CHAPTER 6
EFFECTS OF ACCELERATED AGING OF MILLED RICE ON CHANGES IN AROMA AND VOLATILE COMPONENTS OF FRESHLY HARVESTED RICE CV. KDML 105

6.1 Introduction

Effects of accelerated aging of milled rice on change in physico-chemical properties related to cooking quality (cooking quality in preference to aged rice) of freshly harvested rice cv. KDML 105 has already been discussed and proven in Chapters 3 and 4. However, the consequence of this accelerated aging technique on aroma characteristic of the rice samples has to be investigated and verified. This was to determine whether the accelerated aging technique could change or result in favorable or unfavorable effects on some volatile compounds responsible for the odor character of the aromatic rice.

The volatile compounds that provide aroma characteristic of fragrant rice had been studied by a number of researchers and a relatively large number of compounds from uncooked (Mahatheeranont *et al*., 2001) and cooked (Buttery *et al*., 1983a, 1983b, and 1986; Paule and Powers, 1989; Widjaja *et al*., 1996a; Yang *et al*., 2008) aromatic rice had been identified. Research results also indicated that aroma of the rice was composed of a mixture of numbers of odor-active compounds and these compounds contributed to the unique aroma of aromatic rice (Widjaja *et al*., 1996b; Yang *et al*., 2008). Among the compounds identified, 2-acetyl-1-pyrroline was considered to be the most important odor-active compound in aromatic rice.

Study on the effect of accelerated aging had been carried out previously on KDML 105 paddy and that study was successful to enhance rate of aging of the rice as measured in terms of pasting and cooking property changes (Soponronnarit *et al*., 2008), but such study did not investigate the aroma of the rice sample that would be altered as a result of the technique used. The presence of husk and bran layer incorporated with moistening and the relatively high temperature employed in such accelerated aging process could have impacts on the aroma compound profile and could potentially change the typical aroma characteristic of the rice as well. This current study was thus conducted to investigate changes in aroma and volatile

components after KDML 105 freshly harvested rice was age-accelerated and to test the hypothesis that there was no difference in terms of the aroma constituents of the treated and untreated samples when milled rice was used.

6.2 Materials and Methods

Treatments of the experiment discussed in Chapter 4 and showed good results in terms of rice color and appearance, texture of cooked rice, pasting property and aroma parameters were extracted and adjusted. This was done to optimize for the duration of exposure in the process of accelerated aging. The main goal of doing this was to reduce loss of 2-acetyl-1-pyrroline from the rice during exposure to high temperature condition. At the same time, the duration time in the process had to be sufficient for changing cooking properties of the fresh rice samples to be similar to that of aged rice. In this study, aroma components of the rice that might be altered due to high temperature and exposure time were investigated in detail.

6.2.1 Rice Samples and Preparations

Rice cv. KDML 105 was grown in 2006 season on the same experimental plots of rice samples used in previous experiments at Lampang Agricultural Research and Training Center, Rajamangala University of Technology Lanna, Lampang. Preparation of rice samples was done as mentioned in Chapter 3. The rice was harvested at maturity by hand, left to dry 2 to 3 days in the field and then threshed to paddy having approximately 14% MC. The paddy sample was divided into 2 portions by a Boerner divider (Seedburo Equipment Co., Chicago, IL). One portion was stored in jute sacks in ambient condition and changes in 2-acetyl-1-pyrroline and *n*-hexanal content of rice were monitored at 1-month intervals for a storage period of 6 months. Another portion of the freshly harvested paddy sample was dehulled by a McGill sample sheller and milled for 30 sec in a friction-type miller operating with a 1.0 kg weight positioned at the end of a 25-cm mill lever arm. Milled head rice was separated from the broken kernel by a cylinder grader and used for the following accelerated aging treatments. Protein (N×5.95) and lipid contents of the head rice as determined by AOAC (1999) standard methods were 6.54 and 0.92%, respectively. The apparent amylose content was 17.65% (w/w) as determined by the method of Juliano *et al*. (1981). Prior to accelerated aging treatment, the MC of milled rice

sample was determined by oven method (103^{o}C for 17 hr) and the average value of three replications was 13.13% (wb).

6.2.2 Accelerated Aging Treatments

Three replicates, each of 370 g of fresh milled rice samples were placed in aluminum containers (11 cm height × 8.5 cm diameter) and covered with heavy-duty aluminum foil. The rice samples were then exposed to three different aging treatments, 100°C for 100 min, 110°C for 45 min and 120°C for 25 min in an automatic autoclave (SS-320, Tomy Seico Co. Ltd., Wako, Saitama, Japan). Change in milled rice temperature during accelerated aging treatment was recorded using a data logger. Temperature sensor was set at center of containers and the mean reported was an average of three replications. The temperature profiles of the three accelerated aging treatments are shown in Figure 6.1.

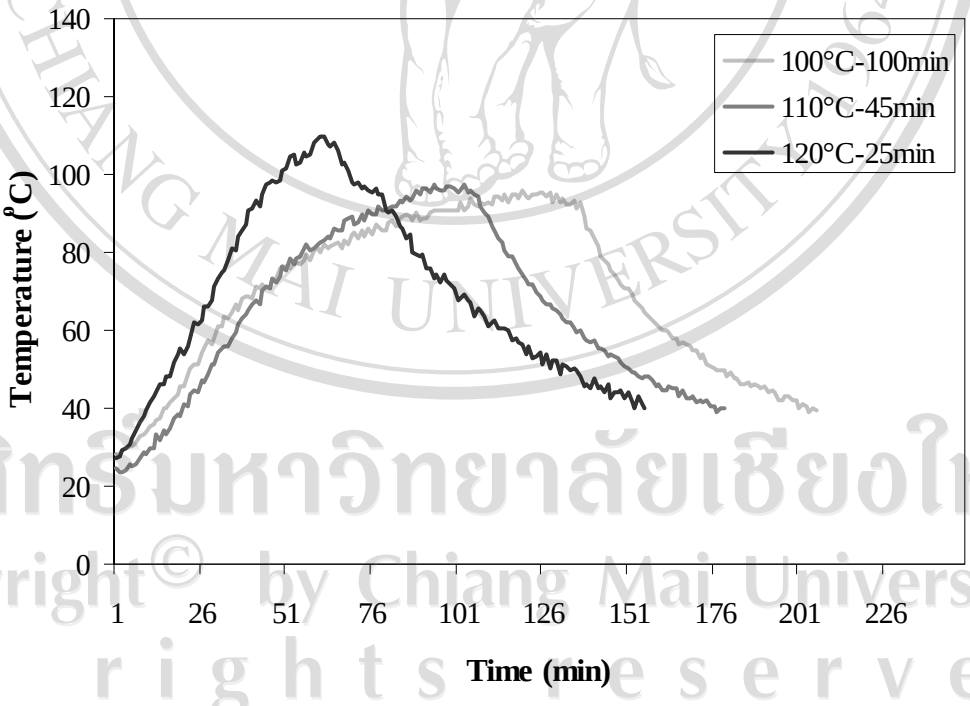

Figure 6.1 Temperature profiles of the three accelerated aging treatments. Data recorded at the center of milled rice containers.

After exposure, the rice samples were left covered in the aluminum containers and cooled for about 2 hr at 21°C. The rice samples were then poured onto aluminum trays and allowed to equilibrate their temperature and moisture content with ambient air for 24 hr. Subsequently, all samples including fresh rice (control) were placed into zip-locked plastic bags and kept at -20^{o}C until the time of analyses.

6.2.3 Analysis of 2-Acetyl-1-pyrroline and *n*-Hexanal

The amounts of 2-acetyl-1-pyrroline and *n*-hexanal of the AA, freshly harvested milled rice and those stored under natural condition in rough rice form were analyzed using the method employing headspace-gas chromatography (HS-GC) developed by Sriseadka *et al*. (2006). Milled rice sample was ground to pass through a 0.5 mm screen and the resulting flour, weighing exactly 1.000 g, was placed into a 20 ml headspace vial. An internal standard (1 µL of 0.50 mg/ml 2,6-dimethylpyridine in benzyl alcohol) was added into the vial which was then immediately sealed with a PTFE/silicone septum (Restek Corp., Bellefonte, PA) and an aluminum cap. Then, the sample vials were placed in the headspace autosampler (Agilent Technologies model G1888) of a gas chromatograph model 6890N (Agilent Technologies, Wilmington, DE) equipped with a fused silica capillary column, HP-5 (5% phenyl 95% dimethylpolysiloxane, 30 m × 0.53 mm i.d., 1.5 µm film thickness; J&W Scientific, Folsom, CA). Sample headspace vial was equilibrated at 120°C for 9 min in the autosampler before the rice headspace was transferred to the injection port of the GC. The GC condition was set as follows: the column temperature program started at 50°C and increased at a rate of 1^{o}C/min to 70°C, the injector and flame ionization detector (FID) temperatures were 230 and 250°C, respectively. Purified helium was used as carrier gas at a flow rate of 7 mL/min. Amounts of 2-acetyl-1-pyrroline in the rice samples were determined by using standard calibration curves and the relative amounts of *n*-hexanal were derived from the ratio of the peak areas of *n*-hexanal and 2,6-dimethylpyridine.

6.2.4 Analysis of Rice Headspace Volatile Components

Volatile components in headspace of the AA and freshly harvested milled rice samples were extracted using a solid-phase microextraction (SPME) device, followed by a qualitative analysis of the volatiles by gas chromatography–mass spectrometry

(GC-MS). Analysis was carried out in an Agilent Technologies (Wilmington, DE) gas chromatograph model 6890N coupled to a HP 5973 mass-selective detector (Agilent Technologies, Palo Alto, CA), and a fused silica capillary column, HP-1MS, with dimethylpolysiloxane as nonpolar stationary phase (30 m × 0.25 mm i.d. and 0.25 µm film thickness; Agilent Technologies, Wilmington, DE) was utilized. Rice flour weighed exactly 5.000 g was sealed in a 27-ml headspace vial fitted with a PTFE/silicone septum (Restek Corp., Bellefonte, PA) and an aluminum cap. The sample vial was incubated at 120 °C for 45 min. A SPME fiber (Supelco, Bellefonte, PA) of 1 cm in length, coated with polydimethylsiloxane (PDMS) at 100 µm thickness mounted in the manual SPME holder (Supelco) was then inserted through the septum of the vial. The fiber was allowed to absorb volatile compounds in the headspace for 15 min while temperature of the sample was still held at 120 °C. The SPME fiber was withdrawn from the sample vial and volatile components were desorbed at 250°C in the GC-MS injection port prior to the component separation and analysis by GC-MS.

The GC-MS condition was set as follows: injection port was in splitless mode; initial column temperature, 45°C; ramped at a rate of 2°C /min to 180°C; mass spectrometer was operated in the electron impact (EI) mode with an electron energy of 70 eV; ion source temperature, 230°C; quadrupole temperature, 150°C; mass range *m/z* 29-550; scan rate, 0.68 s/scan; EM voltage, 1423 V. The GC-MS transfer line was set to 280°C and purified helium gas at a flow rate of 1 mL/min was used as the carrier gas. The volatile compounds were tentatively identified by comparing their mass spectra with those compiled in the Wiley7n and Nist98 libraries of the MS database.

6.2.5 Statistical Analysis

Aroma data were analyzed using analysis of variance (ANOVA) to determine the effect of accelerated aging treatments. Duncan's multiple range test ($P<0.05$) was used to separate the means.

6.3 Results and Discussion

This discussion is mainly focused on aroma quality in terms of quantity of the key volatile compounds (2-acetyl-1-pyrroline and *n*-hexanal) and volatile components

of accelerated aging rice samples. They are discussed in comparison with those of fresh rice samples, and those of naturally-aged rice may also be referred to. For all other results such as textural and pasting properties, they will be discussed in Chapter 7.

6.3.1 Aroma Quality on the Basis of 2-Acetyl-1-pyrroline and *n*-Hexanal Quantity

Quantification of 2-acetyl-1-pyrroline in rice samples using GC-FID indicated that the quantity of this volatile compound in the rice samples decreased after accelerated aging treatment (Figure 6.2). The concentrations obtained from rice accelerated aging with 100°C for 100 min, 110°C for 45 min and 120°C for 25 min

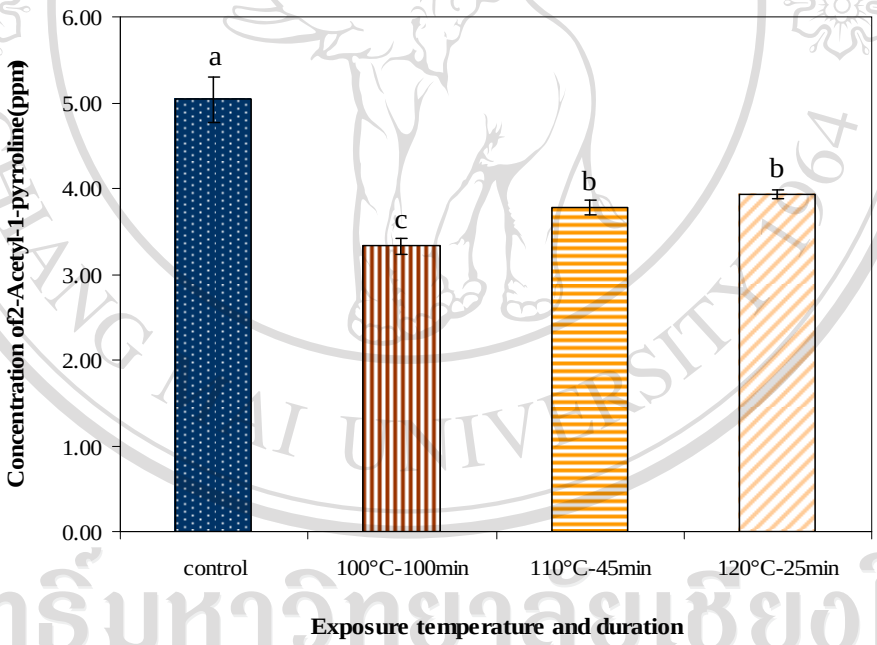

Figure 6.2 Effect of accelerated aging treatments (temperature (°C) and duration (min)) on the concentration of 2-acetyl-1-pyrroline of freshly harvested KDML105 milled rice. Vertical bars (±SD) with the same letters are not significantly different by DMRT (P<0.05).

were 3.33, 3.78 and 3.94 ppm, respectively. 2-Acetyl-1-pyrroline content decreased by 33.9% in rice exposed to 100°C for 100 min and by 21.8% in 120°C for 25 min

treatment, as compared to that of fresh rice (5.04 ppm). However, when compared to rice that was naturally aged, the level of 2-acetyl-1-pyrroline in all samples receiving accelerated aging treatments were found to be higher than the level (2.95 ppm) observed in 3-month naturally-stored sample (Figure 6.3). From the results, it could be noted that reduction of 2-acetyl-1-pyrroline was greater in rice given longer duration treatment, though heating temperature applied to the rice was lower (100°C for 100 min). Therefore, age-accelerated using high temperature and short time (120°C for 25 min) was recommended for the production of aged rice in which its aroma quality is better than that of naturally-stored rice. High 2-acetyl-1-pyrroline in rice aged by this heating condition might be attributed to a comparatively short duration to release the aroma compound from the inner part of rice kernel to its surrounding atmosphere.

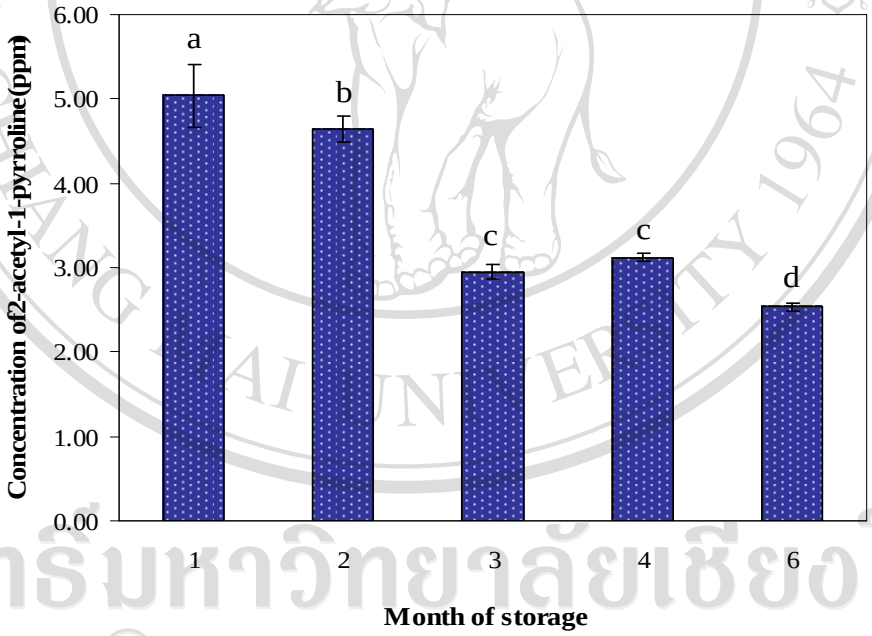

Figure 6.3 Change in 2-acetyl-1-pyrroline concentration of KDML105 milled rice stored as paddy in ambient condition for a period of 6 months. Vertical bars (±SD) with the same letters are not significantly different by DMRT (P<0.05).

2-Acetyl-1-pyrroline was reported to form naturally in rice during growing in paddy field (Yoshihashi *et al*., 2002). The compound is present in starch granule of

milled rice kernel in free and starch bound forms, and its concentration decreases with time of storage (Yoshihashi *et al*., 2005). Analysis of this compound had also revealed that the compound was equally distributed across kernel of aromatic rice (Bergman *et al*., 2000). These findings could support the aforementioned postulation. 2-Acetyl-1-pyrroline in rice kernel does not increase after harvesting or during cooking, and since there are no postharvest techniques currently reported to effectively preserve this highly volatile compound during storage of aromatic rice. These facts suggest the advantage of using accelerated aging to bring the freshly harvested rice to be an advanced stage of aging process giving rice of similar cooking property to that of stored rice (as discussed in Chapters 4 and 7), while still maintaining its high aroma quality.

During processing, the relative amount of *n*-hexanal in accelerated aging samples was reduced significantly with the treatment of 120°C for 25 min having the lowest content (Figure 6.4). Area ratios of *n*-hexanal/DMP of the accelerated-aged

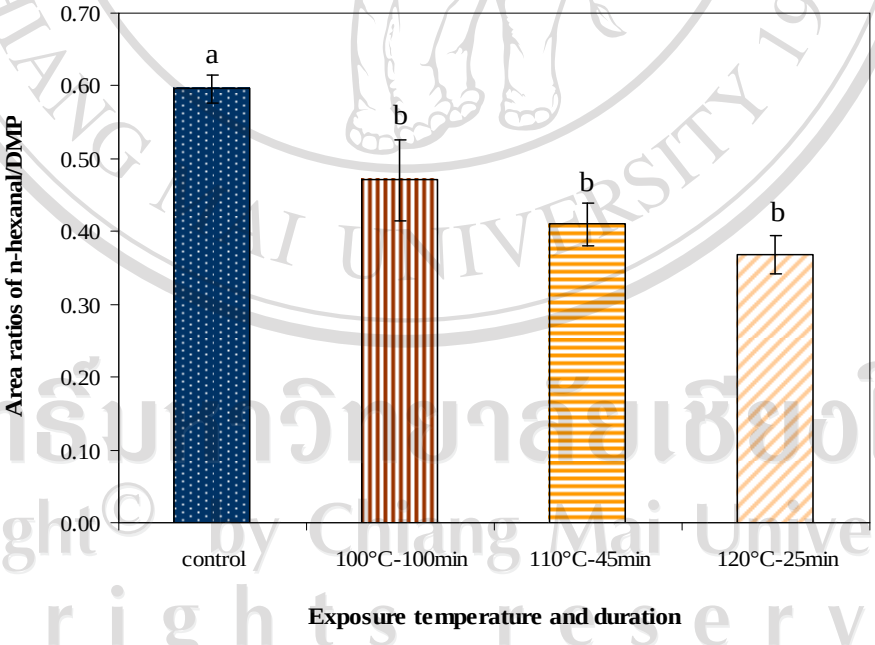

Figure 6.4 Effect of accelerated aging treatments (temperature (°C) and duration (min)) on the area ratios of *n*-hexanal of freshly harvested KDML105 milled rice. Vertical bars (±SD) with the same letters are not significantly different by DMRT (*P*<0.05).

samples were in the range of 0.37 to 0.47 which was lower than that of fresh rice (0.60). Analyses were also made to observe amount of *n*-hexanal generated on those rice that were stored in paddy form under natural condition. The results revealed that the area ratios of *n*-hexanal/DMP in the naturally-stored rice samples were higher than those of rice given accelerated aging treatments (Figure 6.5). The increase in *n*-hexanal of naturally-stored rice was attributed to the degradation of lipid composition of the rice. This rice lipids component was reported to be hydrolyzed and oxidized to free fatty acids or peroxides (Zhou *et al*., 2002) which subsequently resulted in the

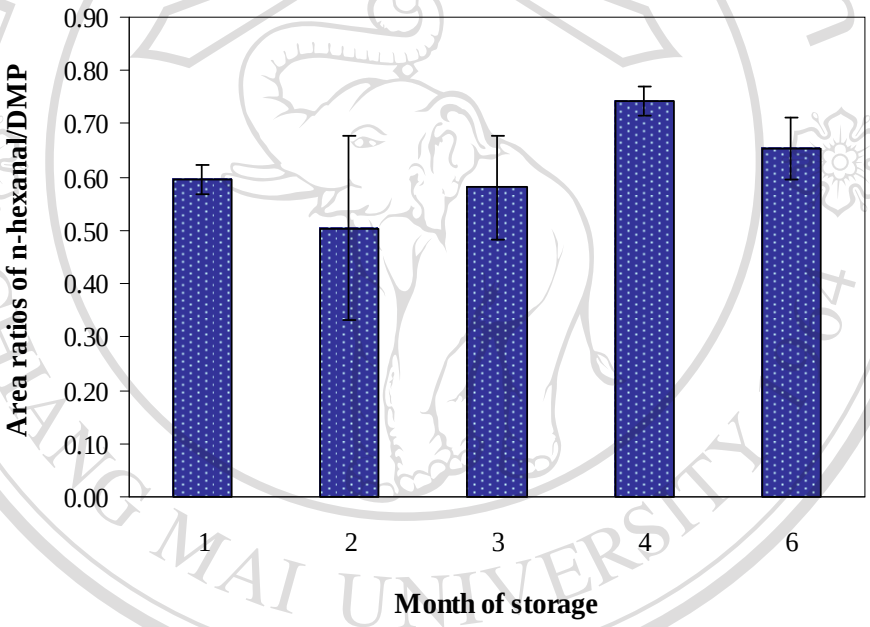

Figure 6.5 Change in area ratios of *n*-hexanal/DMP of KDML105 milled rice stored as paddy in ambient condition for a period of 6 months. Vertical bars (±SD) with the same letters are not significantly different by DMRT (*P*<0.05).

increases in off-odor compounds formation including *n*-hexanal of stored rice. This carbonyl compound, *n*-hexanal, was reported to be a degraded product of linoleic acid (Monsoor and Proctor, 2004). Accelerated aging using high temperature of this study could lower the activity of lipid hydrolysis enzyme and could also enhance the release of this highly volatile compound resulting in less *n*-hexanal content in the age-

accelerated samples. The decrease in *n*-hexanal would indicate that aroma quality of the rice was improved.

6.3.2 Aroma Quality on the Basis of Volatile Components Determined by GC-MS

Gas chromatographic profiles of volatile components of the freshly harvested milled rice and its corresponding accelerated aging samples are illustrated in Figures 6.6, 6.7 and 6.8. These volatile components were extracted from headspace of milled rice samples using SPME which were then separated and identified employing GC-MS. Following the gas chromatographic analysis, aroma quality of accelerated aging samples was assessed on the basis of the similarity of volatile compounds present in their chromatograms as reference to that of the freshly harvested milled rice. It was found that all chromatograms receiving from accelerated aging were qualitatively similar to that of fresh rice regarding to their components. All the volatile compounds typical to KDML105 rice aroma character (based on the chromatogram of fresh rice sample) still remained in the chromatograms of accelerated aging rice samples. There were no new compounds that might generate or form as a subsequence of accelerated aging treatment was observed. These chromatograms showed the same 13 identifiable compounds (Table 6.1) which were classified as aldehydes (*n*-hexanal, *n*-heptanal, benzaldehyde, *n*-nonanal, and *n*-decanal), alcohols (1-octen-3-ol and 1-octanol), hydrocarbons (*n*-dodecane, *n*-tridecane, (*E*)-2-tetradecene and *n*-tetradecane) and heterocyclic compounds (2-pentylfuran and 2-acetyl-1-pyrroline). Among the compounds identified, *n*-nonanal was found to be the most abundant compound in headspace of both accelerated aging and freshly harvested rice samples, followed by benzaldehyde and *n*-hexanal.

During processing, high temperature of accelerated aging could promote oxidation of the rice constituents and concurrently enhance some highly volatile compounds to release from the rice grain. These occurrences led to the reduction in quantities of volatiles in headspace of the milled rice samples as observed by the decreases in peak areas ratio of volatile compounds in the chromatograms of aging samples (Table 6.1). Widjaja *et al*. (1996a) had suggested that the unique aroma

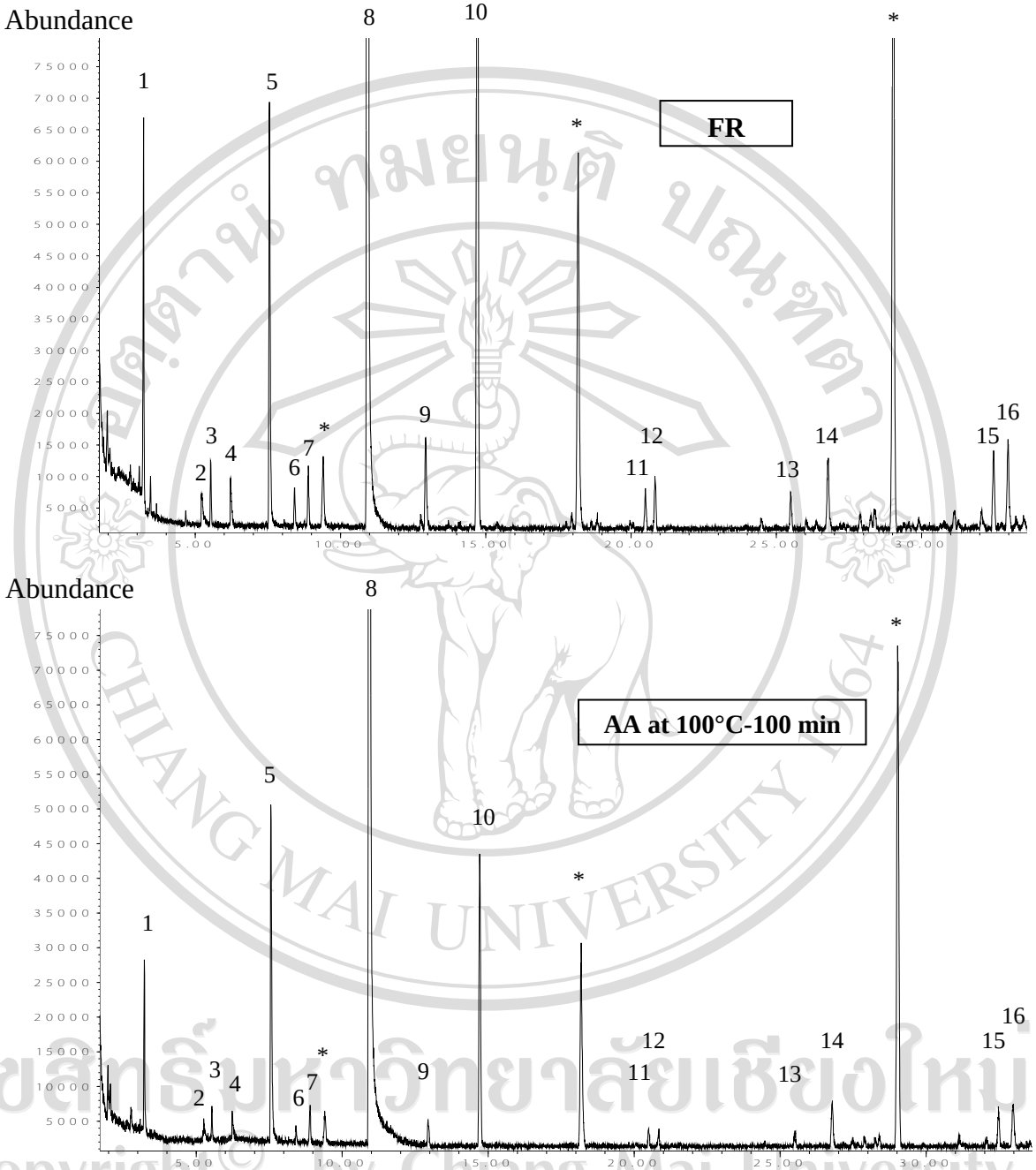

Figure 6.6 GC-MS chromatograms of KDML 105 freshly harvested milled rice (FR) and after given accelerated aging (AA) at 100°C for 100 min. Numbers above the peaks indicate the component identification. Peaks labeled (*) are those resulted from bleeding of the adsorbent.

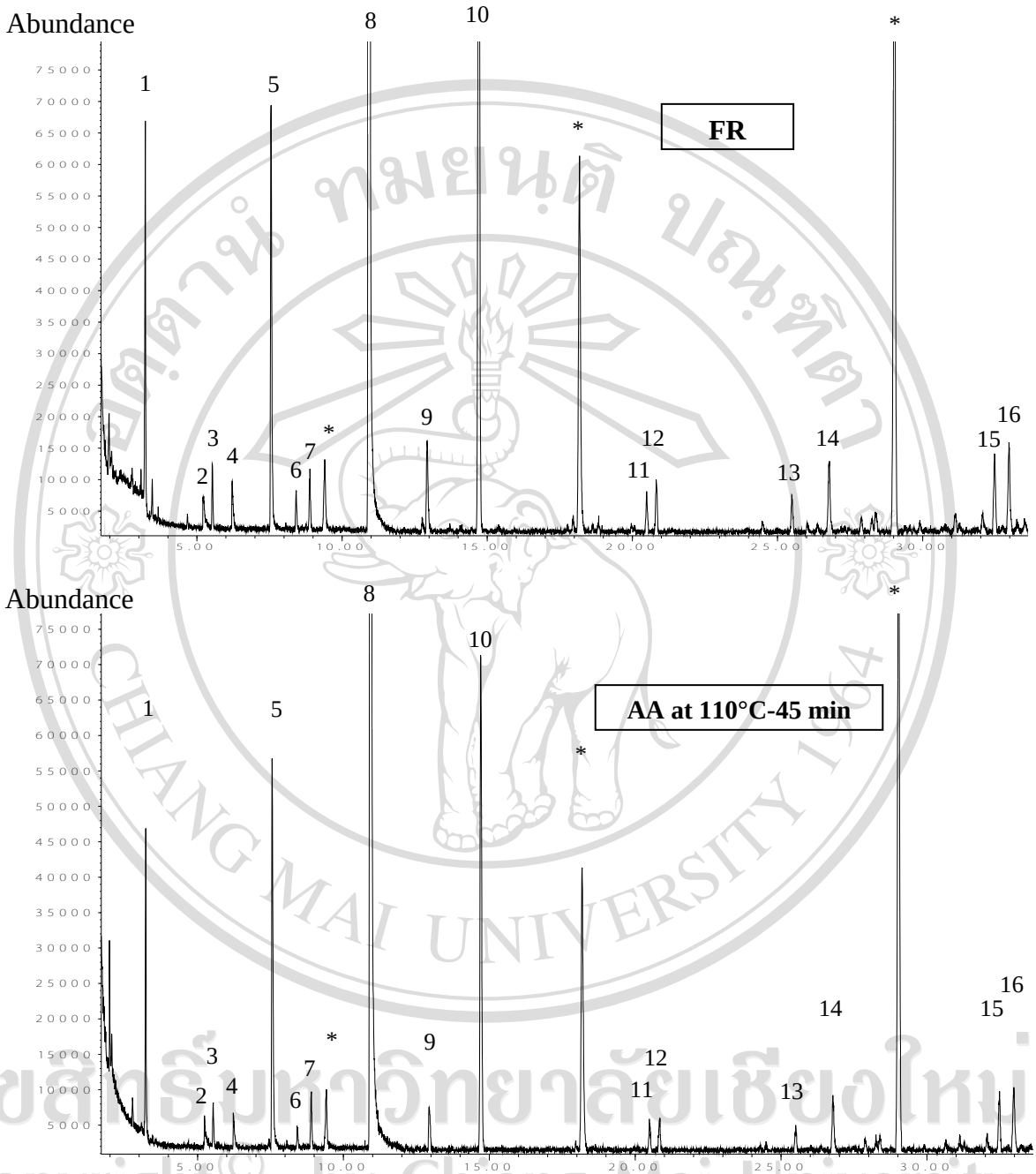

Figure 6.7 GC-MS chromatograms of KDML 105 freshly harvested milled rice (FR) and after given accelerated aging (AA) at 110°C for 45 min. Numbers above the peaks indicate the component identification. Peaks labeled (*) are those resulted from bleeding of the adsorbent.

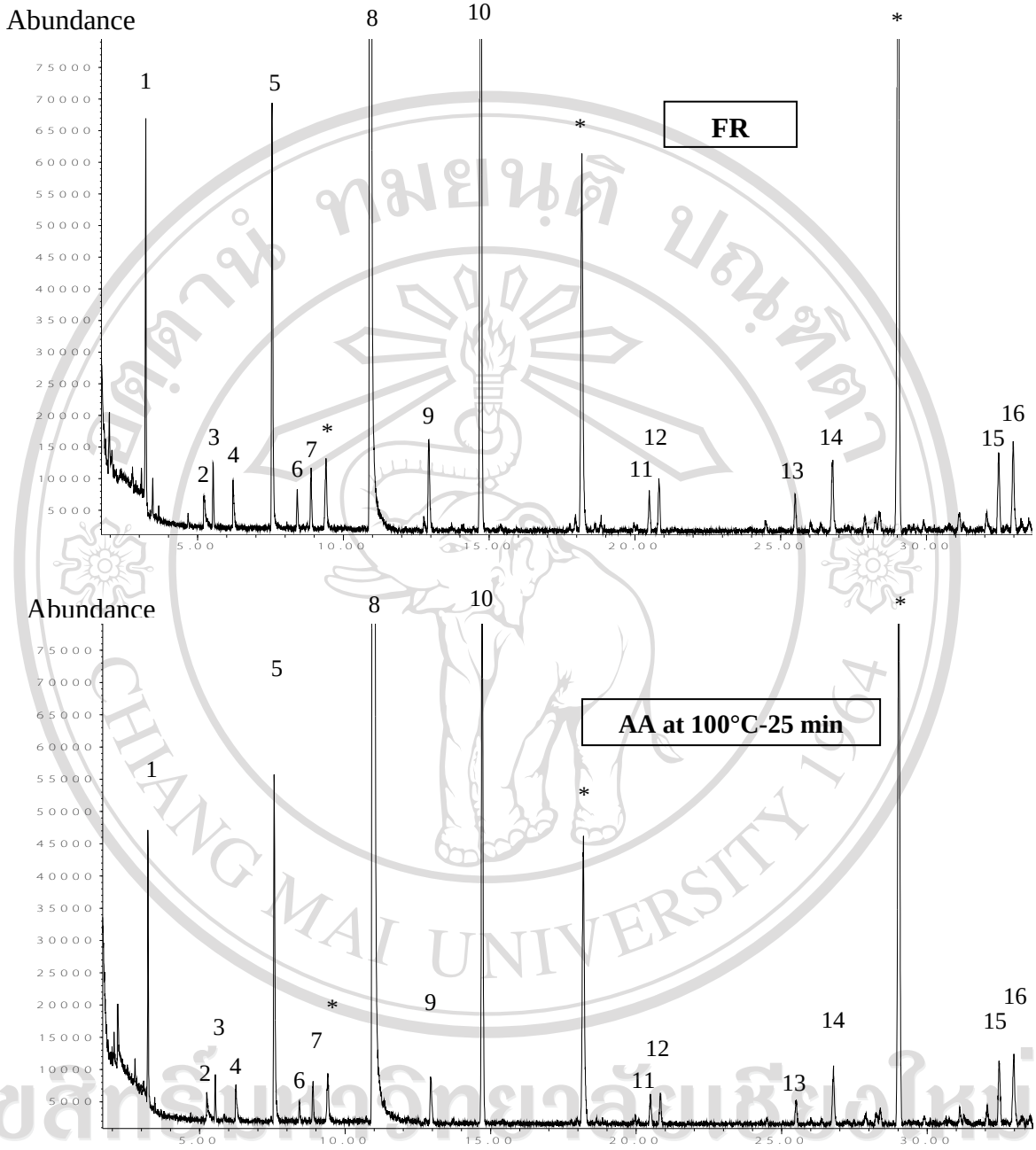

Figure 6.8 GC-MS chromatograms of KDML 105 freshly harvested milled rice (FR) and after given accelerated aging (AA) at 120°C for 25 min.

Numbers above the peaks indicate the component identification.

Peaks labeled (*) are those resulted from bleeding of the adsorbent.

Table 6.1 Identification of volatile components of freshly harvested KDML 105 milled rice and after accelerated aging treatment.

Peak no.	RT[a]	Compound	*m/z*[b]	Match quality (%)	MW[c]	Peak area ratios[d]			
						FR[e]	H100	H110	H120
1	3.22	*n*-hexanal	100(2), 85(4), 82(26), 72(28), 67(16), 57(68), 56(96), **44(100)**	90	100	11.41±0.85	5.43±0.07	7.13±0.09	8.25±0.10
2	5.23	2,6-dimethylpyridine[f]	**107(100)**, 106(45), 92(15), 79(10), 66(19), 44(30), 40(17),	89	107	-	-	-	-
3	5.52	*n*-heptanal	114(3), 96(17), 86(16), 81(33), **70(100)**, 68(17), 57(38), 55(59)	93	114	2.31±0.18	1.14±0.07	1.23±0.08	1.54±0.04
4	6.21	2-acetyl-1-pyrroline	111(24), 83(41), 69(22), 68(21), 55(4), 52(4), **43(100)**, 41(53)	86	111	2.01±0.27	0.90±0.04	1.09±0.18	1.55±0.10
5	7.55	benzaldehyde	**106(100)**, 105(98), 78(17), 77(88), 51(34), 50(20)	97	106	19.35±1.21	15.61±0.96	14.20±0.43	17.16±0.69

[a] RT, Retention time (min); [b] *m/z*, mass/charge ratio; [c] MW, Molecular weight; [d] Peak area ratio of each compound and 2,6-dimethylpyridine (internal standard); [e] FR, Freshly-harvested rice; H100, 100°C-100 min; H110, 110°C-45 min; H120, 120°C-25 min; [f] Internal standard; [g] Solvent of internal standard. Data represent the average ± standard deviation of three determinations.

Table 6.1 continued.

Peak no.	RT[a]	Compound	*m/z*[b]	Match quality (%)	MW[c]	Peak area ratios[d]			
						FR[e]	H100	H110	H120
6	8.42	1-octen-3-ol	128(2), 99(7), 85(12), 82(8), 72(16), 67(10), **57(100)**, 55(16)	90	128	1.67±0.11	0.61±0.03	0.67±0.01	0.91±0.08
7	8.89	2-pentylfuran	138(18), 109(7), 95(7), 82(23), **81(100)**, 53(14), 44(14), 41(14)	92	138	2.72±0.12	1.64±0.14	2.02±0.18	1.90±0.06
8	10.93	benzyl alcohol[g]	108(99), 107(70), 91(17), **79(100)**, 77(64), 65(7), 51(21)	97	108	-	-	-	-
9	12.93	1-octanol	130(1), 112(4), 84(68), 83(49), 70(68), 69(83), 57(46), **56(100)**, 55(85), 43(66), 42(46), 41(92)	89	130	5.11±0.24	1.24±0.14	2.09±0.08	2.92±0.14

Table 6.1 continued.

Peak no.	RT[a]	Compound	*m/z*[b]	Match quality (%)	MW[c]	Peak area ratios[d]			
						FR[e]	H100	H110	H120
10	14.71	*n*-nonanal	142(2), 124(4), 114(9), 98(47), 95(31), 82(36), 70(44), **57(100)**, 44(45), 41(82)	91	142	70.93±3.30	16.66±1.09	22.61±0.55	35.66±0.88
11	20.49	*n*-dodecane	170(12), 141(2), 113(3), 85(47), 71(51), **57(100)**, 43(67)	88	170	2.19±0.08	1.00±0.07	1.29±0.06	1.95±0.08
12	20.82	*n*-decanal	156(2), 112(30), 109(11), 95(42), 82(69), 71(68), 67(57), **57(100)**	90	156	2.98±0.20	0.72±0.01	1.42±0.02	2.00±0.07
13	25.49	unknown	114(1), 85(80), 84(19), **71(100)**, 69(14), 57(99), 43(68)	-	-	2.02±0.16	0.80±0.07	1.18±0.04	1.44±0.07

Table 6.1 continued.

Peak no.	RT[a]	Compound	*m/z*[b]	Match quality (%)	MW[c]	Peak area ratios[d]			
						FR[e]	H100	H110	H120
14	26.79	*n*-tridecane	184(10), 127(8), 112(9), 99(10), 85(45), 71(62), **57(100)**, 43(86)	94	184	4.51±0.12	2.83±0.29	2.84±0.05	4.06±0.10
15	32.47	(*E*)-2-tetradecene	196(16), 111(48), 97(81), 83(93), 69(98), **55(100)**, 41(97)	97	196	5.11±0.17	2.17±0.16	2.80±0.04	4.53±0.22
16	32.97	*n*-tetradecane	198(7), 127(9), 99(10), 85(50), 71(73), **57(100)**, 43(58)	93	198	6.23±0.63	3.27±0.23	3.73±0.04	5.22±0.16

characteristic of fragrant rice is dependent on the levels and the relative proportions of many individual components that make up its fragrance characteristic. Results of this study revealed that the decreases in the levels of volatile components in accelerated-aged rice were indirect proportion with the contents of their respective compounds in freshly harvested rice, and among accelerated aging samples. However, aging at 120°C for 25 min showed minimum reduction of peak areas of the rice volatiles. Although peak areas of rice volatile components were reduced by accelerated aging process, reasonable amounts still remained which indicated that aroma quality of the aged samples was not affected.

6.4 Conclusions

Based on the results investigated from milled rice headspace, it can be concluded that the volatile constituents making up odor character of KDML 105 given accelerated aging were not different from the ordinary fresh rice one. Though there were decreases in peak area of the volatile components, all the identified volatile compounds found in fresh rice were present in the accelerated-aging rice samples. Similar in volatile components between fresh and accelerated aging samples could translate to similar volatile compounds, if determined, in their cooked rice. Moreover, the accelerated-aged rice had greater aroma quality than that of 3-month naturally-aged rice as compared in terms of the amount of 2-acetyl-1-pyrroline and *n*-hexanal present in their samples.