UNIVERSITI PUTRA MALAYSIA

IRON BIOAVAILABILITY FROM SPIRULINA *(ARTHROSPIRA PLATENSIS)* AND ITS INTERACTIONS WITH OTHER DIETARY FACTORS IN VITRO AND IN VIVO

LOH SU PENG

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By

LOH SU PENG

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

June 2004

I lift up my eyes to the hills
Where does my help come from?
My help comes from the LORD
the Maker of heaven and earth

Psalm 121:1-2

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

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June 2004

Chairman : Associate Professor Maznah Ismail, Ph.D.

Faculty : Medicine and Health Sciences

Deficiency of iron is common worldwide. Various approaches have been used to improve iron intake and absorption. These include the use of spirulina, a microalage that is already popular in many Asian countries as a functional food supplement. The main objective of this study was to determine the iron bioavailability from spirulina and its interactions with other dietary factors both *in vitro* and *in vivo*.

In vitro digestion/Caco-2 cell culture system accompanied by either centrifugation or dialysis step was used to assess the availability of iron from spirulina. Using the centrifugation method, the cultured and commercial spirulina yielded significantly higher results ($P < 0.05$) than then dialysis method, both in the form of iron available for uptake and the actual amount of iron being transported across the Caco-2 cells. The amount of available iron and iron being transported from ferrous sulphate ($FeSO_4$) did not differ significantly for both the dialysis and centrifugation method. The effects of

different molar ratios of nutrients (calcium, ascorbic acid, zinc, tannic acid and caffeine) to iron on the availability of iron from cultured spirulina differs in comparison with $FeSO_4$. In the presence of lower concentrations of calcium (1:5, 1:10, 1:15 and 1:20 Fe:Ca molar ratios), iron from spirulina was not significantly inhibited compared to $FeSO_4$ but at higher concentrations (1:37.34, 1:74.67 and 1:149.34 Fe:Ca molar ratios) iron from both spirulina and $FeSO_4$ was significantly inhibited. The availability of iron from spirulina in the presence of ascorbic acid were not significantly enhanced at all the molar ratios tested (1:0.5, 1:1, 1:1.5 and 1:2 Fe:AA molar ratios) whereas iron availability from $FeSO_4$ were significantly higher for all the molar ratios. Both zinc and tannic acid were more inhibiting on iron availability from spirulina in comparison to $FeSO_4$. As for caffeine, it did not show any significant inhibitory effects on both iron availability from spirulina and $FeSO_4$. Two iron pools could coexist in the spirulina, one containing organic iron and another comprising inorganic iron. Organic iron is known to be more bioavailable and less affected by the presence of other nutrients. This could be one of the explanations why the iron from this algae is highly available and its bioavailability is not significantly affected by other nutrients as in $FeSO_4$.

Haemoglobin repletion assay was used to further investigate the effect of calcium on absorption of iron in spirulina and it comparison with $FeSO_4$. In this study, haemoglobin and haematocrit levels of male Sprague-Dawley rats fed both spirulina and $FeSO_4$ were found similar although the dose of $FeSO_4$ used had twice the amount of iron compared to that in spirulina. The presence of calcium did not significantly reduced the haematological value in rats fed spirulina and $FeSO_4$. The percentage of haemoglobin regeneration efficiency (HRE) obtained was significantly higher in rats fed spirulina compared with rats fed $FeSO_4$ indicated that the absorption efficiency were better from iron in spirulina compared to iron in $FeSO_4$.

The distribution study of iron from spirulina and $FeSO_4$ in the presence of calcium was done using iron deficient and iron normal male ICR mice fed either spirulina or $FeSO_4$ tagged extrinsically with ^{59}Fe. The amount of ^{59}Fe being absorbed by the iron deficient mice fed spirulina was comparable with those fed $FeSO_4$ at 6 h and 24 h. However at 7 d, the $FeSO_4$ group showed better absorption than the spirulina group. In the iron normal mice, a significantly lower percentage of ^{59}Fe was observed in mice fed spirulina compared to mice fed $FeSO_4$ at 6 h and 24 h indicating that iron from spirulina were not readily absorbed in iron normal states, which could prevent iron overload and toxicity. The presence of calcium did not significantly inhibit iron absorption in spirulina as shown in the *in vitro* study.

This study indicated that spirulina is a concentrated source of iron for both supplementation and fortification. Iron from spirulina is highly bioavailable and easily absorbed by the body especially in the iron deficient state. Beside providing the necessary iron, it could also prevent iron overload and toxicity in normal iron status and thus making spirulina suitable for both the iron deficient and normal iron status.

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

BIOAVAILABILITI FERUM DARIPADA SPIRULINA (*ARTHROSPIRA PLATENSIS*) DAN INTERAKSINYA DENGAN FAKTOR DIETARI LAIN SECARA IN VITRO DAN IN VIVO

Oleh

Loh Su Peng

Jun 2004

Pengerusi : Profesor Madya Maznah Ismail, Ph.D.

Fakulti : Perubatan dan Sains Kesihatan

Masalah kekurangan ferum merupakan perkara biasa di seluruh dunia. Pelbagai cara telah digunakan untuk meningkatkan pengambilan dan penyerapan ferum. Ini termasuk penggunaan spirulina, sejenis mikroalga yang telah popular di banyak negara di Asia sebagai makanan suplemen berfungsi. Objektif utama kajian ini adalah untuk menentukan bioavailabiliti ferum daripada spirulina dan interaksinya dengan faktor dietari lain secara *in vitro* dan *in vivo*.

Gabungan proses penghadaman *in vitro* dan sel kultur Caco-2 yang diikuti samada dengan langkah emparan atau dialisis telah digunakan untuk menilai keperolehan (*availability*) ferum daripada spirulina. Dengan menggunakan kaedah emparan, spirulina yang dikultur dan spirulina komersial telah memberikan hasil yang lebih signifikan ($P<0.05$) berbanding dengan kaedah dialisis dari segi bentuk ferum yang tersedia untuk penyerapan dan juga jumlah sebenar ferum yang diangkut melalui sel Caco-

2. Jumlah ferum yang tersedia dan ferum yang diangkut dari ferus sulfat ($FeSO_4$) tidak berbeza secara signifikan untuk kedua-dua kaedah dialisis dan emparan. Kesan pelbagai nisbah molar nutrien (kalsium, asid askorbic, zink, asid tanik dan kafein) terhadap kedapatan ferum daripada spirulina yang dikultur bebeza berbanding dengan $FeSO_4$. Kehadiran kalsium pada kepekatan rendah (nisbah molar Fe:Ca 1:5, 1:10, 1:15 and 1:20), tidak merencat penyerapan ferum daripada spirulina secara signifikan berbanding $FeSO_4$ tetapi pada kepekatan tinggi (nisbah molar Fe:Ca 1:37.34, 1:74.67 dan 1:149.34) penyerapan ferum daripada kedua-dua spirulina dan $FeSO_4$ didapati terencat secara signifikan. Keperolehan ferum daripada spirulina dengan kehadiran asid askorbik pada nisbah molar yang diuji (nisbah molar Fe:AA 1:0.5, 1:1, 1:1.5 and 1:2) tidak ditingkatkan secara signifikan manakala keperolehan ferum $FeSO_4$ dapat ditingkatkan secara signifikan untuk semua nisbah molar. Kedua-dua zink dan asid tanik menunjukkan kesan rencatan terhadap keperolehan ferum daripada spirulina berbanding dengan $FeSO_4$. Kafein pula tidak menunjukkan sebarang kesan signifikan terhadap ferum dari kedua-dua spirulina dan $FeSO_4$. Dua tadahan ferum wujud bersama di dalam spirulina, satu mengandungi ferum organik dan satu lagi ferum tak organik. Ferum organik telah dikenalpasti menunjukkan bioavailabiliti ferum yang lebih baik dan tidak dipengaruhi oleh kehadiran nutrien-nutrien lain. Ini mungkin merupakan salah satu penjelasan mengapa keperolehan ferum dari alga ini adalah sangat tinggi dan bioavailabilitinya tidak dipengaruhi secara signifkan oleh kehadiran nutrien-nutrien lain seperti dalam $FeSO_4$.

Asai *haemoglobin repletion* telah digunakan untuk mengkaji secara *in vivo* kesan kalsium terhadap penyerapan ferum daripada spirulina dan perbandingannya dengan $FeSO_4$. Dalam kajian ini, didapati hemoglobin, hematokrit dan paras *mean corpuscular volume* (MCV) tikus jantan Sprague-Dawley yang diberi diet spirulina dan $FeSO_4$ adalah serupa walaupun dos ferum daripada $FeSO_4$ adalah dua kali ganda lebih tinggi dari jumlah ferum spirulina. Kehadiran kalsium tidak merencat secara signifikan nilai hematologi tikus yang diberi spirulina tetapi tidak dalam $FeSO_4$. Peratusan "kecekapan pembaharuan hemoglobin" (*haemoglobin repletion efficiency*) yang didapati adalah lebih tinggi secara signikan dalam tikus yang diberi spirulina berbanding tikus yang diberi $FeSO_4$. Ini menunjukkan kecekapan penyerapan ferum daripada spirulina adalah lebih baik jumlah ferum daripada $FeSO_4$.

Kajian penyebaran ferum daripada spirulina dan $FeSO_4$ dengan kehadiran kalsium telah dijalankan dengan menggunakan mencit jantan ICR yang kekurangan ferum dan normal ferum. Mereka diberi sama ada diet spirulina atau $FeSO_4$ yang telah dilabel ^{59}Fe secara ekstrinsik. Jumlah ^{59}Fe yang diserap oleh mencit kekurangan ferum adalah serupa antara kumpulan yang diberi spirulina dengan kumpulan yang diberi $FeSO_4$ pada jam ke-6 dan ke-24. Walau bagaimanapun, pada hari ke-7, kumpulan $FeSO_4$ menunjukkan penyerapan yang lebih baik berbanding dengan kumpulan spirulina. Dalam mencit normal ferum, peratus ^{59}Fe yang lebih rendah dalam spirulina berbanding dengan $FeSO_4$ pada jam ke-6 dan ke-24 menunjukkan ferum dari spirulina tidak dapat tersedia untuk diserap yang mana dapat menghalang ketoksikan ferum dari berlaku. Kehadiran kalsium tidak menghalang secara signifikan penyerapan ferum daripada spirulina seperti yang ditunjukkan oleh kajian *in vitro*.

Keseluruhan kajian ini menunjukkan spirulina merupakan sumber ferum yang berkepekatan tinggi untuk dijadikan suplemen dan fortifikasi makanan. Bioavailabiliti ferum dari spirulina adalah tinggi dan mudah diserap oleh tubuh terutamanya dalam keadaan kekurangan ferum. Selain dapat memberi ferum yang diperlukan, ia juga dapat menghalang berlakunya kesaratan ferum dan ketoksikan dalam keadaan normal ferum dan oleh itu spirulina adalah sesuai untuk kedua-dua keadaan kekurangan ferum dan normal ferum.

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I certify that an Examination Committee met on 2nd June 2004 to conduct the final examination of Loh Su Peng on her Doctor of Philosophy thesis entitled "Iron Bioavailability from Spirulina (*Arthrospira platensis*) and its Interactions with Other Dietary Factors in vitro and in vivo" 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:

Khor Geok Lin, Ph.D.
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Maznah Ismail, Ph.D.
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Dato' Abdul Salam Abdullah, Ph.D.
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Hishamuddin Omar, Ph.D.
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Rehir Dahalan, Ph.D.
Atomic Energy Licensing Board
(Member)

Dennis D. Miller, Ph.D.
Professor
Department of Food Science
Cornell University
(Independent Examiner)

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 AUG 2004

The thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Maznah Ismail, Ph.D.
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Dato' Abdul Salam Abdullah, Ph.D.
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Hishamuddin Omar, Ph.D.
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Rehir Dahalan, Ph.D.
Atomic Energy Licensing Board
(Member)

AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 20 SEP 2004

DECLARATION

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

LOH SU PENG

Date: 25.08.04

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Iron deficiency is the world's most widespread nutritional disorder, affecting both industrialised and developing countries. Iron deficiency and anaemia affect all age groups, particularly the young children and pregnant women and their impact present a major hurdle to national development. The World Health Organization estimated nearly 2 billion people worldwide are anaemic and over twice that number are iron deficient (WHO, 2000). Globally, 39% of preschool children and 52% of pregnant women are anaemic, of whom more than 90% live in developing countries (Figure 1.1).

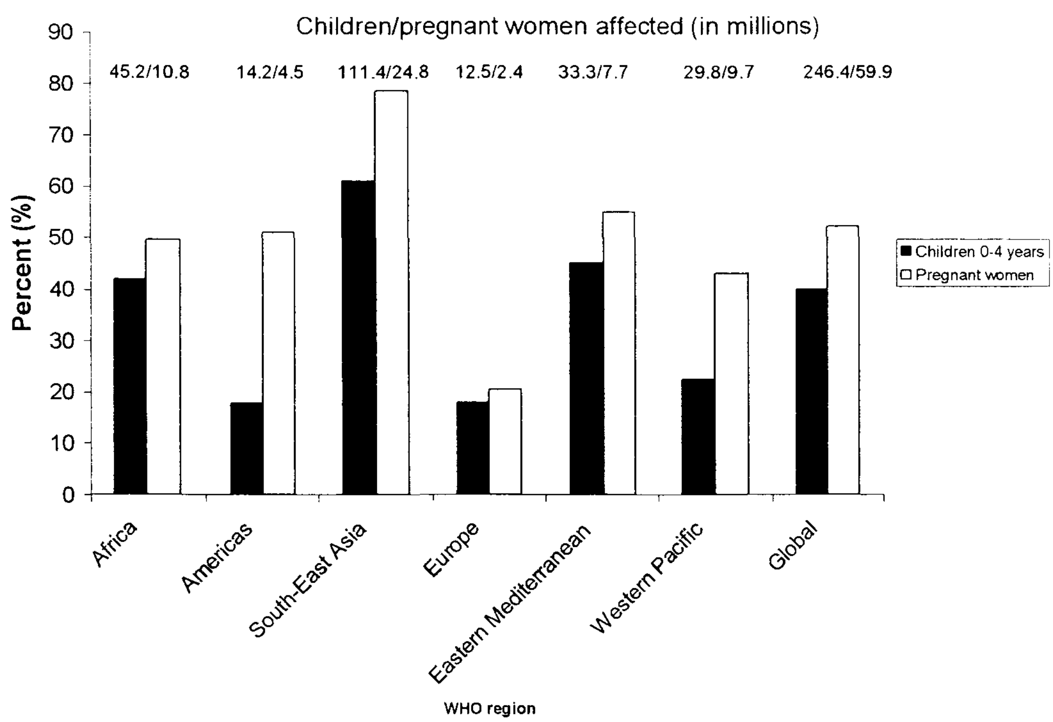

Figure 1.1: Estimated percentage of population affected by iron deficiency anaemia, by WHO region. Source: WHO (2000)