CHAPTER III

MATERIALS AND METHODOLOGY

This chapter describes the materials and methodology of the rice sample preparation for measurement using the HS-GC technique and statistical methods. The chemical profile data and criterion to select the variables were analysed using statistical methods for comparison and classification of the rice varieties.

3.1 Rice Samples and Preparation

In this study, three fragrant rice varieties Kor Kho 15, KDML 105 and Pathum thani 1 were used. The rice samples were obtained from the Pathum thani Rice Research Center in August of 2009. These samples were preserved in polyethylene (PE) bags in a black box at room temperature before the experiment. Four samples were randomly collected for each variety by a different storage duration on two weeks intervals (2, 4, 6, 8, 10 and 12 weeks). All samples were chemically profiled using the headspace gas chromatography (HS-GC) technique. The preparation steps included, firstly, sample gridding and sifting, secondly, three grams of rice powder being added to vials with 1.0μL of 1000ppm 2, 4-dimethyl pyridine (DMP), and lastly, the vials were sealed with PTFE/silicone septum and an aluminum crimp cap before being analyzed by the HS-GC.

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3.2 HS-GC Analysis

An Agilent 6890 equipped with an Agilent G1888 headspace sampler and a FID (Agilent technology, Palo Alto, CA) was utilized to measure chemical profiles in the rice seed extracts. The oven temperature, loop temperature and line temperature

were set at 120, 130 and 140°C, respectively. The vial equilibration time, vial pressurization time, loop fill time, loop equilibration time and injection time were set at 9.00, 0.10, 0.01, 0.60 and 0.40 min, respectively. A fused silica capillary column HP-5, 5% phenyl methyl siloxane, with dimension of 60.0 m. x 0.32 mm. id and 1.0 μm film thickness, was programmed by starting at 45°C. The temperature was ramped to 240°C at a rate of 3°C min^{-1}, resulting in an overall separation time of 70 min. The injector temperature was set at 150°C and was operated in a splitless mode. Purified helium was used as the GC carrier gas at a flow rate of 3.0 ml min^{-1}.

3.3 Chemical Profile Data

The rice chromatograms were obtained by using the HS-GC technique in Figure.3.1 - 3.3. This work was performed in the Rice Chemistry Research Laboratory, Department of Chemistry, Faculty of Science, Chiang Mai University. For the dataset of seventy-two samples, the area under the peak of each component (Table A, Appendix A) was obtained for every peak-area variable at each retention time (p_1, p_2, p_3, …, p_{114}) during time range 5-70 min. The peak-area profile data (Table B1.1-1.6, Appendix B) were then normalized from quantity performance of an internal standard yielding the original peak-area profile data (114 peaks and area data). In each sample measurement, the efficiency in chemical absorption could differ; therefore an internal standard must be used to adjust its value. The normalized data or peak-area adjusted are shown in Table B 2.1-2.6, Appendix B.

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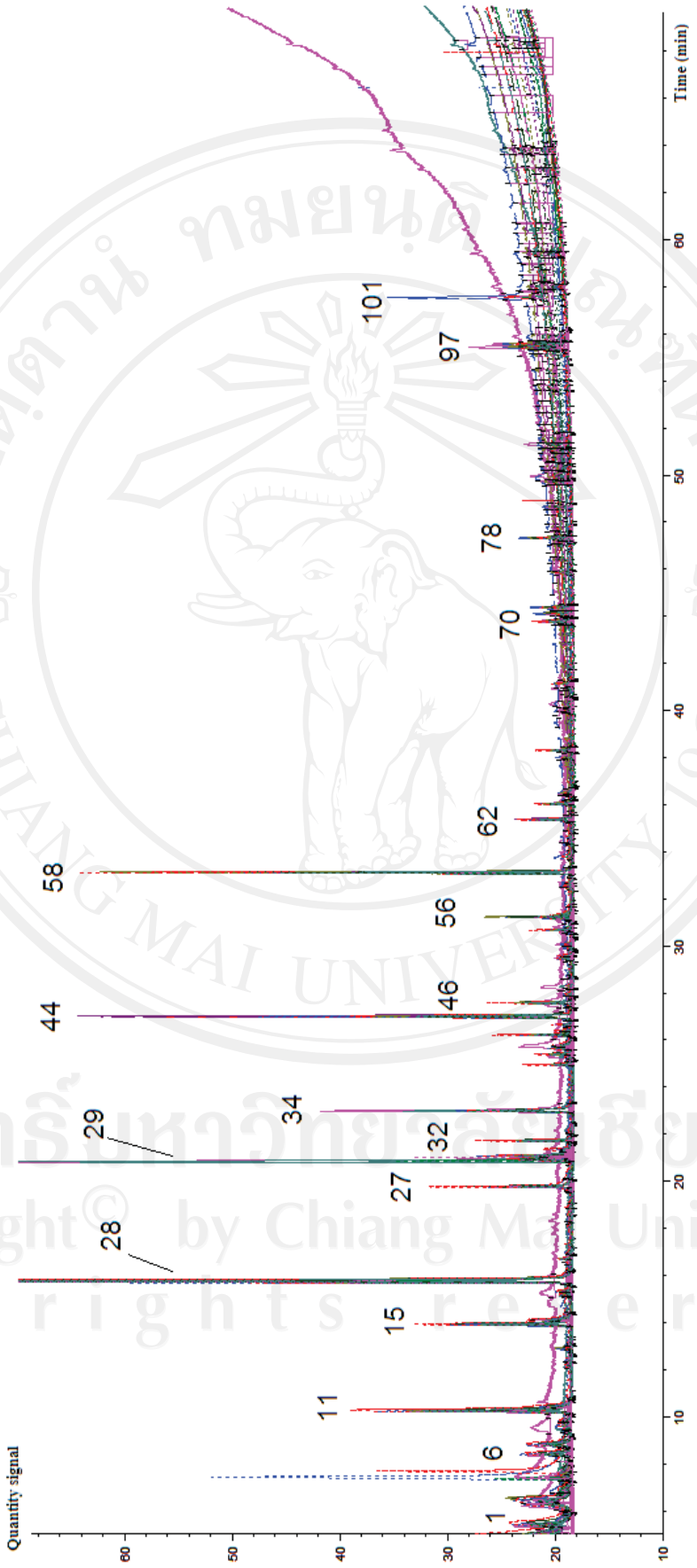

Figure 3.1 HS-GC chromatogram of an extract of Kor Kho 15 rice sample.

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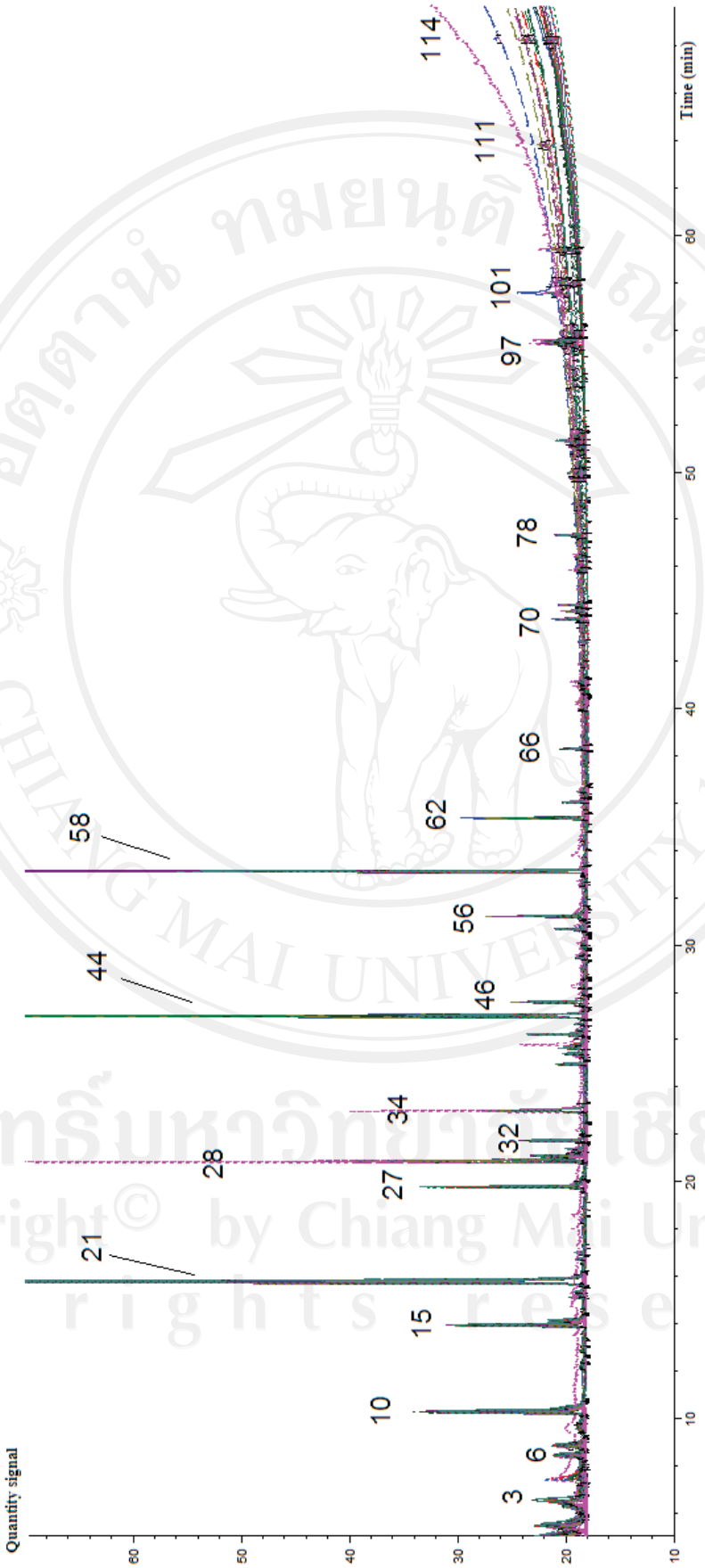

Figure 3.2 HS-GC chromatogram of an extract of KDML 105 rice sample.

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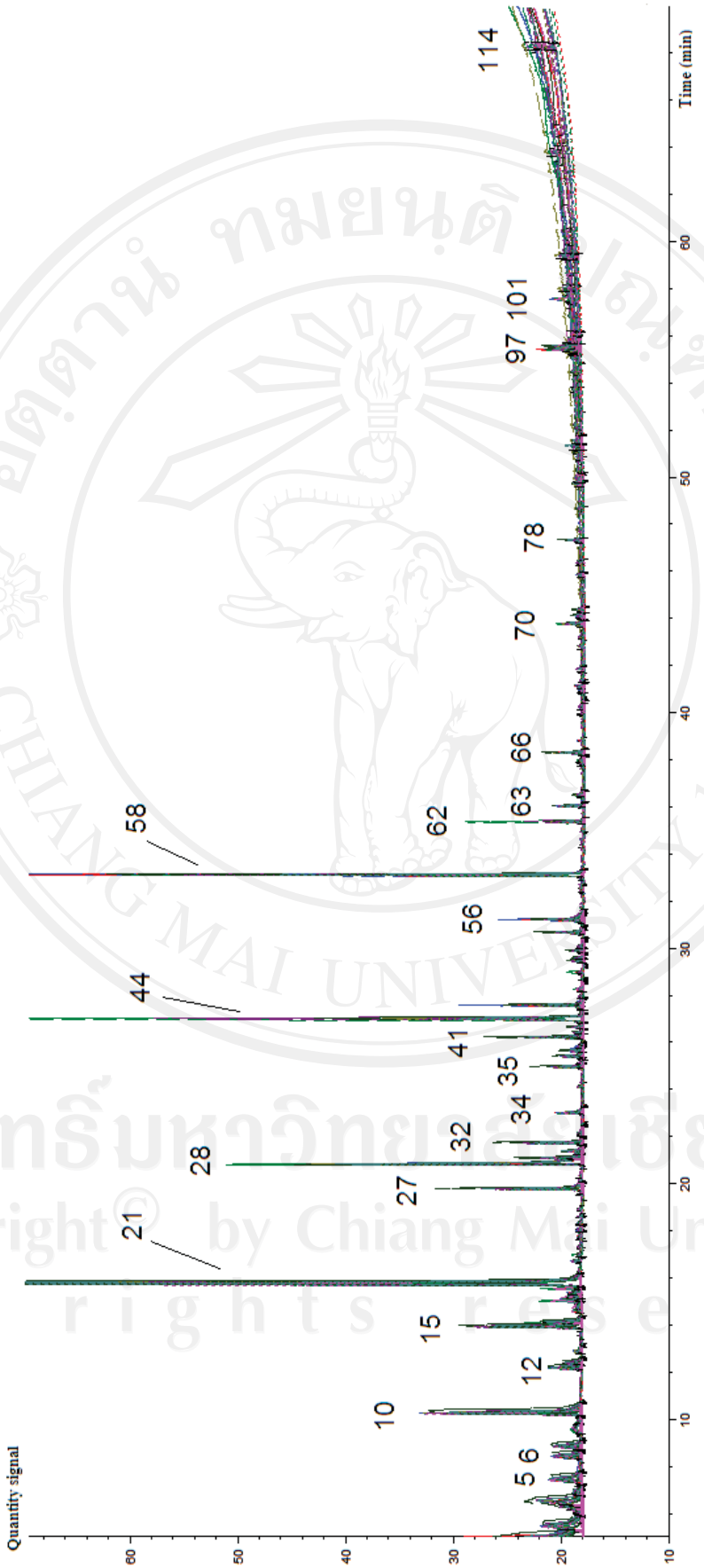

Figure 3.3 HS-GC chromatogram of an extract of Pathum-thani 1 rice sample.

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3.4 Statistical Methods

In order to evaluate the effect of storage durations, the median and box plot of peak-area variables were plotted to compare the peak-area profile between different storage duration for each variety. The fragrant-rice variety classification with discriminant analysis has many limitations such as, the fact that the peak-area variables (114 variables) were higher than the sample numbers (72 samples). Therefore, the numbers of peak-area variables were reduced to construct the classification models and the PCA was formed for the PCs of the 114 variables. Stepwise method in LDA (SLDA) was used to select only variables necessary to perform the classification model. The others conditions were used to select variables into LDA models composed;

- The significant variables using *F*-test in ANOVA

The means of each peak-area variable were compared between three rice varieties using the one-way ANOVA method to extract the significant peak-area data (the different overall mean). The significant level was 0.05. The significant variables were used to construct the classification models by LDA.

- The variables that appeared in a given period of time

In this study, each sample of fragrant rice was analysed by HS-GC during a time period from zero to 70 min for measuring the peak-area profile. From the pattern of the peak-area profiles obtained (Figure 3.1-3.3), a lot of peak-area variables with high quantity were found in the first period more than the last period. The LDA, SLDA and significant variables with LDA models were constructed by using the

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variables in a given periods of time, such as during 5-70 min, 10-70 min, 15-70 min, 5-35 min, 10-35 min, 15-35 min, 5-25 min, 10-25 min and 15-25 min.

- The variables with a given percent of non-zero value

For a little quantity of some peak-area variables in rice, then peak-area adjusted could not be detectable so that it's values are zero. The peak-area variables with a lot of values equal zero could not be appropriate to classify rice varieties. So that, we selected the variables with the percent of non-zero values at least 50, 75 and 95% (64, 47 and 41 variables) in some varieties and for all varieties to construct the classification model by LDA, SLDA and LDA with significant variable using ANOVA.

The validation of all classification models was evaluated by using LOOCV technique.

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