UNIVERSITI PUTRA MALAYSIA

EAVAILABILITY AND ACTIVITY OF COENZYME Q10 IN SELECTED MALAYSIAN FRESHWATER FISH

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Chairman: Associate Professor Muhajir Hamid, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

The present study was done to determine the availability of CoenzymeQ_{10} 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 CoQ_{10} and Ŭ-tocopherol in *in vitro* assay. The presence of Coenzyme Q_{10} (CoQ_{10}) 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 CoQ_{10} at variable amounts in different tissues and species of the Malaysian freshwater fish studied. The total amount of CoQ_{10} present in all the tissues were found in the order of Tilapia Hitam

(8.251 εg/g), Tilapia Merah (*Oreochromis niloticus)* (0.259 εg/g), Keli (*Clarias batrachus)* (3.263 εg/g), Puyu (*Anabas testudineus)* (2.446 εg/g), Lampam Jawa (*Puntius gonionotus)* (1.776 εg/g), Patin (*Pangasius polyuranodon)* (1.436 εg/g), Rohu (*Labeo rohita)* (0.519 εg/g), Lee Koh (*Cyprinus carpio)* (0.362 εg/g) and Lampam Sungai (*Puntius schwanenfeldii)* (0.237 εg/g) wet weight.

In this study, n-hexane/ ethanol method was used to extract the Coenzyme Q_{10}. According to the previous study, the Bligh and Dyer method extracted mainly tocopherol, retinol and small amounts of CoQ. However, in this study, Ŭ-tocopherol was also detected in the n-hexane/ ethanol extract. Four fish species identified to have the highest amount of Coenzyme Q_{10} namely Tilapia Hitam (*Oreochromis mossambicus*) Puyu (*Anabas testudineus*), Tilapia Merah (*Oreochromis niloticus*) and Keli (*Clarias batrachus*) were selected for quantitation of the Ŭ-tocopherol and their antioxidant activities using 2,2-diphenyl-1-picrylhydrazil (DPPH) assay. The total body contents of Ŭ-tocopherol were found to be 12.635 εg/g, 12.631 εg/g, 7.152 εg/g and 4.482 εg/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 Ŭ-tocopherol. As the fish shows low levels of Coenzyme Q_{10} instead of Ŭ-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

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

In this study, mixtures of reduced CoQ_{10} and Ŭ-tocopherol standards were used. It was shown that the percentage of inhibition increased in the presence of both CoQ_{10} and Ŭ-tocopherol suggesting synergistic effect. Meanwhile, Ŭ-tocopherol exhibited a higher percentage with 10.99 ï 60.60 % on its own compared to reduced CoQ_{10} with percentage inhibition ranging from 7.81 ï 49.76 %. This would explain the variation of DPPH activities in the various tissues of fish and it can be concluded that Ŭ-tocopherol contributed more to the antioxidant activity compared to CoQ_{10} in the freshwater fish examined.

In conclusion, as the Coenzyme Q_{10} and Ŭ-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.

mukakan kepada Senat Universiti Putra Malaysia
uhi keperluan untuk ijazah Master Sains

KEHADIRAN DAN AKTIVITI KOENZIM Q_{10} DALAM IKAN AIR TAWAR TERPILIH DI MALAYSIA

Oleh

NUR SUMIRAH BINTI MOHD DOM

MEI 2009

Pengerusi : Prof. Madya Muhajir Hamid, PhD
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Kajian ini dijalankan untuk menetukan kehadiran koenzim Q_{10}(CoQ_{10}) dan juga melihat aktiviti antioksida pada hati, usus, otot dan telur pada ektrak spesis 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 CoQ_{10} dan Ŭ-tokoferol secara *in vitro*. Kehadiran koenzim Q_{10} dalam otot, hati, usus dan telur sembilan spesis 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 CoQ_{10} pada jumlah berbeza pada beberapa jenis tisu dan spesis ikan air tawar di Malaysia yang digunakan.

$_{10}$ yang terkandung di dalam semua tisu adalah mengikut turutan berikut, di mana Tilapia Hitam (*Oreochromis mossambicus)* (8.251 ε g/g), Tilapia Merah (*Oreochromis niloticus)* (6.259 ε g/g), Keli (*Clarias batrachus)* (3.263 ε g/g), Puyu (*Anabas testudineus)* (2.446 ε g/g), Lampam Jawa (*Puntius gonionotus)* (1.776 ε g/g), Patin (*Pangasius polyuranodon)* (1.436 ε g/g), Rohu (*Labeo rohita)* (0.519 ε g/g), Lee Koh (*Cyprinus carpio)* (0.362 ε g/g) dan Lampam Sungai (*Puntius schwanenfeldii)* (0.237 ε g/g) berat basah.

Dalam kajian ini, kaedah n-heksana/ etanol digunakan untuk mengekstrak koenzim Q_{10}. 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 Ŭ-tokoferol. Empat spesis ikan yang dikenalpasti mempunyai jumlah koenzim Q_{10} tertinggi iaitu Tilapia Hitam (*Oreochromis mossambicus*) Puyu (*Anabas testudineus*), Tilapia Merah (*Oreochromis niloticus*) and Keli (*Clarias batrachus*) dipilih untuk mengesan jumlah Ŭ-tokoferol yang terkandung dan juga aktiviti antioksida yang terdapat menggunakan kaedah DPPH. Jumlah keseluruhan Ŭ-tokoferol adalah sebanyak 12.635 ε g/g, 12.631 ε g/g, 7.152 ε g/g and 4.482 ε g/g berat basah pada ikan tersebut di atas.

Daripada penemuan tersebut, diketahui bahawa antioksida yang paling banyak menyumbang kepada aktiviti antioksida pada pelbagai tisu ikan adalah Ŭ-tokoferol. Oleh kerana ikan menunjukkan kuantiti koenzim Q_{10} yang rendah berbanding Ŭ-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 Q_{10}, Ŭ-tokoferol and kombinasi keduanya secara *in vitro* sebagai perbandingan kepada kajian yang dijalankan pada ikan.

Bagi kajian ini, campuran piawai CoQ_{10} terturun dan Ŭ-tokoferol telah digunakan. Ia telah menunjukkan bahawa peratusan perencatan telah meningkat dengan kehadiran CoQ_{10} dan Ŭ-tokoferol, mencadangkan kesan sinergistik antara keduanya. Sementara itu, Ŭ-tokoferol telah menunjukkan peratusan perencatan yang lebih tinggi iaitu 10.99 ï 60.60 % berbanding CoQ_{10} terturun dengan peratusan perencatan terdiri dari 7.81 ï 49.76 %. Ini dapat menjelaskan kepelbagaian aktiviti DPPH yang diperolehi pada beberapa jenis tisu ikan dan dapat disimpulkan bahawa Ŭ-tokoferol lebih banyak menyumbang kepada aktiviti antioksida berbanding CoQ_{10} pada ikan air tawar yang dikajiselidik.

Pada kesimpulannya, disebabkan koenzim Q_{10} dan Ŭ-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.

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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 Universiti Putra Malaysia or at any other institutions.

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NUR SUMIRAH MOHD DOM

Date: 1 April 2009

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ATF	Alpha tocopherol
BHT	Butylated hydroxytoluene
CoQ	Coenzyme Q
DPPH	2,2-diphenyl-1-picrylhydrazil
FeCl2	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 CoQ_{10} has been studied and its efficacy in cardiovascular diseases has been reported (Greenberg and Frishman, 1990). In recent years, CoQ_{10} 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 CoQ_{10} 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, CoQ_{10} 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 CoQ_{10} was observed in beef, chicken and fish. CoQ_9 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 CoenzymeQ_{10} 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 CoQ_{10} and Ŭ-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 CoQ_{10} 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 (·CoQ) and the fully reduced forms; ubiquinols ($CoQH_2$) (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.*, 1986).

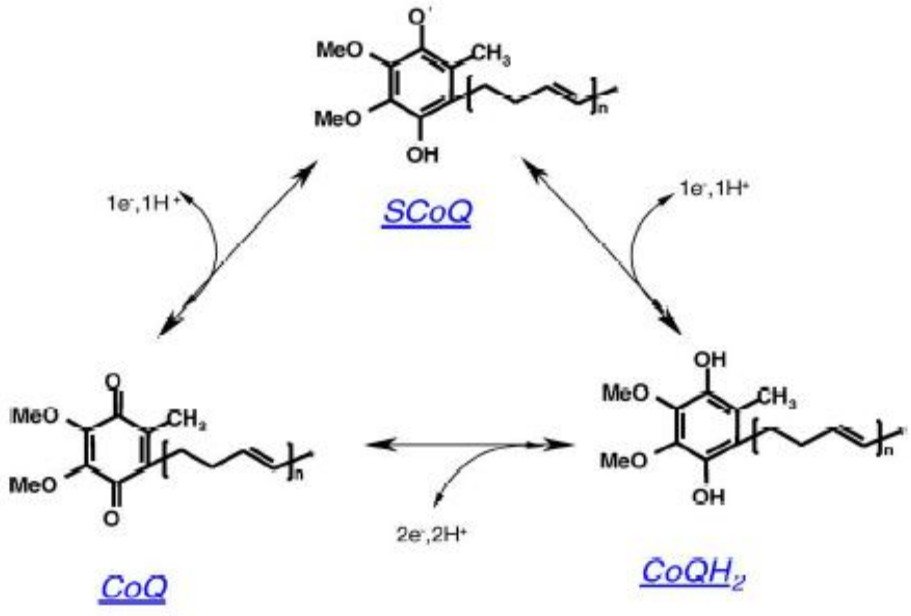

Figure 1: Scheme of CoQ including the fully oxidized form ubiquinone (CoQ), and the fully reduced form ubiquinol ($CoQH_2$), and the intermediate redox form semiubiquinone (SCoQ). Unpaired electron is represented in oxygen linked to carbon 4. The polyprenyl residue in carbon 2 contains isoprene units 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