Effect of Accelerated Aging Treatments on Aroma Quality and Major Volatile Components of Thai Jasmine Rice

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ABSTRACT

The effect of accelerated aging (AA) treatments on aroma quality and major volatile components of freshly-harvested Thai jasmine rice cv. Khao Dawk Mali 105 was investigated. Freshly-harvested milled rice were exposed to three AA conditions which were 100°C for 100 min, 110°C for 45 min and 120°C for 25 min, and then their aroma quality was evaluated. The aroma quality was assessed on the basis of the quantity of aroma-impact compound, 2-acetyl-1-pyrroline (2AP), and an off-odor compound, n-hexanal, using GC-FID. Other volatile components were also analyzed by GC-MS. Results revealed that the quantity of 2AP and n-hexanal decreased in AA samples. However, the AA rice had better aroma quality when compared with that of 3-month naturally-aged rice. Analysis of rice volatile components indicated that the AA treatments did not affect the volatile constituents that make up for odor character of this aromatic rice. Thirteen identified compounds: n-hexanal, n-heptanal, 2-acetyl-1-pyrroline, benzaldehyde, 1-octen-3-ol, 2-pentylfuran, 1-octanol, n-nonanal, n-dodecane, n-decanal, n-tridecane, (E)-2-tetradecene and n-tetradecane, found in freshly-harvested rice, were all present in the AA samples with no addition of new volatiles. From these results, it can be concluded that the AA technique can bring freshly-harvested rice cv. KDML 105 to advanced stage of aging while still maintaining its high aroma quality.

Key words: Aromatic rice, Accelerated aging, 2-acetyl-1-pyrroline, n-hexanal, Volatile components
INTRODUCTION

Aging can improve some of cooking and eating properties of rice that is preferred by Asian consumers. However, aging process takes time and at the same time can reduce some desirable characteristics including aroma of fragrant rice. To shorten time of this conventional aging, a technique called accelerated aging (AA) had been proposed. Accelerated aging of freshly-harvested paddy, using wet or dry heat treatments with suitable grain moisture content had been studied and reported to improve cooking quality of rice which resembled to those of naturally-aged rice (Gujral and Kumar, 2003; Soponronnarit et al., 2008). However, the consequence of this accelerated aging technique on aroma characteristic of rice has not yet been investigated and verified. Such AA practice on freshly-harvested paddy could have impacts on the aroma quality and volatile profile, and could probably change the typical aroma characteristic of rice. This was due to the diffusion of husk and bran components into endosperm of rice during moistening step and the relatively high temperature employed in aging of the moist paddy, as occurred in parboiled rice (Lamberts et al., 2006).

Volatile compounds that provide aroma characteristic of fragrant rice had been studied by a number of researchers. 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 (2AP) and n-hexanal are considered to be the most important odor-active compounds (Buttery et al., 1988; Jezussek et al., 2002). 2AP had been reported to possess very low odor threshold value (a minimum detectable level) which indicates the great important contribution of this compound to aroma of the rice, though it is present in small amount (Harrison and Dake, 2005; Yang et al., 2008). Hexanal, an off-odor compound, had high odor threshold value but it was found to be the most abundant volatile compound in stored rice (Widjaja et al., 1996b; Tava and Bocchi, 1999). During storage of Thai aromatic rice cv. KDML 105, the concentration of 2AP decreased whereas hexanal increased (Laksanalamai and Ilangantileke, 1993; Wongpornchai et al., 2004). Since 2AP and hexanal play an important role in consumer acceptability, alternative postharvest technology should be sought in the way that negative effect on the appearance of these compounds can be minimized.

In this study, aroma quality and volatile components of KDML 105 freshly-harvested milled rice after being given a designed set of AA treatments were investigated whether these AA treatments could change or result in favorable or unfavorable effects on some volatile compounds that are responsible for the odor character of the aromatic rice.
MATERIALS AND METHODS

Rice samples and preparations

The rice cv. KDML 105 used in this study was grown in 2006 season at Lampang Agricultural Research and Training Center, Rajamangala University of Technology Lanna, Lampang. The rice was harvested at maturity by hand, left dry in the field for 2 to 3 days and then threshed to paddy having approximately 14% MC. The freshly-harvested paddy sample was then divided into 2 portions by a Boerner divider (Seedburo Equipment Co., Chicago, IL). One portion was de-hulled 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. The protein ($\text{N} \times 5.95$) and lipid contents of the head rice samples were 6.54 and 0.92%, respectively, as determined by AOAC (1999) standard methods. Apparent amylose content was 17.65% (w/w) as determined by the method of Juliano et al. (1981). Moisture content of milled rice sample, determined prior to the aging treatment using oven method (103°C for 17 hr) was 13.13% (wb). The other portion of paddy sample was stored in jute sacks under ambient condition. Changes in aroma quality as measured by the amounts of 2AP and n-hexanal of its milled rice samples were monitored at 1-month interval for a storage period of 6 months.

Accelerated aging treatments

Three replicates, each of 370 g of freshly-harvested milled rice samples, were placed in aluminum containers (11 cm height $\times$ 8.5 cm diameter) and covered with heavy-duty aluminum foil. The rice samples were then exposed to three different aging treatments, i.e., 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). After exposure, the rice samples were left covered in the aluminum containers and cooled for about 2 hr at 21°C. Samples were then poured onto aluminum trays and their temperature and moisture contents were allowed to equilibrate with ambient air for 24 hr. Subsequently, all samples including freshly-harvested rice (control) were placed into zip-locked plastic bags and kept at -20°C until the time of each experiment.

Analysis of 2-acetyl-1-pyrroline and $n$-hexanal

The amounts of 2AP 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 $\mu$L 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°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 2AP 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.

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. Then, 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 NIST 98 libraries of the MS database.

Statistical analysis

Data regarding the quantities of 2AP and n-hexanal of KDML 105 rice samples were analyzed using analysis of variance (ANOVA) to determine the effect of AA treatments. Differences among samples were determined by least
significant difference test (LSD) at $P<0.05$.

RESULTS AND DISCUSSION

Aroma quality on the basis of 2-acetyl-1-pyrroline and $n$-hexanal contents

The amounts of 2AP in the rice samples decreased after AA treatment (Figure 1). The concentrations obtained from rice aged with 100°C for 100 min, 110°C for 45 min and 120°C for 25 min were 3.33, 3.78 and 3.94 ppm, respectively. The 100°C-100 min treatment had the highest percent of reduction (33.9%) whereas 120°C-25 min had only 21.8% when calculated on the basis of 2AP content (5.04 ppm) of freshly-harvested milled rice. These results revealed that reduction of 2AP was greater in rice given longer duration treatment, although the heating temperature applied to the rice was lower (100°C for 100 min). In comparison with those naturally-aged rice,

![Figure 1](image)

**Figure 1.** Effect of accelerated aging treatments (temperature and duration) on concentration of the aroma compound, 2-acetyl-1-pyrroline, in KDML105 milled rice samples. Control = freshly-harvested KDML105 milled rice. Vertical bars (±SD) with the same letters are not significantly different at $P<0.05$, LSD.

the contents of 2AP in all AA samples were higher than that observed in 3-month naturally-stored sample (2.95 ppm) (Figure 2). Thus, 2AP of the naturally-stored samples decreased rapidly and were lower than those of the AA rice after storage for 3 months.
Figure 2. 2-Acetyl-1-pyrroline concentrations in 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 at \( P<0.05 \), LSD.

Consequently, the age-accelerated treatment using high temperature and short duration (120°C for 25 min) would be recommended for the production of KDML 105 AA rice. The high 2AP content in the rice aged by this heating condition might be attributed to a shorter duration of heating time, being not sufficient for the release of 2AP from inner part of the rice kernel to its surrounding atmosphere. Thus, a large portion of 2AP still remained in the rice kernel. Analysis of 2AP in the rice kernel by the previous studies revealed that the compound was equally distributed across kernel of aromatic rice (Bergman et al., 2000) and it was reported to be present in the starch granule of milled rice kernel in both free and starch-bound forms, with the latter required higher temperature and more time for extraction (Yoshihashi et al., 2005). These research findings could support the aforementioned postulation. 2AP is formed naturally in rice during growth in paddy field (Yoshihashi et al., 2002) and its concentration decreases with time of storage (Wongpornchai et al., 2004; Yoshihashi et al., 2005). Our results suggest the advantage of AA technique to bring the freshly-harvested rice to an advanced stage of aging, yielding rice of similar cooking quality to that of stored rice while still maintaining its high aroma quality.

During processing, the relative amounts of n-hexanal in AA samples were reduced significantly (Figure 3). Area ratios of n-hexanal/DMP of the AA samples were in the range of 0.37 to 0.47 which were lower than that of the freshly-harvested milled rice (0.60). Analysis was also made to observe the amounts of \( n \)-hexanal generated on those rice stored in paddy form under natural condition (Figure 4). The results revealed that the relative contents of \( n \)-hexanal in the naturally-stored rice samples were higher than those of the rice given AA treatments. The increase in \( n \)-hexanal of naturally-stored rice samples was attributed to the degradation of lipid composition of the rice. Lipids in rice were reported to be hydrolyzed and...
Figure 3. Effect of accelerated aging treatments (temperature and duration) on the relative content of n-hexanal in KDML105 milled rice samples. Control = freshly-harvested KDML 105 milled rice. Vertical bars (±SD) with the same letters are not significantly different at $P<0.05$, LSD.

Oxidized to free fatty acids or peroxides (Zhou et al., 2002), which subsequently resulted in the increases in some off-odor compounds, including n-hexanal of the stored rice. This carbonyl compound, n-hexanal, was reported to be a degradation product of linoleic acid (Monsoor and Proctor, 2004). Age-accelerated technique, using high temperature in this study, might affect the activity of lipid hydrolysis enzyme and, at the same time, enhance the release of this compound, resulting in lower content of n-hexanal in the AA samples which indicated that aroma quality of the AA rice was improved.

Figure 4. 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 at $P<0.05$, LSD.
Aroma quality on the basis of volatile components as determined by GC-MS

Gas chromatographic profiles of volatile components of the freshly-harvested milled rice and its corresponding AA samples are illustrated in Figures 5, 6 and 7. These volatile components were extracted from headspace of milled rice samples using SPME technique. Following the analysis of total rice volatiles by GC-MS, the overall aroma quality of the AA rice samples was assessed on the basis of the similarity of their volatile component profiles compared with that of the freshly-harvested milled rice. All of the volatile compounds typically present in the headspace of KDML105 rice showed themselves in the chromatograms of

Figure 5. GC-MS chromatograms of KDML 105 freshly-harvested milled rice (FR) and after being given an accelerated aging (AA) at 100°C for 100 min. Numbers above the peaks indicate the component identification. Peaks labeled (*) are those interferences resulted from degradation of the SPME adsorbent.
Figure 6. GC-MS chromatograms of KDML 105 freshly-harvested milled rice (FR) and after being given an accelerated aging (AA) at 110°C for 45 min. Numbers above the peaks indicate the component identification. Peaks labeled (*) are those interferences resulted from degradation of the SPME adsorbent.

the AA rice samples. There were no additional compounds generated or formed as a consequence of the AA treatments. Each chromatogram showed 13 identified components (Table 1), which were classified as aldehydes (n-hexanal, n-heptanal, benzoaldehyde, 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-acetyl-1-pyrroline and 2-pentylfuran). Among the compounds identified, n-nonanal was found to be the most abundant compound in headspace of both AA and freshly harvested rice samples, followed by benzoaldehyde and n-hexanal.
Figure 7. GC-MS chromatograms of KDML 105 freshly-harvested milled rice (FR) and after being given an accelerated aging (AA) at 120°C for 25 min. Numbers above the peaks indicate the component identification. Peaks labeled (*) are those interferences resulted from degradation of the SPME adsorbent.

During processing, high temperature of AA treatments could likely promote oxidation of the rice constituents and concurrently enhance some portion of these highly-volatile compounds to be released from the rice kernel. 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 some rice volatiles in the chromatograms of AA samples (Table 1). Suggestion had been made that the unique aroma characteristic of fragrant rice is dependent on the levels and the relative proportions of many individual components that make up its fragrance characteristic (Widjaja et al., 1996a). Results of this study revealed that the decreases in the levels of volatile components in AA rice were indirect proportion with the contents of their respective compounds in freshly-harvested rice,
and among AA samples. However, aging at 120°C for 25 min showed minimum reduction of peak areas of the rice volatiles. Although relative contents of the rice volatiles were reduced by the AA process, reasonable amounts still remained which indicated that the overall aroma quality of the AA rice samples was not affected.

Table 1. Identification of volatile components in freshly-harvested and accelerated-aged KDML 105 milled rice.

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Retention time (min)</th>
<th>Compound</th>
<th>m/z[^a]</th>
<th>Match quality (%)</th>
<th>MW[^c]</th>
<th>Peak area ratios[^d]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FR[^e]</td>
<td>H100</td>
<td>H110</td>
</tr>
<tr>
<td>1</td>
<td>3.22</td>
<td>α-hexanal</td>
<td>100(2), 85(4), 82(26), 72(28), 67(16), 57(68), 56(96), 44(100)</td>
<td>90</td>
<td>100</td>
<td>11.41±0.85 5.43±0.07 7.13±0.09 8.25±0.10</td>
</tr>
<tr>
<td>2</td>
<td>5.23</td>
<td>2,6-dimethylpyridine[^f]</td>
<td>107(100), 106(45), 92(15), 79(10), 66(19), 44(30), 40(17)</td>
<td>89</td>
<td>107</td>
<td>- - - -</td>
</tr>
<tr>
<td>3</td>
<td>5.52</td>
<td>β-heptanal</td>
<td>114(3), 96(17), 86(16), 81(33), 70(100), 68(17), 57(38), 55(59)</td>
<td>93</td>
<td>114</td>
<td>2.31±0.18 1.14±0.07 1.23±0.08 1.54±0.04</td>
</tr>
<tr>
<td>4</td>
<td>6.21</td>
<td>2-acetyl-1-pyrroline</td>
<td>111(24), 83(41), 69(22), 68(21), 55(4), 52(4), 43(100), 41(53)</td>
<td>86</td>
<td>111</td>
<td>2.01±0.27 0.90±0.04 1.09±0.18 1.55±0.10</td>
</tr>
<tr>
<td>5</td>
<td>7.55</td>
<td>benzaldehyde</td>
<td>106(100), 105(98), 78(17), 77(88), 51(34), 50(20)</td>
<td>97</td>
<td>106</td>
<td>19.35±1.21 15.61±0.96 14.20±0.43 17.16±0.69</td>
</tr>
<tr>
<td>6</td>
<td>8.42</td>
<td>1-octen-3-ol</td>
<td>128(2), 99(7), 85(12), 82(8), 72(16), 67(10), 57(100), 55(16)</td>
<td>90</td>
<td>128</td>
<td>1.67±0.11 0.61±0.03 0.67±0.01 0.91±0.08</td>
</tr>
<tr>
<td>7</td>
<td>8.89</td>
<td>2-pentylfuran</td>
<td>138(18), 106(7), 95(7), 82(23), 81(100), 53(14), 44(14), 41(14)</td>
<td>92</td>
<td>138</td>
<td>2.72±0.12 1.64±0.14 2.02±0.18 1.90±0.06</td>
</tr>
<tr>
<td>8</td>
<td>10.93</td>
<td>benzyl alcohol</td>
<td>108(99), 107(70), 91(17), 70(100), 77(64), 65(7), 51(21)</td>
<td>97</td>
<td>108</td>
<td>- - - -</td>
</tr>
<tr>
<td>9</td>
<td>12.93</td>
<td>1-octanol</td>
<td>130(1), 112(4), 84(68), 83(49), 70(68), 69(83), 57(46), 56(100), 55(85), 43(66), 42(46), 41(92)</td>
<td>89</td>
<td>130</td>
<td>5.11±0.24 1.24±0.14 2.09±0.08 2.92±0.14</td>
</tr>
<tr>
<td>10</td>
<td>14.71</td>
<td>α-nonanal</td>
<td>142(2), 124(4), 114(9), 98(47), 95(31), 82(36), 70(44), 57(100), 44(45), 41(82)</td>
<td>91</td>
<td>142</td>
<td>7.93±3.30 16.66±1.09 22.61±0.55 35.66±0.88</td>
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<tr>
<td>11</td>
<td>20.49</td>
<td>n-dodecane</td>
<td>170(12), 141(2), 113(3), 85(47), 71(51), 57(100), 43(67)</td>
<td>88</td>
<td>170</td>
<td>2.19±0.08 1.00±0.07 1.29±0.06 1.95±0.08</td>
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<tr>
<td>12</td>
<td>20.82</td>
<td>n-decanal</td>
<td>156(2), 123(30), 109(11), 95(42), 82(69), 71(68), 67(57), 57(100)</td>
<td>90</td>
<td>156</td>
<td>2.98±0.20 0.72±0.01 1.42±0.02 2.00±0.07</td>
</tr>
<tr>
<td>13</td>
<td>25.49</td>
<td>unknown</td>
<td>114(1), 85(80), 84(19), 71(100), 69(14), 57(99), 43(68)</td>
<td>- -</td>
<td>-</td>
<td>2.02±0.16 0.80±0.07 1.18±0.04 1.44±0.07</td>
</tr>
<tr>
<td>14</td>
<td>26.79</td>
<td>n-tridecane</td>
<td>184(10), 127(8), 112(9), 99(10), 85(45), 71(62), 57(100), 43(86)</td>
<td>94</td>
<td>184</td>
<td>4.51±0.12 2.83±0.29 2.84±0.05 4.06±0.10</td>
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<tr>
<td>15</td>
<td>32.47</td>
<td>(E)-2-tetradecene</td>
<td>196(16), 111(48), 97(81), 83(93), 69(68), 55(98), 43(197)</td>
<td>97</td>
<td>196</td>
<td>5.11±0.17 2.17±0.16 2.80±0.04 4.53±0.22</td>
</tr>
<tr>
<td>16</td>
<td>32.97</td>
<td>n-tetradecane</td>
<td>198(7), 127(9), 99(10), 85(50), 71(73), 57(100), 43(58)</td>
<td>93</td>
<td>198</td>
<td>6.23±0.63 3.27±0.23 3.73±0.04 5.22±0.16</td>
</tr>
</tbody>
</table>

[^a]: 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.
CONCLUSION

Based on the results of volatile analysis by HS-GC and SPME-GC-MS of the milled rice samples, it can be concluded that the profiles of volatile constituents making up the odor character of rice cv. KDML 105 given accelerated aging were not different from that of the ordinary freshly-harvested rice. Though there were decreases in relative contents of the volatile components, all the volatile compounds found in freshly-harvested rice were present in the AA rice samples. Moreover, the AA rice had better aroma quality than that of 3-month naturally-aged rice in terms of higher amount of the key aroma compound, 2AP, and lower content of an off-odor, n-hexanal, present in their kernels.

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