UNIVERSITI PUTRA MALAYSIA

BIOAVAILABILITY AND PHARMACOKINETICS STUDIES OF GAMMA ORYZANOL

WAFAA MUSTAFA HASAN HAILAT

FPSK(M) 2004 6
BIOAVAILABILITY AND PHARMACOKINETICS STUDIES OF GAMMA ORYZANOL

By

WAFAA MUSTAFA HASAN HAILAT

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

October 2004
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of
the requirement for the degree of Master of Science

BIOAVAILABILITY AND PHARMACOKINETICS STUDIES OF GAMMA
ORYZANOL

By

WAFAA MUSTAFA HASAN HAILAT

October 2004

Chairman: Associate Professor Maznah Ismail, Ph.D.

Faculty: Medicine and Health Sciences

Rice bran oil was extracted from rice bran collected after four milling breaks that were
used to process rice in Bernas factory, Sekinchan, Malaysia. Two organic solvents were
used, a non-polar solvent that was hexane and a mixture of non-polar and polar, which
were chloroform-methanol. Gamma oryzanol content of rice bran oil was then
quantified, and the total antioxidant activity (TAA) was determined using FTC and TBA
methods. After oil extraction, dietary fiber content was quantified in the four phases of
defatted rice bran. Results showed that rice bran contained around 20% lipid in the
extracts of the two solvents used. Unlike oil yield, γ-oryzanol content was affected by
rice milling and the type of solvent used for extraction. For chloroform-methanol
extract, phase 2 of rice milling contained the highest amount of γ-oryzanol (5280 ± 120
ppm), followed by phase 3 (3820 ± 60 ppm), phase 4 (3400 ± 100 ppm), and phase 1
(3000 ± 80 ppm). The four phases of hexane extracts contained lower amount of γ-
oryzanol than chloroform-methanol extracts. Phase 2 of rice milling contained the
highest γ-oryzanol content (4560 ± 100 ppm), followed by phase 3 (2400 ± 40 ppm),
phase 4 (2080 ± 40 ppm), and phase 1 (1600 ± 60 ppm). TAA studies showed that rice
bran oil extracted from phase 2 of rice milling had significantly higher antioxidant activity than phase 1 (p<0.05). However, no significant differences were found among other phases (p>0.05). It was found that rice bran is a good source of dietary fiber. However, fiber distribution was affected also by milling systems. Phase 2 of rice milling contained the highest amount of TDF which was 51.2 ± 0.9 %, followed by phases 3, 1 and 4 that contained 45.2 ± 1.0 %, 37.6 ± 0.1 % and 35.5 ± 0.8 % respectively.

Caco-2 cell line was used as in vitro model to study γ-oryzanol bioavailability from different formulations that were triolein solution, emulsion, tocotrienol rich fraction (TRF)-γ-oryzanol emulsion, and microspheres. By day 9, cell line showed polarized monolayer properties as was detected from transepithelial electrical resistance (TEER) value (247.2 ± 25.0 Ωcm²) and phenol red diffusion (4.2 ± 0.1 %). However, all experiments were conducted at day 18, to ensure that cells were fully polarized. In vitro digestion of 100 mg dose from each formulation resulted in low micellarization concentrations of γ-oryzanol from both triolein solution and microspheres, that were 21 ± 2 μg/ml digestate, and 20 ± 2 μg/ml respectively. Nevertheless, micellarization concentrations were greatly improved to 5087 ± 147 μg/ml and 5160 ± 228 μg/ml, from emulsion and TRF- γ-oryzanol emulsion, respectively. After 10 h of incubation, only 0.43 ± 0.02 μg (2.03 ± 0.09 %) γ-oryzanol was transported to the lower compartments from triolein solution. Cellular uptake of γ-oryzanol from microspheres after the same period of incubation, increased to 1.25 ± 0.09 μg (6.33 ± 0.44 %). Gamma oryzanol absorption increased further to 114.94 ± 2.02 μg (2.31 ± 0.04 %) and 115.82 ± 4.52 μg (2.24 ± 0.05 %) from emulsion and TRF- γ-oryzanol emulsion, respectively.
Pharmacokinetics of γ-oryzanol was studied using rabbits. Gamma oryzanol emulsion was given as a single intravenous dose. Plasma level of γ-oryzanol was quantified using HPLC. Plasma clearance of γ-oryzanol followed two compartments model, indicating that γ-oryzanol was distributed to the internal tissues. Elimination constant was $0.086 \pm 0.004 \, \mu g/ml.h$, and the half-life was $8.040 \pm 0.360 \, h$.

Rabbits were used as in vivo model to study the bioavailability of γ-oryzanol from triolein solution, microspheres, emulsion and TRF- γ-oryzanol emulsion. The maximum concentration of γ-oryzanol from triolein solution was $6.37 \pm 1.48 \, \mu g/ml$, and improved to $130.30 \pm 30.40 \, \mu g/ml$ upon loading γ-oryzanol in microspheres. However, in both formulations, the maximum concentrations were achieved after 2 h of ingestion. Whereas the maximum concentrations of γ-oryzanol from emulsion and TRF- γ-oryzanol emulsion were $555 \pm 100 \, \mu g/ml$ and $525 \pm 95 \, \mu g/ml$ respectively and the $t_{max}$ was 2 h.

The absolute bioavailability of γ-oryzanol emulsion was $6.61 \pm 0.86 \%$. The oral emulsion was used as a standard, so that the relative bioavailability ($F_{relative}$) values of the other formulations were calculated. While $F_{relative}$ for γ-oryzanol from triolein solution was only $0.51 \pm 0.06 \%$, it was significantly ($p<0.05$) increased to $16.63 \pm 1.71 \%$ upon loading γ-oryzanol in microspheres. Addition of TRF to γ-oryzanol emulsion resulted in an increase of $F_{relative}$ to $109.60 \pm 13.83 \%$. However, this increase could be due to the preservative effect of TRF antioxidants.
In conclusion, the bioavailability of γ-oryzanol was low. However, its absorption increased around 200 times after emulsification and 33 times upon loading in microspheres.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KAJIAN BIOAVAILABILITI DAN FARMAKOKINETIK GAMMA ORIZANOL

Oleh
WAFAA MUSTAFA HASAN HAILAT

Oktober 2004

Pengerusi: Profesor Madya Maznah Ismail, Ph.D.
Fakulti: Perubatan dan Sains Kesihatan

Minyak dedak beras diekstrak daripada dedak beras yang diperolehi melalui empat peringkat proses pengilangan yang digunakan semasa memproses beras di kilang BERNAS, Sekinchan, Malaysia. Dua pelarut organik telah digunakan, pelarut bukan polar iaitu heksana dan pelarut campuran polar dan bukan polar iaitu kloroform-metanol. Kandungan gamma orizanol bagi minyak dedak beras telah ditentukan dan jumlah aktiviti antioksidan (JAA) telah ditentukan menggunakan kaedah FTC dan TBA. Selepas minyak diekstrak, kandungan fiber diet ditentukan dalam empat fasa dedak beras ternyahlemak. Hasil kajian menunjukkan dedak beras mengandungi lebih kurang 20 % lipid bagi kedua-dua pelarut yang digunakan. Berbeza dengan kandungan minyak, kepekatan orizanol dipengaruhi oleh fasa pengilangan dan jenis pelarut yang digunakan semasa pengekstrakan. Bagi ekstrak kloroform-metanol, fasa kedua proses pengilangan beras menunjukkan amaun γ-orizanol yang tinggi (5280 ± 120 ppm), diikuti fasa ketiga (3820 ± 60 ppm), fasa keempat (3400 ± 100 ppm) dan fasa pertama (3000 ± 80 ppm).
Keempat-empat fasa ekstrak heksana mengandungi peratus $\gamma$-orizanol yang lebih rendah daripada ekstrak kloroform-metanol. Bagi ekstrak heksana, sampel daripada fasa kedua proses pengilangan beras menunjukkan peratusan $\gamma$-orizanol yang tinggi (4560 ± 100 ppm), diikuti fasa ketiga (2400 ± 40 ppm), fasa keempat (2080 ± 40 ppm) dan fasa pertama (1600 ± 60 ppm). Kajian menunjukkan JAA minyak dedak beras yang diekstrak daripada fasa kedua adalah lebih tinggi secara signifikan daripada fasa pertama (p<0.05). Namun, tiada perbezaan yang signifikan (p>0.05) diperolehi antara fasa-fasa yang lain. Dedak beras merupakan sumber yang baik bagi fiber diet. Walau bagaimanapun taburan fiber turut dipengaruhi oleh sistem pengilangan. Fasa kedua bagi proses pengilangan beras mengandungi amaun jumlah fiber diet (JFD) yang tinggi iaitu 51.2 ± 0.9 %, diikuti fasa ketiga, fasa pertama dan fasa keempat yang mengandungi 45.2 ± 1.0 %, 37.6 ± 0.1 % dan 35.5 ± 0.8 % masing-masing.

Sel Caco-2 digunakan sebagai model in vitro untuk mengkaji bioavailabiliti $\gamma$-orizanol daripada formulasi yang berbeza, iaitu larutan triolein, emulsi yang kaya dengan pecahan tokotrienol dan mikrosfera. Semasa hari ke-9, titisan sel menunjukkan ciri-ciri ekalapis yang terpolar seperti yang nilai ditunjukkan oleh transepitelial rintangan elektrik (TEER) (247.2 ± 25.0 $\Omega \cdot \text{cm}^2$) dan difusi fenol merah (0.42 ± 0.08 %). Walau bagaimanapun, semua ujikaji yang dijalankan pada hari ke 18 menunjukkan sel-sel berpolar sepenuhnya. Pencernaan in vitro bagi dos 100 mg daripada setiap bentuk menunjukkan peratus pemiselan $\gamma$-orizanol yang rendah bagi kedua-dua larutan triolein dan mikrosfera, iaitu 21 ± 2 $\mu$g/ml dan 20 ± 2 $\mu$g/ml penghadaman masing-masing. Namun begitu, kepekatan pemiselan telah meningkat kepada 5087 ± 147 $\mu$g/ml dan 5160
± 228 μg/ml, daripada emulsi dan emulsi yang kaya pecahan tokotrienol masing-masing. Selepas 10 jam eraman, hanya 0.43 ± 0.02 μg (2.03 ± 0.09 %) γ-orizanol diangkut kepada bahagian yang lebih rendah daripada larutan triolein. Pengambilan selular bagi orizanol daripada mikrosfera selepas tempoh eraman yang sama, meningkat kepada 1.25 ± 0.09 μg (6.33 ± 0.44 %). Penyerapan γ-orizanol semakin meningkat kepada 114.94 ± 2.02 μg (2.31 ± 0.04 %) dan 115.82 ± 4.52 μg (2.24 ± 0.05 %) daripada emulsi dan emulsi yang kaya pecahan tokotrienol masing-masing.

Farmakokinetic bagi γ-orizanol dikaji dengan menggunakan arnab. Emulsi γ-orizanol diberi sebagai dos intravena tunggal. Aras plasma bagi γ-orizanol ditentukan dengan menggunakan HPLC. Pembersihan plasma γ-orizanol mengikuti dua bahagian model, menunjukkan γ-orizanol diagihkan kepada dua tisu dalaman. Pemalar penyingkiran ialah 0.086 ± 0.004 μg/ml.jam dan separuh hayat adalah 8.040 ± 0.360 jam.

Arnab digunakan sebagai model in vivo dalam kajian bioavailabiliti γ-orizanol daripada larutan triolein, mikrosfera, emulsi dan fraksi kaya tokotrienol (FKT) dalam emulsi γ-orizanol. Kepekatan maksimum γ-orizanol daripada larutan triolein ialah 6.37 ± 1.48 μg/ml dan meningkat kepada 130.3 ± 30.4 μg/ml setelah γ-orizanol diberi dalam bentuk mikrosfera. Walau bagaimanapun, dalam kedua-dua bentuk, kepekatan maksimum dicapai selepas 2 jam penghadaman. Kepekatan maksimum γ-orizanol daripada emulsi dan emulsi yang kaya pecahan tokotrienol pula ialah 555 ± 100 μg/ml dan 525 ± 95 μg/ml masing-masing dan masa maksimum bagi kedua-dua bentuk ialah 2 jam.
Bioavailabiliti bagi emulsi γ-orizanol ialah 6.61 ± 0.86 %. Emulsi secara oral digunakan sebagai piawaian, jadi nilai bioavailabiliti relatif (F relatif) bagi setiap bentuk dikira. F (relatif) bagi γ-orizanol daripada larutan triolein hanya 0.51 ± 0.06 %, tetapi ia meningkat secara signifikan (p<0.05) kepada 16.63 ± 1.71 % apabila γ-orizanol diberi dalam bentuk mikrosfera. Penambahan fraksi kaya tokotrienol (FKT) dalam emulsi orizanol menghasilkan peningkatan F (relatif) kepada 109.60 ± 13.83 %. Walau bagaimanapun, peningkatan ini mungkin akibat daripada kesan pengawetan antioksidan FKT.

Kesimpulannya, bioavailabiliti γ-orizanol adalah rendah. Walau bagaimanapun, penyerapannya meningkat kira-kira 200 kali selepas emulsifikasi dan 33 kali selepas pemberian dalam bentuk mikrosfera.
ACKNOWLEDGEMENTS

I would like to thank my God for shedding on me health and keeping my brain working to the extent of completing this research, which I hope will contribute to the welfare of mankind.

I would like to express my most sincere appreciation to my supervisor Associate Professor Dr. Maznah Ismail for her invaluable attention, guidance, and support throughout my graduate studies. I would like to also thank other members of my committee, Professor Dato’ Dr. Abdul Salam Abdullah and Dr. Hishamuddin Omar for their assistance.

I gratefully acknowledge staff, laboratory assistants and graduate students at Department of Nutrition and Health Sciences for their help and cooperation. Finally I would like to convey my appreciation to my parents, husband, brothers and sisters for their support over the course of this thesis.
I certify that an Examination Committee met on 7th October 2004 to conduct the final examination of Wafaa Mustafa Hasan Hailat on her Master of Science thesis entitled "Bioavailability and Pharmacokinetics Studies of Gamma Oryzanol" 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:

Hamdan Mohd Noor, Ph.D.
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Muhammad Nazrul Hakim Abdullah, Ph.D.
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Amsah Haji Yahaya, Ph.D.
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Salmijah Surif, Ph.D.
Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Independent Examiner)

ZAKARIAH ABD. RASHID, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 20 DEC 2004
This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. 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
University Putra Malaysia
(Member)

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

---

[Signature]

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

Date: 13 JAN 2005
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.

WAFAA MUSTAFA HASAN HAILAT

Date: 01 DEC 2004
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>x</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xviii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xx</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION**

2. **LITERATURE REVIEW**

   2.1 Rice Bran
   2.2 Rice Milling Process
   2.3 Health Benefits of Rice Bran
   2.4 Rice Bran Stabilization
      2.4.1 Chemical Stabilization
      2.4.2 Physical Stabilization
         2.4.2.1 Cold Storage
         2.4.2.2 Storage in An Inert Atmosphere
         2.4.2.3 Irradiation
         2.4.2.4 Heat Stabilization
            2.4.2.4.1 Moist Heat
            2.4.2.4.2 Dry Heat
         2.4.2.5 Microwave Stabilisation
   2.5 Rice Bran Oil
      2.5.1 Composition of Rice Bran Oil
         2.5.1.1 Triglycerides
         2.5.1.2 Minor Constituents
      2.5.2 Rice Bran Oil Extraction
      2.5.3 Refining of Crude Oil
      2.5.4 Health Benefits of Rice Bran Oil
   2.6 Gamma Oryzanol
      2.6.1 Overview
      2.6.2 Biological Effects of γ-Oryzanol
      2.6.3 Chromatographic Separation of γ-Oryzanol
         2.6.3.1 Chromatography Principle
         2.6.3.2 HPLC Principle
         2.6.3.3 Separation of γ-Oryzanol By HPLC

xiv
2.7 Bioavailability
2.7.1 Estimating The Bioavailability Using In Vivo System
2.7.2 Caco-2 Cells: In Vitro System For Estimating Intestinal Absorption
2.8 Emulsion As Oral Drug Delivery Form
2.9 Microspheres
2.9.1 Microspheres As Oral Drug Delivery Form
2.9.2 Microspheres Preparation
2.10 Kinetics Models of Intravenously Injected Compounds
2.10.1 One Compartment Model
2.10.2 Two Compartments Model
2.10.3 Three Compartments Model

3 MATERIALS AND METHODS

3.1 Materials
3.1.1 Biological Materials
3.1.2 Chemicals
3.1.3 Instruments and Equipments
3.2 Experimental Methods
3.2.1 Rice Bran Collection and Stabilization
3.2.2 Rice Bran Oil Extraction By Solvent Extraction Method
3.2.3 HPLC Analysis of γ-Oryzanol
3.2.4 Antioxidant Activity Assay
3.2.4.1 Ferric Thiocyanate (FTC) Method
3.2.4.2 Thiobarbituric Acid (TBA) Method
3.2.5 Total Dietary Fibre Analysis
3.2.6 In Vitro Bioavailability Studies of γ-Oryzanol And Its Ferulate Part
3.2.6.1 Preparation of γ-Oryzanol Solution
3.2.6.2 Preparation of γ-Oryzanol Emulsion
3.2.6.3 Preparation of Tocotrienol Rich Fraction (TRF)-γ-Oryzanol Emulsion
3.2.6.4 Preparation of γ-Oryzanol Loaded Microspheres
3.2.6.5 Cultivation of Caco-2 Cells
3.2.6.5.1 Cell Incubation
3.2.6.5.2 Transwell Bicameral Chambers
3.2.6.5.3 Phenol Red Diffusion
3.2.6.5.4 Transepithelial Electrical Resistance (TEER)
3.2.6.6 Cellular Uptake Studies
3.2.6.6.1 In Vitro Digestion
3.2.6.6.2 Cellular Uptake of γ-Oryzanol
3.2.6.6.3 Cells Extraction
3.2.7 In Vivo Bioavailability and Pharmacokinetics Studies of γ-Oryzanol
3.2.7.1 Animals Acclimatization
3.2.7.2 Preparation and Administration of Intravenous Dose
3.2.7.3 Administration of Oral Doses
3.2.7.4 Blood Sampling
3.2.7.5 Extraction of γ-Oryzanol From Plasma

3.3 Statistical Analysis

4 RESULTS AND DISCUSSION

4.1 Oil Yield
4.2 HPLC Analysis of γ-Oryzanol
4.3 Total Antioxidant Activity Studies
  4.3.1 Ferric Thiocyanate Method
  4.3.2 Thiobarbituric Acid Method
4.4 Fibre Content of Rice Bran
4.5 Bioavailability Studies of γ-Oryzanol From Different Formulations
  Using Caco-2 cells
  4.5.1 Cell Growth On The Transwell Bicameral Chambers
  4.5.2 Micellarization of γ-Oryzanol
  4.5.3 Cellular Uptake of γ-Oryzanol
    4.5.3.1 Toxicity Test For γ-Oryzanol Formulations
    4.5.3.2 Cellular Uptake of γ-Oryzanol From Different Formulations
      4.5.3.2.1 Cellular Uptake of γ-Oryzanol From Triolein Solution
      4.5.3.2.2 Cellular Uptake of γ-Oryzanol From Emulsion
      4.5.3.2.3 Cellular Uptake of γ-Oryzanol From TRF-γ-Oryzanol Emulsion
      4.5.3.2.4 Cellular Uptake of γ-Oryzanol From Microspheres
4.6 In Vivo Bioavailability and Pharmacokinetics Studies of γ-Oryzanol
  4.6.1 Pharmacokinetics of γ-Oryzanol Using Single Dose of Intravenous Emulsion
  4.6.2 Bioavailability of γ-Oryzanol From Triolein Solution
  4.6.3 Bioavailability of γ-Oryzanol From Emulsion
  4.6.4 Bioavailability of γ-Oryzanol From TRF-γ-Oryzanol Emulsion
  4.6.5 Bioavailability of γ-Oryzanol From Microspheres

5 GENERAL DISCUSSION

6 CONCLUSION

7 RECOMMENDATIONS

REFERENCES
APPENDICES
BIODATA OF THE AUTHOR
LIST OF TABLES

Table

2.1 Nutrient composition of stabilized full fat rice bran 9
2.2 Comparison of rice bran to other cereal brans 10
2.3 Composition of crude rice bran oil 18
4.1 Rice bran oil yield from bran at four phases of rice milling breaks using two different solvents 62
4.2 γ- Oryzanol content in rice bran oil extracted from four phases of rice milling process using two different solvents 70
4.3 Percentage of antioxidant activity of rice bran oil extracted from rice bran collected after four milling breaks using FTC method 78
4.4 Percentage of antioxidant activity of rice bran oil extracted from rice bran collected after four milling breaks using TBA method 80
4.5 Dietary fibre contents in rice bran collected from four phases of rice milling 82
LIST OF FIGURES

Figure

2.1 Structure of mature rice grain 7
2.2 Flow chart of rice milling by-products 8
2.3 γ-Oryzanol Structures 23
2.4 HPLC chromatogram of crude rice bran oil 31
2.5 Semilog graph for plasma level-time curve for the compound that follow one compartment model 39
2.6 Semilog graph for plasma level-time curve for the compound that follow two compartments model 40
2.7 Semilog graph for plasma level-time curve for the compound that follow three compartments model 41
3.1 Transwell bicameral chamber, 24 mm diameter 53
4.1 HPLC chromatogram of γ-oryzanol standard 67
4.2 Separation of γ-oryzanol components from crude rice bran oil 68
4.3 HPLC chromatogram of γ-oryzanol extracted from crude rice bran oil 69
4.4 Absorbance values of hexane extracted oil from four phases of rice bran using FTC method 75
4.5 Absorbance values of chloroform-methanol (2:1) extracted oil from four phases of rice bran using FTC method 76
4.6 Absorbance values of hexane extracted oil from four phases of rice bran using TBA method 79
4.7 Absorbance values of chloroform-methanol (2:1) extracted oil from four phases of rice bran using TBA method 79
4.8 Phenol red diffusion through transwell bicameral chambers 87
4.9 TEER values across transwell bicameral chambers 88
4.10 Micellarization of γ-oryzanol from different formulations 91
<table>
<thead>
<tr>
<th>4.11</th>
<th>Cellular uptake of γ-oryzanol from triolein solution</th>
<th>93</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.12</td>
<td>Cellular uptake of ferulic acid</td>
<td>95</td>
</tr>
<tr>
<td>4.13</td>
<td>Cellular uptake of γ-oryzanol from emulsion</td>
<td>97</td>
</tr>
<tr>
<td>4.14</td>
<td>Cellular uptake of γ-oryzanol from TRF- γ-oryzanol emulsion</td>
<td>99</td>
</tr>
<tr>
<td>4.15</td>
<td>Cellular uptake of γ-oryzanol from microspheres</td>
<td>102</td>
</tr>
<tr>
<td>4.16</td>
<td>Plasma level of γ-oryzanol after intravenous administration</td>
<td>104</td>
</tr>
<tr>
<td>4.17</td>
<td>Logarithm plasma level of γ-oryzanol after the administration of intravenous dose</td>
<td>105</td>
</tr>
<tr>
<td>4.18</td>
<td>Elimination phase of γ-oryzanol after the administration of intravenous dose</td>
<td>106</td>
</tr>
<tr>
<td>4.19</td>
<td>Plasma level of γ-oryzanol from triolein solution during 72 h</td>
<td>108</td>
</tr>
<tr>
<td>4.20</td>
<td>Plasma level of γ-oryzanol from emulsion during 72 h</td>
<td>111</td>
</tr>
<tr>
<td>4.21</td>
<td>Plasma level of γ-oryzanol from TRF- γ-oryzanol emulsion during 72 h</td>
<td>113</td>
</tr>
<tr>
<td>4.22</td>
<td>Plasma level of γ-oryzanol from microspheres during 72 h</td>
<td>115</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

TAA  Total Antioxidant Activity
FTC  Ferric Thiocyanate Method
TBA  Thiobarbituric Acid Method
TRF  Tocotrienol Rich Fraction
TC   Total Cholesterol
HDL-C High Density Lipoprotein Cholesterol
LDL-C Low Density Lipoprotein Cholesterol
VLDL-C Very Low Density Lipoprotein Cholesterol
FFA  Free Fatty Acid
LH   Leuteinizing Hormone
TSH  Thyroid Stimulating Hormone
GH   Growth Hormone
PRL  Prolactin Releasing Hormone
PDA  Photodiode Array
HPLC High Performance Liquid Chromatography
F    Bioavailability
TDF  Total Dietary Fibre
IDF  Insoluble Dietary Fibre
SDF  Soluble Dietary Fibre
PLGA Poly (D,L-lactide-co-glycolide)
TEER Transepithelial Electrical Resistance
DMEM Dulbecos Modified Eagle Medium
IV   Intravenous
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>Butylated Hydroxy Toluene</td>
</tr>
<tr>
<td>RBO</td>
<td>Rice Bran Oil</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Rice is a staple food for about 60% of the world population. About 90% of the world's rice is produced and consumed in Asia. It is second to wheat in terms of annual production. World rice production in 1991 was 466 million metric tonnes (Sayre, 1991), and it has been increasing faster than other grains. For example, by 2002 it increased to 602 million metric tonnes (FAO, 2002). As a result of continuous growth in rice production and consumption, rice research and development activity has become important.

In order to produce edible white rice, it is milled to produce hull, bran, germ, and the white rice. Rice hulls have no nutritional value, but rice bran and germ are rich in protein, lipids, vitamins, and trace minerals (Saunders, 1985). Currently the majority of rice bran is used as animal feed. The naturally occurring enzymatic activity of rice bran leads to the hydrolysis of the oil after milling. However, immediate stabilization of rice bran could convert it to a useful and a healthy product (Ramezanzadeh et al., 1999).

Due to its composition, nutritional profile, functional characteristics and hypoallergenicity, rice bran is added to provide a healthy diet, high in dietary fiber and low in saturated fat (Marshall and Wadsworth, 1994). In addition, Kahlon et al. (1990) found that rice bran was as effective as oat bran at lowering serum cholesterol in hypercholesterolemic hamsters. There are strong indications that the consumption of rice bran may be specifically beneficial in reducing the risk of cardiovascular disease, which is now the major cause of mortality in many countries (Marshall and
Wadsworth, 1994; Khor, 1997). The mortality rate has been on the decline since 1960s in countries such as Australia, New Zealand and Japan. However, in Malaysia and China mortality due to cardiovascular disease is increasing and reaching 30-40% in 1997 (Khor, 1997). Although it had been found that rice bran could protect from cardiovascular diseases, this effect was suggested mainly due to its antioxidants (Marshall and Wadsworth, 1994).

Locally planted rice in Sekinchan is milled through two stage mills to remove first the hulls, and then the bran. However, bran is removed through four millers to produce four phases of rice bran. Few of previous research studied the milling conditions that affect the concentrations of rice bran antioxidants. Most studies focused on the extraction of rice bran oil and the isolation of its antioxidants. Others studied the health benefits of rice bran. Wells (1993) and Martin et al. (1993) studied the effect of heat or the stabilization process of rice bran on its tocopherols and tocotrienols levels.

In comparison with other cereal brans, rice bran contains high oil content, which ranged from 16-32% (Marshall and Wadsworth, 1994). In 1988, only 450,000 metric tonnes of rice bran oil were produced in the world, despite the potential of 2 million metric tonnes. Japan is the leading producer of rice bran oil with an average annual production of 100,000 metric tonnes (Sayre, 1988). Rice bran oil extraction began in Korea using the hydraulic press method. After that hexane extraction was carried out (Sayre, 1988). Supercritical fluid extraction is now investigated as alternative for organic extraction since organic solvents could be hazardous and expensive (Xu and Godber, 2000).
Due to the low content of linolenic acid and high antioxidants content, including tocopherols, tocotrienols, γ-oryzanol and phenolic compounds, rice bran oil has the ability to adjust cholesterol serum level (Marshall and Wadsworth, 1994). Rice bran oil contains 4.2% of unsaponifiable matter, which is higher than any other common vegetable oils (Sayre, 1991). The unsaponifiable matter comprised mostly of sterols along with γ-oryzanol. Crude rice bran oil can be refined to give a number of beneficial products, including edible rice bran oil, tocopherols and γ-oryzanol.

Gamma oryzanol is a mixture of ferulic acid esters of triterpene alcohols and plant sterols (Rogers et al., 1993). It had been found that crude rice bran oil contains 0.96-2.9% γ-oryzanol (Marshall and Wadsworth, 1994). Studies proved that γ-oryzanol possesses curative functions for many human diseases, including a reduction of cholesterol level in human and inhibition of platelet aggregation (Seetharamaiah and Chandrasekhara, 1988; Cicero and Gaddi, 2001). In addition, it had been found that γ-oryzanol had the ability to promote skin capillary circulation, and had anti-itching and anti-dandruff action, and has been used in cosmetics (Seetharamaiah and Prabhakar, 1986). However, plant sterols have limited bioavailability. As such, the benefits offered by γ-oryzanol could be limited by its bioavailability.

There are not many studies about the bioavailability and the pharmacokinetics of γ-oryzanol. Fujiwara et al. (1983) determined the amount of radioactivity in rat's blood after the administration of radiolabelled γ-oryzanol. The measurement of radioactivity in the blood could lead to overestimation of γ-oryzanol since metabolites could also be detected. Fujiwara et al. (1983) reported that 10-20% of