CHAPTER 4
CONCLUSIONS

In this research, the development of an automated headspace-gas chromatographic (SHS-GC) technique with a nitrogen-phosphorous detector (NPD) for the determination of an aroma compound, 2-acetyl-1-pyrroline, in rice leaves was performed. The preliminary investigation on the amounts of 2AP in rice leaves found its tendency of increasing from seedling (10-20 days) to booting stage and the highest amounts of 2AP was found in booting stage (50 days). This is in agreeable with the basic knowledge that secondary metabolites are mostly produced in rice plant during booting stage. The decrease of peak area of 2AP from the booting stage until the end of grain development stage (100-120 days) is probably due to the translocation of 2AP from leaves to grain as well as the lower rate of secondary metabolite production during these lately stages of rice plant. The leaf age at 20 days of KDML105 rice was selected for further use in this method and the amount of rice leaf sample as low as 0.2000 g was required for this developed method.

The optimum conditions of SHS-GC/NPD were oven temperature at 120°C, which the loop temperature and transfer line temperature were set 10°C and 20°C higher than the oven temperature, vial equilibration time of 5 min, vial pressurization time of 0.05 min, loop fill time of 0.01 min, loop equilibration time of 0.40 min, and inject time of 0.40 min. These optimum conditions gave high sensitivity

and good separation most appropriate for determination of the aroma compound, 2AP, in rice leaf samples.

The authentic 2AP was synthesized in this study as a standard solution in order to create a standard calibration curve used for quantitative analysis. The concentration of 2AP in this product solution was determined by using NMR technique. 2AP standard calibration curve obtained exhibited excellent linearity in the range of 0.5 - 50.0 ppm with a correlation coefficient of 0.9998 for 2AP in rice leaves. Method validation performed for this developed SHS-GC/NPD method demonstrated the limits of detection and limits of quantitation at 0.1 µg of 2AP and 0.01 g of rice leaves sample, respectively. The intraday and interday coefficients of variation determined using KDML105 rice leaves as samples were 1.93% RSD (n=10) and 2.40% RSD (n=25), respectively. The percentage recovery of 2AP in the first headspace extraction step was 65.84%. Overall, the validation data confirm high sensitivity and usefulness of the developed SHS-GC/NPD system.

The experiment on application of the developed SHS-GC/NPD method to quantitative analysis of 2AP was performed using the leaves of five rice varieties, KDML105 (organic), KDML105, RD15, RD33 and RD6. The concentrations of 2AP in all rice leaf samples were higher than those found in their rice grains. The decreasing concentration of 2AP in rice leaves at booting stage until the end of grain production is probably due to the translocation of 2AP from leaves to grains as well as the lower rate of secondary metabolite production during these lately stages of rice plant.

This developed SHS-GC/NPD method can be another choice for determination of the rice aroma compound, 2AP, in rice leaves which is more convenient, rapid and requires less amount of sample, compared with that applied to the rice grain. The method is also more appropriate for analysis of 2AP in various hybrid rice samples, which are normally obtained from the rice breeding programs.