx
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	3
2.1 Coenzyme Q	3
2.1.1 Functions of CoQ	4
2.1.2 Food sources rich in CoQ	5
2.2 Biosynthesis and Distribution	8
2.2.1 Biosynthesis of Coenzyme Q	8
2.2.2 Distributions of Coenzyme Q	11
2.3 Intracellular transport	15
2.4 Regulation of Coenzyme Q	15
2.5 Catabolism of Coenzyme Q/ Half life	16
2.6 Free radical	18
2.6.1 Lipid peroxidation	18
2.7 Coenzyme Q ₁₀ in fish	21
2.8 Food antioxidants	22
2.9 Other lipid soluble antioxidants in aquatic organism	23
2.9.1 Vitamin E	23
2.9.2 Vitamin A	25
2.10 Involvement of Coenzyme Q	27
2.11 The Implications of Coenzyme Q in human	29
2.12 Dietary Coenzyme Q and Medications	29
2.13 Malaysian Freshwater Fish	31
2.14 Determination of The Levels of Coenzyme Q	32
2.14.1 Extraction Procedures	32
2.14.2 Antioxidant Activity of CoQ	33
2.14.3 High Performance Liquid Chromatography (HPLC)	35

ETHODS

		39
	3.1.1 Experimental design, Fish, Rearing system and diets	39
	3.1.2 Chemical and Equipments	39
	3.1.3 Statistical Analysis	40
3.2	Extraction of CoQ ₁₀ in the fish samples	43
	3.2.1 n-hexane/ ethanol method	43
	3.2.2 2-propanol method	43
	3.2.3 Bligh and Dyer Extraction Method	44
	3.2.4 Extraction Efficiency and Recovery	45
3.3	Extraction and quantification of Coenzyme Q ₁₀ and -tocopherol using High Performance Liquid Chromatography (HPLC)	45
	3.3.1 Preparation of sample	45
	3.3.2 Preparation of standards	46
	3.3.3 HPLC Analysis	46
3.4	Determination of antioxidant activity in various tissues of Malaysian Freshwater Fish	47
	3.4.1 DPPH radical scavenging activity	47
	3.4.2 Thiobarbituric Acid (TBA) method	48
3.5	<i>In vitro</i> study of CoQ ₁₀ , reduced CoQ ₁₀ and -tocopherol standards	50
	3.5.1 Reducing method of CoQ	50
	3.5.2 Stability of reduced Coenzyme Q ₁₀	50
3.6	DPPH scavenging activities of different concentrations of Coenzyme Q ₁₀ and -tocopherol studied <i>in vitro</i>	51
	3.6.1 Synergistic effects between reduced CoQ ₁₀ and -tocopherol using DPPH assay	51
3.7	Thiobarbituric Acid (TBA) test on reduced CoQ ₁₀ , -tocopherol and combination of CoQ ₁₀ and -tocopherol	52
	3.7.1 Synergistic effects between reduced CoQ ₁₀ and -tocopherol using TBA assay	53
	3.7.2 Study of antioxidative activities among reduced CoQ ₁₀ , -tocopherol, oxidized CoQ ₁₀ and oxidized -tocopherol	53
4	RESULTS AND DISCUSSION	
4.1	Extraction procedure of Coenzyme Q	55
4.2	Recovery of Coenzyme Q ₁₀ for liver tissue of Keli (<i>Clarias batrachus</i>)	56

	and quantitation of Coenzyme Q ₁₀	
	of selected Malaysian	
	freshwater fish using High Performance	
	Liquid Chromatography (HPLC)	57
4.4	HPLC analysis of α -tocopherol and	
	determination of DPPH radical scavenging	
	activities of four selected fish	
	contains high levels of Coenzyme Q ₁₀	66
4.5	Determination of antioxidant activities of	
	synthetic Coenzyme Q ₁₀ and alpha-tocopherol	
	in <i>in vitro</i> studies	75
4.5.1	Antioxidative effect of reduced	
	Coenzyme Q ₁₀ and its stability	
	using DPPH scavenging activity assay	76
4.5.2	Effects of administration of reduced	
	Coenzyme Q ₁₀ , α -tocopherol, alone or	
	together on DPPH scavenging assay	79
4.5.3	Determination of antioxidative activities	
	using Thiobarbituric Acid (TBA) Assay	80
4.6	Synergistic effects of reduced CoQ ₁₀ and	
	α -tocopherol on DPPH scavenging	
	activity assay	83
4.7	Synergistic effects of reduced CoQ ₁₀	
	and α -tocopherol on	
	Thiobarbituric acid (TBA) assay	85
4.8	Antioxidative activities of reduced CoQ ₁₀ ,	
	freshly prepared α -tocopherol, oxidized CoQ ₁₀	
	and oxidized α -tocopherol on	
	DPPH scavenging activity assay	87
5	CONCLUSIONS	90
	REFERENCES	93
	APPENDICES	110
	BIODATA OF STUDENT	123

LIST OF TABLES

Table		Page
1	Contents of Coenzyme Q ₁₀ in foods	7
2	The comparison studies of CoQ ₁₀ contents in foods	8
3	Amount CoQ ₁₀ in tissues of human, rat, bovine brain and fish	13
4	Age-related changes of ubiquinone content of human organ	16
5	Half life of ubiquinone in rat tissues and spinach	17
6	Levels of CoQ ₁₀ in various tissues and species in the previous studies	21
7	The determination of CoQ samples using various conditions of HPLC	37
8	List of chemicals used in this study	41
9	List of equipments used in this study	42
10	Nine categories of tests done on the reaction mixture	55
11	HPLC analysis of Coenzyme Q ₁₀ by various extraction procedures on liver and muscle of Keli (<i>Clarias batrachus</i>)	56
12	Recovery of Coenzyme Q ₁₀ added to the samples before applying the n-hexane/ ethanol method	57
13	Amounts of CoQ ₁₀ in the tissues of muscle, liver, intestine and egg of Malaysian freshwater fish	59
14	Comparison amount of Coenzyme Q ₁₀ with the previous studies	65
15	Levels of α -tocopherol ($\mu\text{g/g}$ wet weight) and antioxidant activity (%) in the various tissues of Keli (<i>Clarias batrachus</i>).	68

	herol ($\mu\text{g/g}$ wet weight) and ty (%) in the various tissues of Paya (<i>Anabas testudineus</i>)	68
17	Levels of -tocopherol ($\mu\text{g/g}$ wet weight) and antioxidant activity (%) in the various tissues of Tilapia Merah (<i>Oreochromis niloticus</i>)	69
18	Levels of -tocopherol ($\mu\text{g/g}$ wet weight) and antioxidant activity (%) in the various tissues of Tilapia Hitam (<i>Oreochromis mossambicus</i>)	69

LIST OF FIGURES

Figure		Page
1	Scheme of CoQ including the fully oxidized form ubiquinone (CoQ), and the fully reduced form ubiquinol (CoQH ₂), and the intermediate redox form semiubiquinone (SCoQ)	4
2	Mevalonate biosynthesis pathway of ubiquinone	9
3	A simplified scheme of the terminal reactions in CoQ biosynthesis	10
4	Sites of action of CoQ, vitamin E and ascorbate on lipid peroxidation	20
5	Structures of tocopherols and tocotrienols	24
6	Structure of vitamin A (retinol acetate)	26
7	The protonmotive Q cycle	27
8	The reduction of ubiquinone to ubiquinol	28
9	Effect of different concentrations of reduced form of ubiquinone (ubiquinol) towards DPPH scavenging activity assay	79
10	Effect of stability of reduced ubiquinone towards DPPH scavenging activity assay within 10 days observations	79
11	Effect of different concentrations of reduced Coenzyme Q ₁₀ (CoQ ₁₀) and α -tocopherol (ATF) ranging from 6.0 to 30.0 nmol on the DPPH scavenging activity assay	81
12	Effect of different concentrations of ubiquinol and α -tocopherol on Thiobarbituric acid (TBA) assay from 6.0 to 30.0 nmol	82
13	Effects of ubiquinol, α -tocopherol (ATF) and combination of both towards DPPH scavenging activity assay	84

	ol and -tocopherol at 6.0 and 12.0 nmol towards TBA assay	86
15	Effects of antioxidative activities on reduced CoQ ₁₀ , freshly prepared -tocopherol (ATF), oxidized CoQ ₁₀ and oxidized -tocopherol on DPPH scavenging activity assay	88

LIST OF ABBREVIATIONS

ATF	Alpha tocopherol
BHT	Butylated hydroxytoluene
CoQ	Coenzyme Q
DPPH	2,2-diphenyl-1-picrylhydrazil
FeCl ₂	Ferrous chloride
HPLC	High Performance Liquid Chromatography
TAA	Total Antioxidant Activity
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid

CHAPTER 1

INTRODUCTION

In recent years, Coenzyme Q (CoQ) has received great attention as the only endogenously synthesized lipid soluble antioxidant. In animal cells, CoQ functions include electron carrier in the mitochondrial respiratory chain, regulation of mitochondrial permeability transition pores, an activator of mitochondrial uncoupling proteins (Dallner *et al.*, 2003) and can be scavengers of Reactive Oxygen Species (ROS) or lipid radicals. The health benefit of CoQ₁₀ has been studied and its efficacy in cardiovascular diseases has been reported (Greenberg and Frishman, 1990). In recent years, CoQ₁₀ has become popular as a dietary supplement and easily available in the market in various product forms (Evans *et al.*, 2009). The main sources of CoQ₁₀ in human diet are fish, meat, oil, nuts and wheat with daily intake ranges between 3 to 5 mg/ day (Wajda *et al.*, 2007).

The fish species with the highest Coenzyme Q can be mass cultured not only as a source of cheap protein, but also as a source of this novel compound to be used as an antioxidant for health purposes (Ernster and Dallner, 1995). Referring to Mattila and Kumpulainen in 2001, CoQ₁₀ was found highest in meat, rapeseed oil and fish followed by dairy products, vegetables, fruits and berries. Meanwhile, Kubo *et al.* in 2008 has reported the highest content of CoQ₁₀ was observed in beef, chicken and fish. CoQ₉ was found most in mouse tissues (Tang *et al.*, 2004) and cereals (Mattila and Kumpulainen, 2001).

mination of Coenzyme Q studies has been done on rats, plants, human, microbes and only a few in fish (Kamei *et al.*, 1986; Weber *et al.*, 1997; Mattila and Kumpulainen, 2001; Kubo *et al.*, 2008). The previous studies have been done only on marine fish (Pennock *et al.*, 1962; Diplock and Hasselwood, 1967; Farbu and Lambertsen, 1979; Giardina *et al.*, 1997) and only one on freshwater fish (Lokman, 2006). Thus, the objectives of this present study were:

- 1) To identify and determine the availability of CoenzymeQ₁₀ in various tissues of selected Malaysian freshwater fish
- 2) To screen the antioxidant activity of the liver, intestine, muscle and egg of selected Malaysian freshwater fish extracts using DPPH assay
- 3) To study the interactions of CoQ₁₀ and α -tocopherol in *in vitro*.

CHAPTER 2

LITERATURE REVIEW

2.1 Coenzyme Q

In this universe, almost all living creatures including humans can produce ubiquinone and it was first thought to be a vitamin (Ernster and Dallner, 1995). Coenzyme Q (CoQ) or ubiquinone (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone) is a lipid soluble compound which is present in all tissues, cells and membranes in highly variable amounts (Dallner and Sindelar, 2000). The polyprenyl side chain of CoQ varies from 6 to 12 isoprene units in different cell types. Usually, higher animals contain CoQ₁₀ as the sole endogenous quinone (Ramasarma, 1985). It was isolated and characterized by Festenstein *et al.* in 1955 and it was established in 1957 by Crane *et al.* that this compound functions as a member of the mitochondrial respiratory chain. Meanwhile in 1958, Wolf *et al.* determined its complex structure noting that the redox-active benzoquinone ring is connected to a long isoprenoid side chain (Dallner *et al.*, 2003).

Coenzyme Q, which is preferably located in the mitochondria comprises the fully oxidized form; ubiquinone (CoQ) homologues, the partially reduced form, also a free radical; semiubiquinone (\cdot -CoQ) and the fully reduced forms; ubiquinols (CoQH₂) (Turrens *et al.*, 1985; Lang *et al.*, 1986). Due to their antioxidant properties, ubiquinols can be regarded as another class of endogenous

ubiquinones are potential prooxidants (Lang *et al.*,

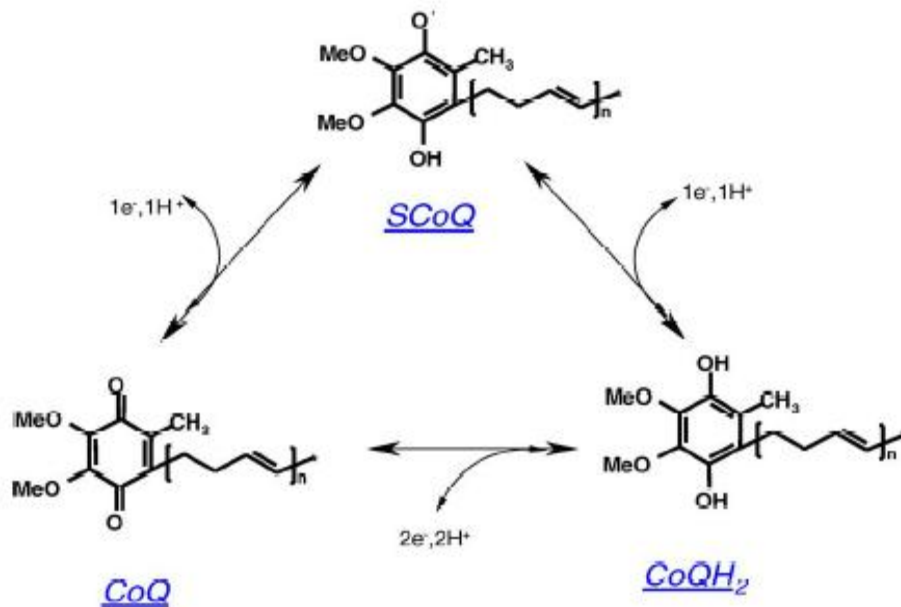


Figure 1: Scheme of CoQ including the fully oxidized form ubiquinone (CoQ), and the fully reduced form ubiquinol (CoQH₂), and the intermediate redox form semiubiquinone (SCoQ). Unpaired electron is represented in oxygen linked to carbon 4. The polyprenyl residue in carbon 2 contains isoprene units repeated several times (n) (Rodriguez-Aguilera *et al.*, 2004).

2.1.1 Functions of Coenzyme Q

In mitochondria, CoQ serves three well-characterized functions; (i) it shuttles electrons from complex I (NADH-ubiquinone reductase) and II (succinate-ubiquinone reductase) to complex III (ubiquinol-cytochrome c reductase) of the electron transport chain (ETC), while releasing protons into the intermembrane region (Ernster and Dallner, 1995; James *et al.*, 2004); (ii) in concert with vitamin E, CoQ acts in its reduced form (ubiquinol) as an antioxidant to stem lipid peroxidation in the biological membrane and in serum low-density lipoprotein