# CONTENTS

|             |  | Page |
|-------------|--|------|
| TITLE PAG   | E S  | i    |
| APPROVAL    | SHEET  | ii   |
| ACKNOWL     | EDGEMENTS                                      | iii  |
| ABSTRACT    | (ENGLISH)                                      | iv   |
| ABSTRACT    | (THAI)   | vi   |
| LIST OF TA  | BLES   | xiv  |
| LIST OF FIG | GURES  | xvi  |
| ABBREVIA'   | TIONS AND SYMBOLS                              | xix  |
|             |  |      |
| CHAPTER     | 1: INTRODUCTION                                |      |
| 1.0         | Rice   | 1    |
|             | 1.1.1 Aromatic rice                            | 2    |
|             | 1.1.2 Aromatic rice of Thailand                | 3    |
|             | 1.1.3 History of KDML 105                      | 3    |
|             | 1.1.4 Aroma                                    | 4    |
| 1.2         | Introduction to headspace sampling             | 8    |
| 1.3         | Introduction to headspace gas chromatography   | 9    |
| 1.4         | Type of Headspace Sampling                     | 11   |
| 1.5         | Principles and instrumentation of static HS-GC | 12   |
| 1.6         | Theory of Headspace Analysis                   | 16   |
| 1.7         | Gas Chromatography                             | 21   |

|           |        |   | Page       |
|-----------|--------|---|------------|
|           | 1.7.1  | Introduction to gas chromatography                            | 21         |
|           | 1.7.2  | Principles of gas chromatography                              | 22         |
|           |        | 1.7.2.1 Some important terms in chromatography                | 23         |
|           |        | 1.7.2.2 Column and stationary phase                           | 27         |
|           | 1.7.3  | Instrument of gas chromatography                              | 28         |
|           |        | 1.7.3.1 Carrier gas and flow regulation                       | 29         |
|           |        | 1.7.3.2 Sample introduction                                   | 30         |
|           |        | 1.7.3.3 Column and column oven                                | 31         |
|           |        | 1.7.3.4 Detector  | 32         |
| 1.8       | Qualit | ative Analysis  | 36         |
| 1.9       | Quant  | itative Analysis  | 37         |
|           | 1.9.1  | Internal standard method                                      | 37         |
|           | 1.9.2  | External standard method                                      | 38         |
| 1.10      | The so | cope and aims of this research                                | 38         |
|           |        |   |            |
| CHAPTER 2 | : EXP  | ERIMENTAL   |            |
| 2.1       | Appar  | ratus and Chemicals   | 40         |
|           | 2.1.1  | Apparatus   | 40         |
|           | 2.1.2  | Chemicals   | 41         |
| 2.2       | Prepa  | ration of 1.0 M sodium hydroxide (NaOH)                       | 41         |
| 2.3       | Prepa  | ration of 0.1 M Hydrochloric acid                             | 42         |
| 2.4       | Prepa  | ration of the internal standard, 2,4,6-trimethylpyridine (TMF | <b>'</b> ) |
|           | soulti | on  | 42         |

|      |        |               |   | Page |
|------|--------|---------------|---|------|
|      | 2.4.1  | Preparation   | n of TMP 100 ppm solution                         | 42   |
|      | 2.4.2  | Preparation   | n of TMP 0.25 ppm solution                        | 42   |
| 2.5  | Prepar | ation of star | ndard solutions                                   | 42   |
|      | 2.5.1  | Preparatio    | n of 2-AP stock solution (10 ppm)                 | 42   |
|      | 2.5.2  | Preparatio    | n of 2-AP stock solution (0.5-8.0 ppm)            | 43   |
| 2.6  | Extrac | tion of 2-A   | P in standard solutions by acidic solvent         | 43   |
| 2.7  | Extrac | tion of 2-A   | P in rice seed extract solution by acidic solvent | 45   |
| 2.8  | Identi | fication of 2 | 2-AP and internal standard (TMP) in rice seed     |      |
|      | extrac | t             |   | 46   |
| 2.9  | HS-G   | C instrume    | ntal conditions                                   | 46   |
| 2.10 | Optin  | nization      |   | 48   |
|      | 2.10.1 | Optimizat     | tion of extraction conditions                     | 48   |
|      |        | 2.10.1.1      | Extraction time                                   | 49   |
|      |        | 2.10.1.2      | Volume of 1.0 M NaOH added into the extract       |      |
|      |        |               | solution  | 49   |
|      |        | 2.10.1.3      | Equilibrium time after 1.0 M NaOH was added       |      |
|      |        |               | into rice seed extract                            | 49   |
|      | 2.10.  | 2 Optimiza    | tion of automated headspace sampler parameters    | 49   |
|      |        | 2.10.2.1      | Optimization of temperature of vial, transfer lin | ne   |
|      |        |               | and sample loop                                   | 50   |
|      |        | 2.10.2.2      | Optimization of vial equilibration time           | 50   |
|      |        | 2.10.2.3      | Optimization of pressurizing time                 | 50   |
|      |        | 2.10.2.4      | Optimization of loop filling time                 | 51   |

|         |                   |   | Page |
|---------|-------------------|---|------|
|         | 2.10.2.5          | Optimization of loop equilibration time         | 51   |
|         | 2.10.2.6          | Optimization of injection time                  | 51   |
| 2.11    | Construction of c | calibration curve                               | 52   |
| 2.12    | Validation        |   | 52   |
|         | 2.12.1 Detection  | limit   | 52   |
|         | 2.12.2 Linearity  |   | 53   |
|         | 2.12.3 Precision  |   | 54   |
|         | 2.12.4 Recovery   | assay   | 54   |
|         | 2.12.4.1          | Preparation of standard solution for recovery   |      |
|         |                   | assay   | 54   |
|         | 2.12.4.2          | Preparation of rice sample for recovery         |      |
|         |                   | assay   | 54   |
| 2.13    | Analysis of real  | samples   | 55   |
|         |                   |   |      |
|         |                   |   |      |
| CHAPTER | 3: RESULTS AN     | D DISCUSSION                                    |      |
| 3.1     | Identification of | f 2-AP and internal standard (TMP) on           |      |
|         | chromatogram o    | of the rice seed extract when was used for      |      |
|         | optimization of   | extraction method.                              | 57   |
|         | 3.1.1 Identific   | cation of 2-AP and TMP in headspace of the rice |      |
|         | seed ext          | tract when DB-1701 column was used              | 57   |
|         | 3.1.2 Identific   | cation of 2-AP and TMP in headspace of the rice |      |
|         | seed ex           | stract when DB-17MS column was used             | 59   |

|     |   | Page  |
|-----|---|-------|
| 3.2 | Optimization of extraction conditions                       | 61    |
|     | 3.2.1 Effect of extraction time                             | 62    |
|     | 3.2.2 Effect of volume of 1.0 M NaOH                        | 64    |
|     | 3.2.3 Effect of equilibrium time                            | 66    |
| 3.3 | Summary of optimized extraction conditions                  | 68    |
| 3.4 | Optimization of the automated headspace sampler conditions  | 69    |
|     | 3.4.1 Effect of temperature of vial, loop and transfer line | 69    |
|     | 3.4.2 Effect of vial equilibration time                     | 74    |
|     | 3.4.3 Effect of pressurization time                         | 76    |
|     | 3.4.4 Effect of loop filling time                           | 78    |
|     | 3.4.5 Effect of loop equilibration time                     | 80    |
|     | 3.4.6 Effect of loop injection time                         | 82    |
| 3.5 | Summary of optimized automated headspace sampler condition  | ns 84 |
| 3.6 | Construction of Calibration Curves                          | 86    |
| 3.7 | Validation  | 87    |
|     | 3.7.1 Detection limit                                       | 88    |
|     | 3.7.2 Linearity   | 89    |
|     | 3.7.3 Precision   | 90    |
|     | 3.7.4 Recovery assay  | 91    |
|     | 3.7.4.1 Recovery assay of standard extract solutions        | 91    |
|     | 3.7.4.2 Recovery assay of rice extract solutions -          | 92    |
| 3.8 | Analysis of Real Samples                                    | 93    |

|                       | Page |
|-----------------------|------|
| CHAPTER 4: CONCLUSION |      |
| REFERENCES            | 100  |
| APPENDIX A            | 103  |
| APPENDIX B            | 106  |
| APPENDIX C            | 110  |
| APPENDIX D            | 115  |
| VITA                  |      |

## LIST OF TABLES

|       |   | ъ.   |
|-------|---|------|
| Table |   | Page |
| 2.1   | Composition of the standard solutions                                 | 43   |
| 2.2   | Conditions of automated headspace sampler                             | 47   |
| 2.3   | Conditions of GC  | 48   |
| 2.4   | Information of rice samples   | 55   |
| 3.1   | Retention times and peak areas of 2-AP and TMP at various extraction  |      |
| ·     | times   | 63   |
| 3.2   | Retention times and peak areas of 2-AP and TMP at various volumes     |      |
|       | of 1.0 M NaOH   | 65   |
| 3.3   | Retention times and peak areas of 2-AP and TMP at various equilibrium |      |
|       | times   | 67   |
| 3.4   | Optimized extraction conditions for extract 2-AP in rice seeds        | 68   |
| 3.5   | Retention times and peak areas of 2-AP and TMP at various             |      |
|       | temperatures  | 70   |
| 3.6   | Retention time and peak areas of 2-AP and TMP at various vial         |      |
|       | equilibration times   | 74   |
| 3.7   | Retention times and peak areas of 2-AP and TMP at various             |      |
|       | pressurization times  | 77   |
| 3.8   | Retention times and peak areas of 2-AP and TMP at various loop        |      |
|       | filling times   | 79   |
|       |   | 1/   |

| Table |   | Page |
|-------|---|------|
| 3.9   | Retention times and peak areas of 2-AP and TMP at various           |      |
|       | loop equilibrium times  | 81   |
| 3.10  | Retention time and peak areas of 2-AP and TMP at various loop       |      |
|       | injection times   | 83   |
| 3.11  | Optimized automated headspace sampler conditions for analysis of    |      |
|       | 2-AP in rice seed extract   | 85   |
| 3.12  | Peak area ratio of 2-AP to TMP of each standard solutions           | 87   |
| 3.13  | The detection limit of 2-AP in rice analysis                        | 88   |
| 3.14  | Peak area ratio of 2-AP to TMP of each standard solutions 89        |      |
| 3.15  | Reproducibility of peak area ratio of 2-AP to TMP                   | 91   |
| 3.16  | Recovery assay of standard extract solutions                        | 92   |
| 3.17  | Recovery assay of rice extract solutions                            | 93   |
| 3.18  | Results of HS-GC determination of 2-AP in rice samples              | 96   |
| C1    | Calculation data of concentration on the linear regression equation | 113  |
| DI    | Data for calculation of concentration of 2-AP                       | 115  |
| D2    | Data for calculation of concentration of 2-AP in rice samples       | 116  |

#### LIST OF ILLUSTRATIONS

| Figur | e  | Page |
|-------|--|------|
| 1.1   | Varieties of rice in the world                                     | 2    |
| 1.2   | The structure of 2-acetyl-1-pyrroline                              | 5    |
| 1.3   | Illustration of headspace sampling techniques                      | 11   |
| 1.4   | Schematic of the 'pressure/loop' headspace sampling system         | 13   |
| 1.5   | Schematic of the 'balanced pressure' headspace sampling system in  |      |
|       | splitless configuration  | 14   |
| 1.6   | Schematic diagram of HP 7694 Headspace Sampler                     | 15   |
| 1.7   | A headspace vial containing a liquid sample                        | 17   |
| 1.8   | Schematic of a gas chromatograph                                   | 28   |
| 1.9   | Optimum linear velocity of carrier gas                             | 29   |
| 1.10  | Schematic diagram of HS-GC   | 30   |
| 1.11  | Structure of DB-1701 liquid stationary phase                       | 32   |
| 1.12  | Structure of DB-17MS liquid stationary phase                       | 32   |
| 1.13  | Flame ionisation detector  | 33   |
| 2.1   | Scheme for extraction of standard solutions                        | 44   |
| 2.2   | Scheme for extraction of rice seed                                 | 45   |
| 3.1   | HS-GC chromatographic pattern of rice seed extract containing 2-AP |      |
|       | and TMP  | 57   |
| 3.2   | HS-GC chromatogram of the nonspiked rice seed extract              | 58   |
| 3.3   | HS-GC chromatogram of the spiked rice seed extract                 | 59   |

| Figure | e  | Page |
|--------|--|------|
| 3.4    | HS-GC chromatographic pattern of rice seed extract containing 2-AP       |      |
|        | and TMP  | 60   |
| 3.5    | HS-GC chromatogram of the nonspiked rice seed extract                    | 61   |
| 3.6    | HS-GC chromatogram of the spiked rice seed extract                       | 61   |
| 3.7    | Peak area of 2-AP and TMP and ratio of peak areas of 2-AP/TMP            |      |
|        | obtained at various extraction times                                     | 63   |
| 3.8    | Ratio of peak areas of 2-AP/TMP obtained at various volumes of           |      |
|        | 1.0 M NaOH   | 65   |
| 3.9    | Ratio of peak areas of 2-AP/TMP obtained at various equilibrium          |      |
|        | times  | 67   |
| 3.10   | Ratio of peak areas of 2-AP/TMP obtained at various temperatures         | 70   |
| 3.11   | Chromatogram of temperature of oven, loop and transfer line was          |      |
|        | 100,120,140 °C   | 72   |
| 3.12   | Chromatogram of temperature of oven, loop and transfer line was          |      |
|        | 110,130,150 °C   | 73   |
| 3.13   | Chromatogram of temperature of oven, loop and transfer line was          |      |
|        | 120,140,160 °C   | 73   |
| 3.14   | Ratio of peak areas of 2-AP/TMP obtained at various equilibration times  | 75   |
| 3.15   | Ratio of peak areas of 2-AP/TMP obtained at various pressurization times | 77   |
| 3.16   | Ratio of peak areas of 2-AP/TMP obtained at various loop filling times   | 79   |
| 3.17   | Ratio of peak areas of 2-AP/TMP obtained at various loop equilibrium     |      |
|        | times  | 81   |
| 3.18   | Ratio of peak areas of 2-AP/TMP obtained at various loop injection times | 83   |

## xviii

| Figure | e  | Page |
|--------|--|------|
| 3.19   | Chromatogram of rice extract obtained with HS-GC under optimum |      |
|        | conditions   | 85   |
| 3.20   | Calibration curve of 2-AP                                      | 87   |
| 3.21   | Calibration of 2-AP in the range 0.37 – 11.57 ppm.             | 90   |
| 3.22   | Chromatogram of rice extract of KDML 105 Chiang mai            | 94   |
| 3.23   | Chromatogram of rice extract of KDML 105 Tungkularonghai       | 94   |
| 3.24   | Chromatogram of rice extract of Howm Supanburi                 | 95   |
| 3.25   | Chromatogram of rice extract of Howm Patumtani                 | 95   |
| A1     | Scheme for synthesis of 2-AP                                   | 104  |
| Δ2     | Scheme of apparatus for hydrogenation reaction                 | 105  |

#### ABBREVIATIONS AND SYMBOLS

peak area Α alumina Αl the concentration of A in a unit volume of the mobile phase  $A_{m}$ analytical reagent AR the concentration of A in a unit volume of the stationary phase  $A_s$ 2A 2-acetylpyrrole 2-acetyl-1-pyrroline 2-AP intercept a В butt connector slope of the straight line b  $\mathbf{C}$ concentration capillary column CC CG carrier gas the concentration of analyte in gas phase  $C_{G}$ the concentration of a component in mobile phase  $C_{M}$  $C_{0}$ original concentration Conc. concentration the concentration of analyte in sample phase  $C_{S}$ the concentration of a component in stationary phase  $C'_{S}$ 

D

DF

density

detection frequency

FID flame ionisation detector

GC gas chromatography

GC-O gas chromatography olfactometry

GLC gas-liquid chromatography

GSC gas-solid chromatography

g gram

H plate height

HAS headspace analysis

HETP height equivalent to a theoretical plate

HG headspace gas

HP high purity

HS headspace sample

HS-GC headspace gas chromatography

HV headspace vial

hr hour

I.D. internal diameter

K distribution constant

KDML Khao Dawk Mali

k' capacity factor

k<sub>A</sub> the capacity factor for A

k<sub>B</sub> the capacity factor for B

L column length

M molarity

MW molecular weight

m

meter

mg

milligram

min

minute

mm

millimeter

Ν

number of theoretical plate

N

noise

n

number of points on the calibration line

n

number of measurement

 $P_{A}$ 

peak area

ppm

part per million

**PTFE** 

polytetrafluoroethylene

**PLOT** 

porous layer open tubular

Rh

rhodium

Rs

resolution

**RSD** 

relative standard deviation

S

signal

 $S_{B}$ 

blank signal standard deviation

**SCOT** 

support-coated open tubular

**SDE** 

steam distillation / solvent extraction

SHS-GC

static headspace gas chromatography

SHA/GC/MS static headspace analysis / gas chromatography / mass spectrometry

SN

sampling needle

**SPME** 

solid phase microextraction

S

standard deviation

#### xxii

T temperature

TMP 2,4,6-trimethylpyridine

t<sub>M</sub> hold-up time

t<sub>R</sub> retention time

t'<sub>R</sub> adjusted retention time

 $t_{RA}$  retention time of component A

 $t_{RB}$  retention time of component B

V volume

V solenoid valve

V<sub>G</sub> volume of gas phase

V<sub>M</sub> void volume

 $V_{M}$  volume of mobile phase

Vo volume of original sample

V<sub>R</sub> retention volume

V'<sub>R</sub> adjusted retention volume

V<sub>S</sub> volume of sample phase

V's volume of stationary phase

V<sub>V</sub> total volume of vial

W<sub>BA</sub> peak width at baseline of component A

W<sub>BB</sub> peak width at baseline of component B

W<sub>b</sub> peak width at baseline

WCOT wall coated open tubular

W<sub>O</sub> the analyte in sample

wt weight

## xxiii

| X                               | mean measured value   |
|---------------------------------|---|
| x                               | normally are concentrations                                       |
| Xi                              | individual measured value   |
| у                               | instrument signals  |
| $Y_L$                           | lowest detectable instrument signals                              |
| $Y_B$                           | blank signal  |
| $Y_i$                           | response value from the instrument corresponding to the           |
|                                 | individual x – values   |
| $\hat{\mathbf{Y}}_{\mathbf{i}}$ | value of y on the calculated regression line corresponding to the |
|                                 | individual x values   |
| % R.S.D.                        | percent of relative standard deviation                            |
| %                               | percent   |
| α                               | selectivity factor  |
| β                               | phase ratio   |
| °C                              | degree Celsius  |
| hl 🧼                            | microliter  |
| μm                              | micrometer  |
|                                 |   |