

## CONTENTS

|   | Pages |
|---|-------|
| <b>ACKNOWLEDEMENTS</b>                        | iii   |
| <b>ABSTRACT (ENGLISH)</b>                     | v     |
| <b>ABSTRACT (THAI)</b>                        | vii   |
| <b>CONTENTS</b>                               | x     |
| <b>LIST OF TABLES</b>                         | xiii  |
| <b>LIST OF FIGURES</b>                        | xvi   |
| <br>  |       |
| <b>CHAPTER 1 INTRODUCTION</b>                 |       |
| 1.1 Statement and significance of the problem | 1     |
| 1.2 Research objectives                       | 4     |
| 1.3 Potential application advantages          | 4     |
| <br>  |       |
| <b>CHAPTER 2 LITERATURE REVIEW</b>            |       |
| 2.1 Red yeast rice (RYR)                      | 5     |
| 2.2 Manufacturing process                     | 7     |
| 2.3 Rice ( <i>Oryza sativa</i> L.)            | 8     |
| 2.4 <i>Monascus</i> species                   | 12    |
| 2.5 Metabolites of <i>Monascus</i> spp.       | 22    |
| 2.5.1 Pigments                                | 23    |

**CONTENTS (CONTINUED)**

|   | <b>Pages</b> |
|---|--------------|
| 2.5.2 Monacolin K                                   | 29           |
| 2.5.3 Antioxidant properties                        | 35           |
| 2.5.4 Flavor and aroma of red yeast rice            | 48           |
| 2.5.5 Mycotoxin (citrinin)                          | 58           |
| 2.6 Application of <i>Monascus</i> metabolites      | 72           |
| <br><b>CHAPTER 3 MATERIALS AND METHODS</b>          |              |
| 3.1 Chemicals                                       | 80           |
| 3.1.1 Microorganism                                 | 80           |
| 3.1.2 Raw material                                  | 80           |
| 3.1.3 Nitrogen source nutrient                      | 81           |
| 3.2 Methods   | 81           |
| 3.2.1 Isolation of <i>Monascus purpureus</i>        | 81           |
| 3.2.2 Identification of <i>M. purpureus</i>         | 82           |
| 3.2.3 Preparation of red yeast rice                 | 83           |
| 3.2.4 Measuring the content of red pigments         | 84           |
| 3.2.5 Analysis of monacolins by HPLC and LC-MS      | 85           |
| 3.2.6 Determination of antioxidant properties       | 86           |
| 3.2.7 Analysis of volatile aroma compounds by GC-MS | 91           |
| 3.2.8 Analysis of citrinin by HPLC                  | 93           |

**CONTENTS (CONTINUED)**

|   | <b>Pages</b> |
|---|--------------|
| <b>CHAPTER 4 RESULTS AND DISCUSSION</b>   |              |
| 4.1 Isolation of <i>M. purpureus</i>  | 94           |
| 4.2 Identification of fungal strain   | 96           |
| 4.3 Preparation of red yeast rice   | 99           |
| 4.4 Measuring the content of red pigment  | 106          |
| 4.5 Monacolins in red yeast rice  | 108          |
| 4.6 Antioxidant activity in red yeast rice  | 116          |
| 4.6.1 Total antioxidant capacity  | 116          |
| 4.6.2 $\beta$ -carotene bleaching method (BCB)                                      | 118          |
| 4.7 Volatile aroma compounds of red yeast rice prepared from<br>Thai glutinous rice | 121          |
| 4.8 Mycotoxin (citrinin) in red yeast rice  | 130          |
| <b>CHAPTER 5 GENERAL CONCLUSION</b>   | <b>137</b>   |
| <b>REFERENCES</b>   | <b>140</b>   |
| <b>APPENDIX A</b>   | <b>163</b>   |
| <b>APPENDIX B</b>   | <b>169</b>   |
| <b>APPENDIX C</b>   | <b>185</b>   |
| <b>APPENDIX D</b>   | <b>196</b>   |
| <b>CURRICULUM VITAE</b>   | <b>203</b>   |

## LIST OF TABLES

| Table  | Pages |
|--|-------|
| 2.1 Rice classification based on amylose content.  | 11    |
| 2.2 Properties of <i>Monascus</i> .  | 19    |
| 2.3 Different enzymatic activities for some <i>Monascus</i> strains  | 20    |
| 2.4 The different proteinase activity of <i>Monascus</i> strains   | 21    |
| 2.5 Metabolites from <i>Monascus</i> .   | 22    |
| 2.6 Concentration of ACPY found in different varieties of cooked rice.   | 51    |
| 2.7 Compound involved in the discrimination between Azucena, Basmati, and Thai rice: results of canonical variate analysis             | 54    |
| 2.8 Concentrations ( $\mu\text{g.kg}^{-1}$ , wet wt) and odor description of major volatile compounds in various types of cooked rice. | 56    |
| 2.9 Natural occurrence of citrinin in commodities.   | 60    |
| 2.10 Application of <i>Monascus</i> pigments.  | 72    |
| 3.1 The volume of chemical reagents for standard total antioxidant capacity graph.   | 88    |
| 3.2 Reagents and preparation for determining the rate of the reaction in $\beta$ -carotene bleaching activity of standard (BHT).       | 89    |
| 3.3 Reagents and preparation of standard calibration curve for $\beta$ -carotene bleaching activity of standard BHT.                   | 91    |

## LIST OF TABLES (CONTINUED)

| <b>Table</b>   | <b>Pages</b> |
|--|--------------|
| 4.1 Number of rice grains with growth of strain.   | 94           |
| 4.2 Morphological and cultural characterisation of <i>M. purpureus</i> Went and <i>M. purpureus</i> CMU001.  | 97           |
| 4.3 Percentage yield of red yeast rice.  | 100          |
| 4.4 Red pigment contents from fermented 1 cultivar of normal rice and 3 cultivar of glutinous rice with <i>M. purpureus</i> .  | 106          |
| 4.5 Data of chemical profiling of monacolins in fermented red yeast rice (peak area) from non-glutinous rice and glutinous rice without soybean milk for 3 weeks.  | 111          |
| 4.6 Data of chemical profiling of monacolins in fermented red yeast rice ( peak area ) from non-glutinous rice and glutinous rice with soybean milk for 3 weeks.   | 112          |
| 4.7 Compactin, Monacolin K content in 3 weeks old red yeast rice.  | 113          |
| 4.8 Total antioxidant capacity in red yeast rice (GAE(mg/ml)).   | 118          |
| 4.9 The half-inhibition concentration (IC <sub>50</sub> ) values of the antioxidant activity measured using $\beta$ -carotene bleaching method of standard BHT ( $\mu$ g/ml) and red yeast rice samples. | 119          |
| 4.10 Sensory characteristics of red yeast rice prepared from Thai glutinous rice without an addition of soybean milk (2weeks solid fermentation).  | 121          |

**LIST OF TABLES (CONTINUED)**

| <b>Table</b>   | <b>Pages</b> |
|--|--------------|
| 4.11 Sensory characteristic of red yeast rice prepared from Thai glutinous rice with an addition of soybean milk(2weeks solid fermentation). | 122          |
| 4.12 Volatile compounds of red yeast rice without an addition of soybean milk.   | 124          |
| 4.13 Volatile compounds of red yeast rice with an addition of soybean Milk.  | 126          |
| 4.14 Compounds associated with volatile aroma of unprocessed (raw) rice <sup>a</sup>   | 128          |
| 4.15 Citrinin content in red yeast rice fermented without soybean milk.  | 133          |
| 4.16 Citrinin content in red yeast rice fermented with soybean milk.   | 133          |
| 5.1 Conclusion on the study of red yeast rice  | 137          |
| 5.2 Citrinin contents in red yeast rice  | 138          |

## LIST OF FIGURES

| <b>Figures</b>  | <b>Pages</b> |
|---|--------------|
| 2.1 Red yeast rice  | 5            |
| 2.2 Rice plants   | 8            |
| 2.3 Khaw Hom Mali 105, (a) non-glutinous rice grains,<br>(b) non-glutinous steamed rice.  | 9            |
| 2.4 Glutinous rice, (a) Purple rice ( Kam), (b) Kor Kho 6 (RD6)<br>(c) Sanpathong1(SPT1)  | 10           |
| 2.5 (a) Glutinous rice grain, (b) Stream glutinous rice   | 10           |
| 2.6 <i>Monascus purpureus</i> colony  | 12           |
| 2.7 Life cycle and morphological structure of <i>Ascomycetes</i>  | 17           |
| 2.8 Chemical structure of pigments from <i>Monascus</i>   | 25           |
| 2.9 Scheme of the hypothetic metabolic routes leading to the final<br>structure of the water-soluble red pigment<br><br><i>N</i> -glutarylmonascorubramine in <i>M. ruber</i> . | 26           |
| 2.10 Isolation of pure pigments from red yeast rice.  | 28           |
| 2.11 Structural data of monacolins in fermented rice.   | 30           |
| 2.12 Structural of monacolin K.   | 32           |
| 2.13 Mechanism of monacolin K synthesis.  | 33           |
| 2.14 Pathway of cholesterol synthesis.  | 34           |
| 2.15 Structure of vitamin E.  | 37           |

### LIST OF FIGURES (CONTINUED)

| <b>Figures</b>  | <b>Pages</b> |
|---|--------------|
| 2.16 Reduced glutathione (GSH).   | 38           |
| 2.17 Phenolic compounds.  | 39           |
| 2.18 Basic structure and numbering system for flavonoids.   | 41           |
| 2.19 Flavonoids structure.  | 41           |
| 2.20 Some examples of alkaloids.  | 43           |
| 2.21 A peroxidation pathway from linoleic acid to hexanal.  | 48           |
| 2.22 Structural formular of citrinin isomers.   | 63           |
| 2.23 Structural of citrinin H <sub>1</sub> and H <sub>2</sub> .   | 65           |
| 2.24 Scheme of the biosynthesis of citrinin by <i>M. ruber</i> . The start of<br>the condensing reaction is indicated by the bent arrow in the<br>upper left panel. Intermediates are numbered. Enrichment of C-1 (▲),<br>C-3 (□), C-9 (*), and C-4 (•) | 67           |
| 2.25 Biosynthesis of citrinin and red pigment in <i>M. ruber</i> . The toxin<br>pathway in <i>Aspergillus</i> and <i>Penicillium</i> is indicated by the dashed<br>arrow.   | 68           |
| 3.1 Surface sterilization.  | 82           |
| 3.2 Overall used to prepare red yeast rice in this study  | 84           |

## LIST OF FIGURES (CONTINUED)

| Figures  | Pages   |
|--|---------|
| 4.1 Isolation of <i>Monascus purpureus</i> from commercial Chinese red yeast rice available in local market.                 | 95      |
| 4.2 <i>Monascus purpureus</i> strain CMU001.   | 98      |
| 4.3 Starch hydrolysis activities of <i>M. purpureus</i> strain CMU001 after one  | 99      |
| 4.4 Product of red yeast rice.   | 101-105 |
| 4.5 Chromatographic chemical profiling of monacolins in fermented red yeast rice from non-glutinous rice and glutinous rice. | 108-110 |
| 4.6 Monacolin K in lactone form and acid form  | 115     |
| 4.6 Chromatographic chemical profiling of aroma in fermented red yeast rice from non-glutinous rice and glutinous rice.      | 129     |
| 4.7 Chromatogram of volatile aroma compounds of red yeast rice fermented for 2 weeks without an addition of soybean milk.    | 123     |
| 4.8 Chromatogram of volatile aroma compounds of red yeast rice fermented for 2 weeks with an addition of soybean milk        | 125     |
| 4.9 The structure of (a) 2,3-butanediol and (b) acetoin  | 129     |
| 4.10 Chromatographic chemical profiling of citrinin in fermented red yeast rice from non-glutinous rice and glutinous rice.  | 130-132 |