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The Correlation between 2-Acetyl-1-pyrroline Content, Biological Compounds and Molecular Characterization to the Aroma Intensities of Thai Local Rice

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Abstract: Aroma intensities of rice are correlated with the mixture of aroma compounds it contains. 2-acetyl-1-pyrroline (2AP) has been reported as a major aroma compound and as a characteristic compound in fragrant rice. In this study, Thai local cultivars were classified into fragrant and non-fragrant rice based on the 2AP content and molecular characterization. Local rice cultivars were also examined for their proline content and volatile compounds profile, which are important factors in determining aroma. The results suggested that 43 Thai local rice cultivars were classified into 25 fragrant rice cultivars and 18 non-fragrant cultivars. The type of fragrant rice cultivars included 16 non-colored and 9 colored rice cultivars, while the type of non-fragrant rice cultivars included 14 non-colored and 4 colored rice cultivars. The proline content of local rice cultivars was determined and showed no correlation with the 2AP content; however, the proline level appears to be associated with the environmental stress in the rice cultivation area. One hundred and forty volatile compounds were identified from local rice cultivars. Among the detected compounds, 18 volatile compounds, including hexanal 1-pentanol octanal (*E*)-2-heptenal 6-methyl-5-hepten-2-one 1-hexanol nonanal 2-butoxy-ethanol (*E*)-2-octenal 1-tetradecene 1-octen-3-ol decanal benzaldehyde (*E*)-2-nonenal 1-nonanol benzyl alcohol isovanillin and vanillin contributed to the aroma intensities of both fragrant and non-fragrant rice. Aroma compounds were more abundant in fragrant than in non-fragrant rice. Moreover, the levels of aroma compounds recorded in non-colored cultivars were higher than those in colored rice cultivars. In contrast, the 2AP content of colored rice cultivars was higher than that in non-colored rice cultivars. Our findings may assist rice breeding programs in producing a new aromatic genotype rice with high potential aroma intensities.

Key words: 2-acetyl-1-pyrroline, aroma compound, local rice cultivars, proline content

1 Introduction

The aroma intensities in rice are due to a mixture of aroma compounds[1−5]. The characteristic compound in fragrant rice is 2-acetyl-1-pyrroline (2AP)[5−9]. This compound is synthesized via L-proline metabolism by the protein encoded by the betain aldehyde dehydrogenase gene (*badh2*), located on chromosome 8[10, 11]. An 8-base pair (bp) deletion has been reported in exon 7 of *badh2* gene, which encodes the inactive betaine aldehyde dehydrogenase enzyme and is only found in fragrant rice[10, 12]. Based on this study, a method was developed for the analysis of fragrant and non-fragrant rice genotypes by electrophoresing the products of specific allele amplifications on an agarose gel and identifying the genotypes by their different molecular sizes, with the upper size identified as the non-fragrant genotype and the lower size identified as the fragrant rice genotype[12].

In plants, proline is one of the amino acids whose levels vary in response to environmental stresses, such as high salt levels and drought[13−17]. Proline is the first precursor

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molecule in the 2AP synthesis biochemical pathway[4, 10, 18]. Previous reports suggest that the level of 2AP is related to the level of proline. Mo *et al.*[19] found that a decrease in solar intensity (shade treatment) leads to an increased proline content and 2AP content in rice. The 2AP content was also correlated with the proline content of Aychade rice under salt condition[20].

The aroma of rice is due to a mixture of volatile compounds. Buttery *et al.*[21] identified nine compounds, namely, 2AP; (*E*,*E*)-2,4-decadienal; nonanal; hexanal; (*E*)-2-nonenal; octanal; decanal; 4-vinylguaiacol; and 4-vinylphenol which are classified as major contributors to the aroma intensities of cooked rice. It is well known that 2AP is the characteristic compound for fragrant rice, while octanal is also associated with aroma intensities; these compounds have been studied extensively in rice[19, 21, 22]. Hinge *et al.*[23], characterized the volatile compounds of scented and non-scented rice and found that 26 volatile compounds can be classified as major volatile compounds. Among these, 14 volatile compounds, including, 2AP; 1-octanol; 1-octen-3-ol; (*E*)-3-octen-2-one; octanal; (*E*)-2-nonenal; nonanal; heptanal; hexanal; decanal; (*E*)-2-octenal; 2-pentylfuran; phenylacetaldehyde and pentanal were identified to be associated with the odor activity value of aroma intensity of rice.

Thailand was ranked sixth among the top ten countries for rice production in 2017 (United States Department of Agriculture (USDA)). The rice cultivated in Thailand can be classified into two types the lowland rice cultivars and the upland rice cultivars. Among these rice cultivars, the most well-known is Thai jasmine rice (KDML 105), due to its characteristic aroma. Although Thai jasmine rice is known worldwide, another local rice cultivar, Thai local rice, has similar characteristics, especially with respect to the aroma. This rice has been cultivated in areas which are suitable only for this particular cultivar, and many cultivars have not been evaluated till date. Therefore, in the present study, the 43 Thai local rice cultivars were classified as fragrant or non-fragrant, based on the content of active compounds associated with aroma intensities; the active compound content of these cultivars was also compared with that of Thai jasmine rice.

2 EXPERIMENTAL

2.1 Plant material

Grains of 43 Thai local rice cultivars (13 colored and 30 non-colored rice cultivars, the local rice names of which are shown in **Fig. 1**) were obtained from rice fields in Northeastern Thailand during the harvest season (December–February 2015). KDML 105 grains were used as the

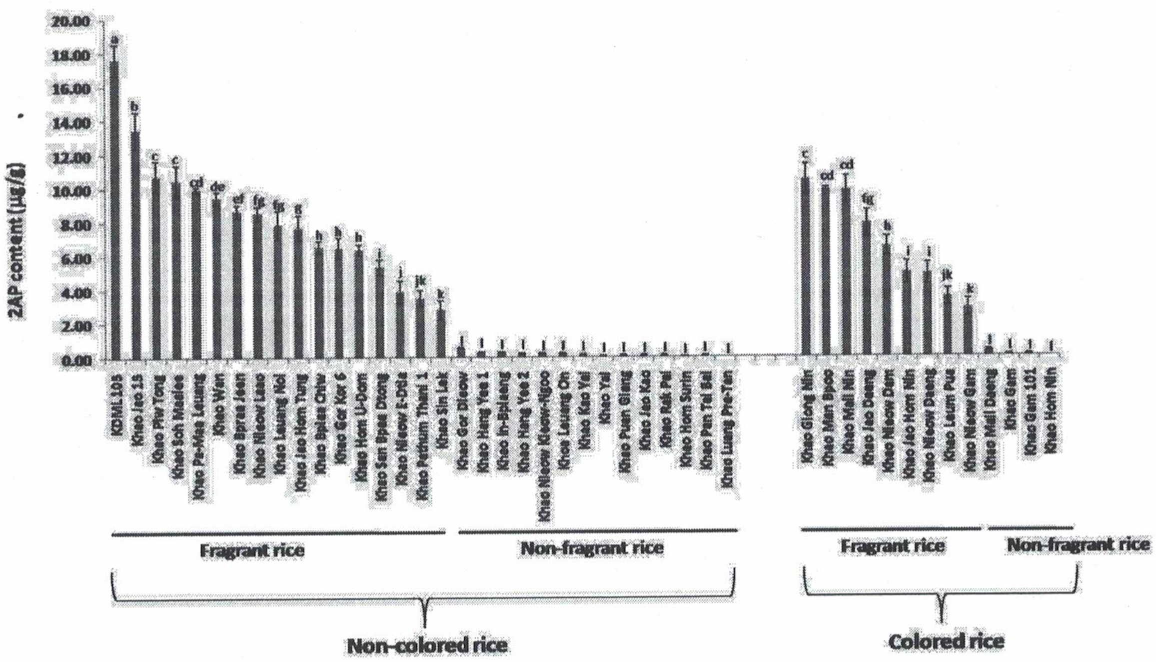

Fig. 1 2AP content of 43 local rice cultivars and KDML105. The figure showed 30 non-colored local rice cultivars and 13 colored local rice cultivars. Among non-colored local rice cultivars, 16 rice cultivars were classified as fragrant rice, while 14 rice cultivars were classified as non-fragrant rice. While, in the group of colored local rice cultivars, 9 rice cultivars were classified as fragrant rice, while 4 rice cultivars were classified as non-fragrant rice. The error bars indicate the standard deviation of means (n = 3); the same letters indicate no significant difference (Duncan, $p > 0.05$).

standard fragrant rice; this was harvested during the same season as the other local rice. Approximately 50 g of all local rice and KDML 105 rice grains were sterilized by soaking in 0.1% NaClO for 30 min. The grains were then rinsed with distilled water and dried in a hot air oven (60℃) to reduce the moisture content to less than 13%. The sterilized rice grains were homogenized to fine pieces using a CryoMill (Retsch, Germany) with liquid nitrogen cooling. The homogenized samples were stored at 4℃ for the analysis of 2AP, proline, and aroma compound content.

2.2 2-acetyl-1-pyrroline determination

The 2AP content was determined using our previous protocol (Sansenya *et al.*[24]) and with some modifications to the method. 2AP with a purity of 95% was purchased from BOC Sciences (New York, USA). A 5 mg/mL stock solution was prepared by adding 2 mL of methanol-toluene (1:1) to 10 mg of 2AP; this was then diluted to 0.01, 0.05, 0.1, 0.5, 1.0, and 2.5 mg/mL with ethanol. One local white rice without 2AP detected was used as blank for 2AP analysis. The calibration standards were prepared by adding 1.25, 2.5, 5.0, 10.0, 25.0, 50.0, and 100.0 μg of the 2AP standard to a 20 mL headspace vial containing 1 g of rice blank. The rice samples were prepared by weighing about 1 g of powder samples into 20 mL headspace vials and capped immediately.

The rice samples were analyzed using a headspace technique. The GC Autosampler 120 from Agilent (CA, USA) was used. Rice samples were incubated at 120℃ for 15 min with shaking, and then 1 mL of gas was withdrawn with a gas tight syringe (2.5 mL) and injected into the GC injector port. Separation of the volatile compounds was achieved by gas chromatography–mass spectrometry (Agilent 7890A GC-7000 Mass Triple Quad) using equipment fitted with a capillary column DB-Wax (60 m × 0.25 mm i.d. × 0.25 μm film thickness, J & W Scientific, Folsom, CA) and a quadrupole mass detector. The injector was set at 240℃, and the split mode was applied with a split ratio of 5:1. Helium gas was used as the carrier gas at a constant flow rate of 1.5 mL/min. The following oven temperature program was used: the column temperature was isothermally maintained at 40℃ for 2 min, programmed at a rate of 10℃/min to 100℃, then at a rate of 5℃/min to 150℃; and finally at a rate of 30℃/min to 250℃, and the column temperature was then maintained isothermally at 250℃ for 15 min. The mass spectrometer was used in the electron ionization mode with the ion source temperature set at 230℃ and ionization energy set at 70 eV. The multi reaction monitoring (MRM) mode was used to analyze the 2AP content. Helium gas was used as the quenching gas at a flow rate of 2.35 mL/min. Nitrogen gas was used as the collision gas at a flow rate of 1.5 mL/min. The precursor ion *m/z* 111 and the product ion *m/z* 83 were selected for 2AP with a collision energy of 5 v. The MS detection dwell time was 30 ms. Quantification was performed by measuring the peak area of ions at *m/z* 83. The amount of 2AP was calculated from the calibration curve, $y = 14.51x + 9.88$ ($R^2 = 0.999$).

2.3 Volatile compounds determination

Volatile compounds were analyzed by gas chromatography-mass spectrometry using the same capillary column DB-Wax (60 m × 0.25 mm i.d. × 0.25 μm film thickness, J &W Scientific, Folsom, CA) according to our previously described protocol (Sansenya *et al.*[25]). Identification of volatile compounds was performed by comparing the mass spectra with mass spectral libraries of the National Institute of Standards and Technology (NIST, 2011 version). The volatile compound content was calculated from the peak areas.

2.4 Proline determination

The proline content was determined using the modified method of Mo *et al.*[19]. Homogenized rice grain (3 g) was homogenized in 3% sulfosalicylic acid for 15 min followed by centrifugation at 10,000 rpm for 15 min and sample filtration using a syringe filter (0.45 μm).

A mixture of 2 mL of the sample, 3 mL of ninhydrin solution, and 2 mL of glacial acetic acid was boiled for 20 min. The reaction mixture was extracted with 4 mL of toluene followed by centrifugation at 4000 rpm for 15 min. The extracted solution was measured at 520 nm, and the amount of proline was quantified by comparing the value of absorption with the standard curve of proline: $y = 0.413x - 0.014$ ($R^2 = 0.999$).

2.5 Fragrant gene determination

Primers were designed using PrimerX (http://www.bioinformatics.org/primerx/cgi-bin/DNA_1.cgi), as follows: forward, 5' TCCTCTCAATACATGGTTTATG- 3' and reverse, 5' TTGGAAACAAACCTTAACCATAG 3'. The nucleotide sequence of the *badh2* gene used for designing the primers was obtained from NCBI, GenBank Accession Number AJ746297.

The polymerase chain reaction (PCR) was performed using the following program: denaturationat 95℃ for 5 min, 35 cycles of 95℃ for 1 min, 53℃ for 1 min, and 72℃ for 1 min, with a final extension step at 72℃ for 5 min. The PCR products were analyzed by horizontal electrophoresis on 2.5% agarose gel and stained with 1.0 μg/mL of ethidium bromide. The estimated size of the PCR product for fragrant rice is 110 bp and 124 bp for non-fragrant rice.

2.6 Statistic analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) 22.0 software for Windows. Quantitative data are presented as mean values with standard deviation values obtained from three replicates. All analyses were processed by one-way analysis of variance (ANOVA). A Duncan's multiple range test was used to determine significant differences. The level of significance was set at $p < 0.05$.

3 RESULTS AND DISCUSSION

3.1 2AP content of 43 local rice cultivars

The 2AP content of 43 local rice cultivars (13 colored and 30 non-colored rice cultivars) and Thai jasmine rice (KDML 105) are shown in **Fig. 1**. The 2AP content of KDML 105 was 17.60 ± 0.90 μg/g, while the 2AP content of local rice cultivars was in the range of 0.09 ± 0.019 μg/g to 13.47 ± 1.050 μg/g. KDML 105 is well known as a fragrant rice variety with a high 2AP content[26−30]. Our results show that the 2AP content of each of the 43 local rice cultivars was lower than that of KDML 105. The highest and lowest 2AP content were obtained for the Khao Jao 15 and Khao Pan Tai Bai local rice cultivars, respectively. The 2AP content of these rice cultivars was lower than that of KDML 105 by approximately 1.30-fold and 195.50-fold, respectively. It was previously reported that the 2AP content of 56 local rice cultivars from Lao People's Democratic Republic (PDR) was found to be in the range of 0.074 to 0.688 μg/g[31]. Our results showed that the 2AP content in the Thai cultivars was higher than that in Lao PDR local rice by approximately 1.2- to 19.5-fold. The disparity in the calculated2AP content might be due to the various quantification methods used, the nature of the different rice cultivars, and differences in the cultivation regions.

2AP has been reported as a major contributor to the characteristic aroma compounds of colored rice[22]. In this study, we have identified the 2AP content of 13 colored local rice cultivars (**Fig. 1**) and found that the 2AP concentration was in the range of 0.11 ± 0.015 to 10.41 ± 0.920 μg/g. The 2AP content for colored cultivars was relatively lower than that for non-colored cultivars (**Fig. 1**). Our findings agree with those from a study by Grimm *et al.*[32], who reported that the 2AP content of non-colored rice was higher than that of colored rice. For example, cultivars, such as Sierra rice and Dellrose rice, showed a higher 2AP content than Black forbidden rice and IAC600 rice, which are colored rice cultivars.

3.2 Fragrant and non-fragrant rice classification

2AP has been reported in both fragrant and non-fragrant rice cultivars, but is only found in small amounts in non-fragrant rice[8, 31, 33, 34]. 2AP is formed during L-proline metabolism, which is associated with the recessive from of the *badh2* gene[10, 12, 35]. We have classified 43 fragrant and non-fragrant local rice cultivars based on the 2AP content and presence or absence of this recessive *badh2* allele. Our results indicate that the 25 local rice cultivars classified as fragrant rice have a 2AP content in the range of 2.79 ± 0.560 μg/g to 13.47 ± 1.050 μg/g, while the 2AP concentration in18 non-fragrant rice cultivars is in the range of 0.09 ± 0.016 μg/g to 0.54 ± 0.042 μg/g. The difference between the highest 2AP content (0.54 ± 0.042 μg/g) of non-fragrant rice and the lowest 2AP content (2.79 ± 0.560 μg/g) of fragrant rice is approximately 5.16-fold. A similar result was observed for the 2AP content of 56 rice cultivars from Lao PDR, with the highest 2AP content (0.074 μg/g) of non-fragrant rice measuring approximately 5.40-fold less than the lowest 2AP content (0.405 μg/g) of fragrant rice[31]. Prior studies have shown that the 2AP content of 13 Indian local rice cultivars differed approximately by 1.92-fold between non-fragrant and fragrant rice[34]. Furthermore, in two local rice cultivars, Khao Luang Pra-Tan and Khao Hom Nin, both classified as non-fragrant rice, the 2AP content could not be detected. Previously published studies have reported that the 2AP content cannot be detected in some other types of non-fragrant rice, such as IR-64 rice[23]. In addition, Grimm *et al.* reported that the 2AP content could not be detected in 6 non-fragrant rice cultivars, including Drew, Giant Embryo, Watermaid, Uncle Ben's, Bhutanese Red, and Himalayan Red32. Rice has been classified as fragrant or non-fragrant, based on the presence of the recessive *badh2* allele, as first reported by Bradbury *et al.*[12]. In this study, KDML 105 rice was used as the standard fragrant rice cultivar, and the PCR product or the recessive *badh2* gene of this rice was approximately 110 base pairs (bp) (**Fig. 2**). Khao Gor Dieow exhibited a 2AP content of 0.54 ± 0.042 μg/g, and a PCR product measuring approximately 124 bp. The PCR productsfor Khao Gor Kor 6, Khao Pathum Thani 1, and Khao Nieow Gam were also approximately 110 bp, which was comparable to that for KDML 105 (**Fig. 2**). Based on the 2AP content of Khao Gor Dieow, which was the highest compared to that of other local rice cultivars including Khao Hang Yee 1, Khao In-Bplaeng, Khao Hang Yee 2, Khao Nieow Kieow-Ngoo, Khoa Leuang On, Khao Kao Yai, Khao Gam, Khao Yai, Khao Puan Glang, Khao Gam 101, Khao Rak Pai, Khao Jao Kao, Khao Pan Tai Bai, Khao Hom Surin, Khao Hom Nin, Khao Luang Pra-Tan (0.09 ± 0.016 μg/g to 0.54 ± 0.042 μg/g). Thus, the local rice cultivars with a 2AP content lower than that of Khao Gor Dieow might be expected to show a PCR product similar in size to that of Khao Gor Dieow. On the other hand, All three local rice cultivars (Khao Gor Kor 6, Khao Pathum Thani 1, and Khao Nieow Gam) had a 2AP content in the range for fragrant rice cultivars (2.79 ± 0.560 μg/g to 13.47 ± 1.050 μg/g). Therefore, other local rice cultivars with a 2AP concentration in this range could be expected to have a PCR product similar in size to that of KDML 105. Our findings are in agreement with those in the report of Bounphanousay *et al.*[31], who showed that the different sizes of the recessive *badh2* gene products could be used to classify the local rice cultivars from Lao PDR. The authors found that the molecular analysis results for all local rice cultivars correlated with the 2AP content, with

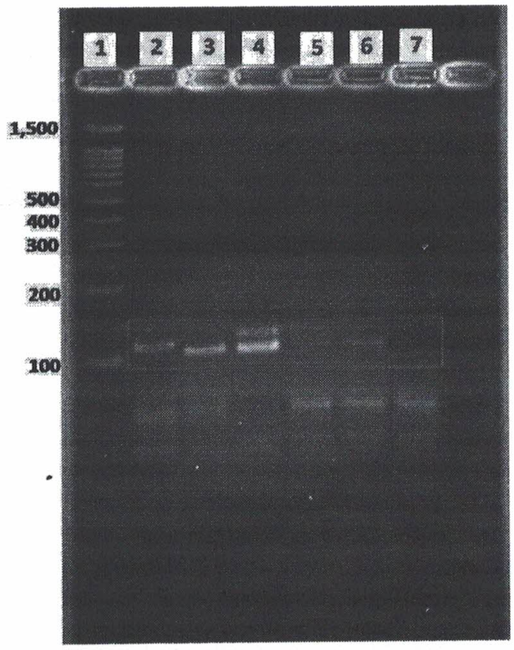

Fig. 2 . The 2.5% agarose gel of 5 rice cultivars. Lane 3; corresponding to standard fragrant rice cultivars KDML 105 with the PCR product approximately 110 bp. Lane 2; the PCR product of Khao Gor Dieow with the PCR product approximately 124 bp and this rice cultivars was classified as non-fragrant rice. Lane 4, 5 and 6; the PCR product of three rice cultivars including of Khao Gor Kor 6, Khao Pathum Thani 1 and Khao Nieow Gam and all three rice cultivars were classified as fragrant rice. Lane 7; indicated the negative control.

fragrant rice yielding smaller PCR products. The fragrant rice cultivars analyzed in this study showed a 2AP content similar to that of the standard fragrant rice. In contrast, non-fragrant rice yielded larger PCR products and had a 2AP content lower than that of standard fragrant rice. To investigate this further, the two sizes of PCR products for Khao Gor Kor 6 were analyzed, and the smaller product was found to be similar to that of KDML 105, while the larger product was similar in size to the PCR product of Khao Gor Dieow (Fig. 2). Khao Gor Kor 6 rice is generated from KDML 105 by mutations induced on exposure to gamma irradiation. The mutations in Khao Gor Kor 6 may affect the *badh2* gene, yielding a PCR product similar in size to those from non-fragrant rice (Fig. 2). The PCR product size for Khao Gor Kor 6 was correlated with its 2AP content, and a decreased 2AP content of Khao Gor Kor 6 was observed when compared to the 2AP content of KDML 105 (Fig. 1). Our results are concordant with those reported by Ahmadikhah *et al.*[36], who showed that the PCR products of *badh2* in mutant rice (the mutant rice were generated by crossing fragrant and non-fragrant genotype rice) were of two different sizes. The larger product was similar in size to the PCR product of non-fragrant rice, while the smaller product approximated the PCR product size of fragrant rice.

3.3 Correlation between proline content and 2-acetyl-1-pyrroline content

The proline content was determined for grains of fragrant and non-fragrant local rice cultivars (Table 1). The proline content ranged from 1.52 ± 0.09 to 4.64 ± 0.34 μg/g. Proline is the precursor for the 2AP synthesis pathway[4, 10, 18]. Our results indicate that the 2AP content was not correlated with the proline content of the grains of local rice. For example, Khao In-Bplaeng was classified as non-fragrant rice and has a high proline content, while KDML 105 was the standard fragrant rice but showed a low proline content. Thus, our results indicate that the accumulation of proline might not affect the accumulation of 2AP in grains of local rice. Previously published work has reported the association between proline content and 2AP content in rice. Poonlaphdecha *et al.*[20] found that the 2AP content increases and is correlated with the proline content under salt stress. Recently, Mo *et al.*[19] reported that an increased 2AP content in grains is correlated with a higher proline concentration under low light intensity conditions.

2AP is formed in rice grains by two mechanisms. 2AP can be synthesized from other parts of rice and can be translocated to the grain during grain formation. 2AP synthesis can occur inrice grains after translocation of the proline precursor from another part of the rice into the grains[19, 20, 23, 37]. 2AP accumulation may be affected by environmental factors. Yoshihashi *et al.*[28] reported that during grain formation ina drought-affected area, the 2AP content of rice grains was higher than that in areas not affected by drought. Proline content in rice can also be affected by environmental factors. In rice grains, proline accumulation depends on the stress factors in different areas of rice cultivation[17, 38]. Moreover, Aspinall *et al.*[13] reported that during grain formation, the proline content was maintained by its incorporation into proteins. The 2AP and proline content in grains of the 43 local rice cultivars analyzed in this study were obtained during the same harvest season but from different cultivation areas. The lack of a correlation between the contents of the two compounds in rice grains analyzed in this study may be explained by the differential accumulation of 2AP and proline in grains depending on environmental conditions in each cultivation area; in addition, during grain formation the proline residues were incorporated into proteins.

3.4 Aroma compounds of fragrant and non-fragrant rice

The volatile compounds present in 14 local rice cultivars (9 fragrant rice and 5 non-fragrant rice) were identified and are listed in Table 2. One hundred forty volatile compounds were identified. The number of volatile compounds

Table 1 Proline content obtained from the grains of fragrant (F) and non-fragrant (N) local rice cultivars.

Rice cultivar	Genotype	Proline content (μg/g)
Khao In-Bplaeng	N	4.64 ± 0.34^{a}
Khao Jao Hom Tung	F	3.91 ± 0.18^{b}
Khao Nieow E-Dtia	F	3.83 ± 0.33^{bc}
Khao Nieow Laao	F	3.68 ± 0.14^{bcd}
Khao Bplaa Chiw	F	3.65 ± 0.06^{bcdf}
Khao Hang Yee 1	N	3.59 ± 0.24^{cdf}
Khao Nieow Kieow-Ngoo	N	3.45 ± 0.07^{dfg}
Khao Jao 15	F	3.39 ± 0.23^{fgh}
Khao Puan Glang	N	3.27 ± 0.10^{gh}
Khao Sin Lek	F	3.13 ± 0.17^{hi}
Khao Jao Kao	N	2.97 ± 0.16^{ij}
Khao Piw Tong	F	2.91 ± 0.20^{ijk}
Khao Hang Yee 2	N	2.88 ± 0.12^{ijk}
Khao Jao Daeng	F	2.78 ± 0.08^{jk}
Khao Bpraa Jeen	F	2.66 ± 0.10^{kl}
Khao Hom U-Dom	F	2.65 ± 0.24^{kl}
Khao Man Bpoo	F	2.64 ± 0.08^{klm}
Khao Pa-Maa Leuang	F	2.45 ± 0.06^{lmn}
Khao Pathum Thani 1	F	2.44 ± 0.29^{lmn}
Khao Leuang Noi	F	2.39 ± 0.08^{lmno}
Khao Gor Kor 6	F	2.35 ± 0.09^{mno}
Khao Gor Dieow	N	2.31 ± 0.10^{nop}
Khao Leum Pua	F	2.11 ± 0.11^{opq}
Khao Pan Tai Bai	N	2.07 ± 0.07^{pq}
Khao Wan	F	2.06 ± 0.20^{pq}
Khoa Leuang On	N	1.98 ± 0.08^{qr}
Khao Luang Pra-Tan	N	1.95 ± 0.07^{qr}
KDML 105	F	1.92 ± 0.04^{qr}
Khao Yai	N	1.85 ± 0.08^{rs}
Khao Rak Pai	N	1.76 ± 0.14^{rs}
Khao Soh Maalee	F	1.75 ± 0.07^{rs}
Khao Kao Yai	N	1.52 ± 0.09^{s}

Values are means ± standard deviation (n=3). Values in the same column with the same superscript letter are not significantly different (Duncan, $p >$ 0.05). F, means fragrant rice and N, means non- fragrant rice.

in non-fragrant rice cultivars was greater than that in fragrant rice cultivars (Fig. 3). The highest number of volatile compounds was obtained from Khao Mali Daeng, Khao Gam, and Khao Hom Nin rice cultivars, all 3 of which contain 53 compounds and are classified as non-fragrant rice. In contrast, the lowest number of volatile compounds (43) was obtained from Khao Jao 15 (fragrant rice). Our results contradict data from prior studies, such as the study by Hinge *et al.*[23], who found that the number of volatile compounds present in fragrant rice was higher than that in non-fragrant rice. In addition, Bryant *et al.*[39] reported that the number of volatile compounds in non-fragrant rice (Cocodrie) was similar to that in fragrant rice (Dellrose). Previously published studies and our results indicate

Table 2 Volatile compounds of fragrant and non-fragrant rice cultivars which analyzed from rice grain.

No.	Compounds	Retention time (min)	Peak Area (%)[2] Rice genotype I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
			F	F	F	F	F	F	F	F	F	N	N	N	N	N
1	2-Pentanone	14.615	1.6	3	–	–	–	–	–	–	–	–	–	–	–	–
2	Pentanal	14.656	–	–	–	–	–	1.12	–	–	–	1.17	–	–	–	–
3	Acetonitrile	16.047	2.5	1.5	–	–	1.15	–	–	–	–	–	1.28	–	1.35	1.26
4	Trichloromethane	17.426	6.5	–	4.24	2.75	3.87	2.28	3.46	3.66	5.33	1.83	15.73	2.98	15.07	15.55
5	Toluene	18.286	3.5	1.5	1.60	1.11	2.45	–	0.95	0.87	3.06	0.93	1.75	2.01	1.38	1.28
6	Hexanal	21.113	4.4	10.0	11.7	7.79	10.35	14.02	14.68	12.28	7.34	12.56	3.58	11.19	1.75	1.95
7	4-methyl-2-hexanone	23.396	0.9	4.8	–	1.79	2.16	0.87	0.94	–	2.47	–	0.93	0.53	–	–
8	Ethylbenzene	23.571	–	–	–	–	–	–	–	–	–	–	1.01	–	1.03	0.83
9	2-Pentanol	24.052	5.1	–	–	–	–	–	–	–	–	–	–	–	–	–
10	(R)-(-)-2-Pentanol	24.054	–	–	–	–	–	–	–	0.67	–	–	–	–	–	–
11	2-n-Butyl furan	24.163	–	1.0	–	–	1.15	0.73	0.80	0.56	–	–	–	0.92	–	–
12	1-Methoxy-2-propanol	24.200	0.9	–	–	–	–	–	–	–	–	–	–	–	–	–
13	o-Xylene	24.371	–	–	–	–	–	–	–	–	–	–	–	–	–	0.69
14	1,3-Dimethyl-benzene	24.402	–	–	–	–	–	–	–	–	–	–	0.75	–	0.77	–
15	2-Heptanone	27.047	0.7	0.9	0.94	0.86	1.88	1.74	1.11	1.28	2.97	2.37	–	1.51	–	–
16	Heptanal	27.163	1.2	1.5	2.18	1.56	1.55	1.54	2.54	1.77	1.60	1.91	1.14	1.59	0.83	0.57
17	Dodecane	28.021	1.0	0.8	0.67	0.87	0.73	0.77	1.01	0.46	1.05	0.72	2.00	0.69	1.69	1.54
18	2-Pentyl-furan	29.691	4.9	9.0	6.57	5.23	7.23	7.74	5.88	6.61	4.71	3.47	3.00	8.62	2.47	2.14
19	2,7,10-Trimethyl-dodecane	30.385	–	–	–	–	–	–	–	–	0.67	–	–	–	0.77	0.76
20	Styrene	30.916	6.1	3.0	0.89	0.85	1.94	0.96	1.01	0.82	3.28	0.62	12.81	1.58	16.34	16.54
21	1-Pentanethiol	30.987	–	1.7	1.57	2.01	–	–	1.67	1.22	1.60	–	–	1.84	–	–
22	1-Pentanol	30.998	–	–	–	–	1.94	1.79	–	–	–	2.02	–	–	–	–
23	2-Octanone	32.503	–	–	–	0.52	0.60	–	–	–	–	–	–	0.50	–	–
24	4-Methyl-2-hexanol	32.496	–	0.9	–	–	–	–	–	–	–	–	–	–	–	–
25	Octanal	32.669	1.7	2.1	3.40	3.08	2.63	2.65	3.61	4.17	2.17	3.15	1.40	2.76	1.22	0.86
26	2,6,11-Trimethyl-dodecane	33.097	–	–	–	–	–	–	–	–	–	–	–	–	0.63	0.44
27	Tridecane	33.194	1.8	1.4	0.85	1.38	0.85	0.81	1.10	0.76	1.16	0.66	5.49	0.94	5.16	4.48
28	(Z)-2-Heptenal	34.379	–	2.2	1.83	1.94	1.56	1.96	2.32	2.25	1.19	1.98	1.02	1.73	–	0.70
29	(E)-2-Heptenal	34.387	1.3	–	–	–	–	–	–	–	–	–	–	–	–	–
30	(2R,4R)-2,4-Dimethylheptan-1-ol	34.391	–	–	–	–	–	–	–	–	–	–	–	–	0.95	–
31	2-hexyl-furan	34.732	–	–	–	–	–	–	–	0.68	–	–	–	–	–	–
32	2,6,10-Trimethyl-dodecane	35.073	–	–	–	–	–	–	–	–	–	–	1.42	–	–	–
33	2,2,4,4,6,8,8-Heptamethyl-nonane	35.073	–	–	–	–	–	–	–	–	–	–	–	–	0.92	–
34	6-Methyl-5-hepten-2-one	35.079	1.4	1.7	1.26	1.00	0.83	0.79	0.94	0.61	1.19	0.79	–	0.58	–	0.75
35	5-(2-Methylpropyl)-nonane	35.645	–	–	–	–	–	–	–	–	–	–	0.69	–	–	–
36	2,6,10-Trimethyl-pentadecane	35.916	–	–	–	–	–	–	–	–	–	–	0.58	–	–	–
37	2,6,8-Trimethyl-decane	35.916	–	–	–	–	–	–	–	–	–	–	–	–	0.76	–
38	1-Hexanol	36.011	5.3	6.6	6.48	8.72	7.63	7.56	4.53	3.50	3.12	1.36	–	6.87	2.56	1.93
39	3-Methyl-tridecane	36.384	–	–	–	–	–	–	–	–	–	–	1.40	–	1.14	0.82
40	Carbonic acid, 2-ethoxyethyl 2-methoxyethyl ester	36.805	–	–	–	–	–	–	–	–	–	–	–	–	–	1.08
41	2-Nonanone	37.528	–	–	–	–	–	–	–	–	–	–	–	0.48	–	–
42	Nonanal	37.711	5.1	5.8	8.19	7.81	5.04	7.12	8.61	8.96	6.38	13.91	5.69	6.49	3.84	2.85
43	2-Methyl-tridecane	37.814	0.8	–	–	–	–	–	–	–	–	–	–	–	–	–
44	3-Methyl-tetradecane	37.818	–	–	–	–	–	–	–	–	–	–	–	–	–	0.57
45	6-Propyl-tridecane	37.821	–	–	–	–	–	–	–	–	–	–	–	–	0.92	–
46	5,5-Dibutylnonane	37.822	–	–	–	–	–	–	–	–	–	–	1.09	–	–	–
47	Tetradecane	37.908	1.7	1.5	1.34	1.60	1.34	1.30	1.96	1.21	1.77	–	4.08	1.69	4.63	0.69
48	2-Butoxy-ethanol	38.301	–	–	–	–	–	–	–	–	–	–	0.60	–	–	–
49	3,5-Octadien-2-ol	38.384	–	–	–	–	–	1.26	1.01	–	0.92	0.63	–	–	–	–
50	3-Octen-2-one	38.390	–	1.5	0.99	1.36	0.90	–	–	1.25	–	–	–	1.65	–	–
51	2-Decanone	39.024	–	–	–	–	1.06	0.45	–	0.57	–	0.99	–	–	–	–
52	6-Methoxy-2-hexanone	39.031	0.6	–	–	–	–	–	–	–	–	–	–	0.47	–	–
53	1-Methyl-4-(1-methylethyl)-cis-cyclohexanol	39.032	–	–	0.52	0.94	0.86	0.86	–	0.71	–	–	–	–	–	–
54	3-Furaldehyde	39.247	–	1.0	–	–	–	–	1.27	–	1.17	–	–	–	–	–
55	Furfural	39.249	0.9	–	0.90	0.60	0.57	0.55	–	0.67	–	1.05	0.98	0.84	0.93	0.85
56	(E)-2-Octenal	39.351	–	1.5	1.96	2.18	1.18	2.11	1.93	2.86	1.06	2.29	–	1.57	–	–
57	(E)-2-dodecenal	39.353	–	–	–	–	–	–	–	–	–	–	–	–	–	0.90
58	(Z)-3-Hexadecene	39.359	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–
59	(E)-9-Eicosene	39.367	–	–	–	–	–	–	–	–	–	–	0.92	–	–	–
60	Pentadecane	39.789	–	–	–	–	–	–	–	–	–	–	–	–	0.88	0.89
61	2-Hexyl-1-decanol	39.916	–	–	–	0.53	–	–	–	–	–	–	–	–	0.58	–
62	1-Tetradecene	39.923	0.8	–	–	–	–	0.55	–	–	–	–	–	–	–	–
63	3-Tetradecanol	39.926	–	–	–	–	–	–	–	–	–	–	0.55	–	–	–
64	Acetic acid	40.262	1.9	2.0	3.64	2.54	2.61	1.34	2.39	1.98	5.22	3.99	2.00	1.40	2.25	4.30
65	1-Octen-3-ol	40.342	2.7	3.4	2.82	3.06	3.78	4.11	3.35	2.84	2.78	2.75	1.79	3.02	1.75	1.42
66	1-Heptanol	40.635	–	1.5	1.32	2.11	1.34	1.62	1.51	1.15	1.05	1.73	–	1.67	–	–
67	5-Methyl-1-hexanol	40.639	1.6	–	–	–	–	–	–	–	–	–	–	–	–	–
68	2-Butyl-1-octanol	40.639	–	–	–	–	–	–	–	–	–	–	0.72	–	0.55	–
69	2-Ethyl-1-decanol	40.639	–	–	–	–	–	–	–	–	–	–	–	–	1.18	–
70	(R,S)-5-Ethyl-6-methyl-3E-hepten-2-one	40.950	0.6	–	0.75	–	0.60	–	–	–	–	–	–	–	–	–
71	2-Ethyl-1-hexanol	42.102	–	–	–	–	–	–	–	0.68	–	–	2.12	–	–	–
72	Undecyl-cyclopentane,	42.156	0.9	–	–	–	–	–	–	–	–	–	1.19	–	–	1.03
73	1-Hexadecanol	42.159	–	–	–	0.98	0.93	–	–	–	1.22	–	1.73	1.06	1.23	1.28
74	Nonyl-cyclopentane	42.160	–	–	–		–	–	–	–	–	–	–	–	1.64	–
75	Decanal	42.381	1.2	1.3	1.81	1.53	2.69	2.48	1.80	2.24	–	1.11	0.87	1.59	0.93	0.52

Table 2 Continued.

No.	Compounds	Retention time (min)	Peak Area (%)[2] Rice genotype													
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
			F	F	F	F	F	F	F	F	F	N	N	N	N	N
76	Benzaldehyde	43.403	4.6	7.7	3.55	2.62	3.03	3.54	6.19	5.26	8.51	4.70	3.62	3.26	4.02	4.61
77	4-Methyl-hexadecane	43.910	–	–	–	–	–	–	–	–	–	–	0.45	–	–	–
78	7-Methyl-pentadecane	43.911	–	–	–	–	–	–	–	–	–	–	–	–	0.69	–
79	2-Dodecenal	43.967	–	–	–	–	–	–	–	–	–	0.69	–	–	–	–
80	(E)-2-Nonenal	43.974	0.7	1.2	1.10	1.07	0.58	0.84	0.77	1.41	–	–	0.55	0.77	0.43	–
81	3,7-dimethyl-1,6-Octadien-3-ol	44.451	–	2.1	0.60	0.83	0.60	0.78	1.15	3.84	3.68	0.83	–	0.76	–	–
82	4-Ethyl-tetradecane	44.454	0.7	–	–	–	–	–	–	–	–	–	0.73	–	0.57	0.55
83	1-Octanol	44.967	1.8	2.2	2.02	2.94	1.79	2.44	2.21	1.52	1.59	3.85	1.19	2.36	1.70	1.05
84	3-Methyl-pentadecane	45.085	–	–	–	–	–	–	–	–	–	–	0.48	–	0.66	0.60
85	2-Dodecanol	45.773	–	–	–	–	1.49	–	–	–	–	–	–	–	–	–
86	2,3-Butanediol	45.781	–	–	–	1.59	–	–	–	–	–	–	–	–	–	–
87	Bornyl acetate	46.173	–	–	–	–	–	–	–	–	–	–	–	0.46	–	–
88	Isoborneol acetate	46.177	–	0.6	–	–	–	–	–	–	0.61	–	–	–	–	–
89	Isobornyl acetate	46.178	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–
90	2-Methyl-pentadecane	46.301	0.6	0.7	–	–	–	–	–	–	0.80	–	0.77	0.43	0.92	0.82
91	2-Methyl-heptadecane	46.307	–	–	–	–	–	–	–	–	–	–	0.72	–	0.83	0.75
92	Hexadecane	46.418	–	–	–	–	–	–	–	–	0.80	0.66	1.55	–	0.98	0.54
93	n-Nonylcyclohexane	46.734	–	–	–	–	–	–	–	–	–	–	–	–	–	0.57
94	(E)-2-Decenal	48.375	–	–	0.81	1.02	–	0.50	–	3.42	–	0.70	–	–	–	–
95	Acetophenone	48.661	–	–	–	–	–	–	–	–	–	–	0.59	–	0.80	0.93
96	2-Ethyl-2-methyl-tridecanol	48.864	–	–	–	–	–	–	–	–	–	–	0.74	–	–	–
97	1-Nonanol	49.045	1.9	1.5	0.81	2.11	1.25	1.59	1.31	1.00	0.92	1.50	0.81	1.00	1.33	0.81
98	Sulfurous acid, octyl 2-pentyl ester	49.209	1.6	–	–	–	–	–	–	–	–	–	–	–	–	–
99	2-Butyl-2-octenal	49.312	–	–	–	–	2.89	2.49	–	0.76	–	–	–	1.60	–	–
100	(Z)-3,7-Dimethylocta-2,6-dienal	49.871	–	1.2	–	–	0.53	–	–	–	0.82	–	–	–	–	–
101	6,10-Dimethyl-2-undecanone	50.057	–	–	–	–	–	–	–	–	–	0.56	–	–	–	–
102	(E,E)-2,4-Nonadienal	50.624	–	1.4	0.69	0.76	–	0.56	–	0.59	–	–	–	0.56	–	–
103	(Z)-3-Decen-1-ol	50.627	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–
104	2,4-Dodecadienal	50.627	–	–	–	–	–	–	0.78	–	–	–	–	–	–	–
105	1,2-Dimethoxy-Benzene	51.390	–	–	–	–	–	–	–	–	–	–	–	1.81	–	–
106	(E)-3,7-Dimethyl-2,6-octadienal	51.779	–	1.4	–	–	0.71	–	–	–	0.93	–	0.61	–	–	–
107	Pentanoic acid	51.939	–	–	–	–	–	–	–	–	–	0.54	–	–	–	–
108	Pentanoic acid, 3-hydroxy-2-methyl-, propyl ester	52.099	1.1	–	–	–	–	–	–	–	–	–	–	–	–	–
109	2-Undecenal	52.518	–	–	0.53	0.71	–	–	–	0.73	–	–	–	–	–	–
110	Methoxy-phenyl-oxime	52.694	1.4	–	1.77	1.37	1.10	1.55	1.86	1.01	2.67	2.23	–	0.89	–	–
111	3-Tridecanone	53.333	–	–	–	–	–	–	–	–	–	–	0.60	–	–	–
112	Hexanoic acid	55.796	1.9	2.3	3.32	4.37	5.05	3.77	3.64	3.11	3.95	5.22	1.93	6.48	1.07	2.14
113	trans-Geranylacetone	56.135	0.6	0.9	0.58	0.48	–	0.57	0.58	0.47	0.75	0.42	0.55	–	0.56	0.58
114	2-methoxy-phenol	56.311	–	–	–	–	–	–	1.29	–	–	–	–	–	–	–
115	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	56.631	–	–	0.96	1.12	0.66	0.77	0.79	0.47	–	0.63	–	–	–	–
116	Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	56.631	–	–	–	–	–	–	–	–	–	–	–	–	–	0.96
117	Benzyl alcohol	56.906	1.6	1.3	1.39	1.04	1.91	1.00	0.94	0.70	1.36	1.16	0.91	0.87	1.41	1.84
118	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	57.222	–	–	–	0.78	–	–	–	–	–	–	–	–	–	–
119	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	57.224	–	–	0.65	–	–	0.49	–	–	–	–	–	–	–	0.67
120	2-Methyl-2-pentenoic acid	58.267	0.9	–	–	–	–	–	–	–	–	–	–	–	–	–
121	2,3-Dimethyl-2-pentenoic acid	58.512	4.3	–	–	–	–	–	–	0.70	–	–	–	–	–	–
122	Heptanoic acid	59.207	–	–	–	0.80	0.64	0.54	–	–	0.80	0.64	–	0.69	–	–
123	1,2-Dimethoxy-4-chloro-benzene	60.361	–	–	–	–	–	–	–	–	–	–	–	0.46	–	–
124	Dihydro-5-pentyl-2(3H)-furanone	61.614	–	–	–	1.07	–	–	–	–	–	–	–	0.95	–	–
125	Octanoic Acid	62.272	0.7	0.8	0.83	2.09	1.18	0.98	1.02	1.18	1.20	1.58	0.54	1.50	–	–
126	6,10,14-trimethyl-2-Pentadecanone	64.010	1.3	1.6	1.01	1.34	1.05	0.86	1.20	0.91	1.80	1.02	0.54	0.48	0.84	0.91
127	Octasiloxane	64.510	–	–	–	–	–	–	–	–	–	0.47	–	–	–	–
128	Nonanoic acid	65.059	1.6	1.2	1.44	2.69	1.13	1.59	1.67	3.62	3.48	4.38	0.99	1.93	0.99	0.73
129	Hexadecanoic acid, methyl ester	66.296	–	–	–	–	–	–	–	–	–	–	–	–	–	0.58
130	n-Decanoic acid	67.642	–	–	–	–	–	–	–	0.62	0.62	0.48	–	–	–	–
131	2-Ethylhexyl salicylate	68.429	–	–	–	–	–	–	–	–	–	–	–	–	0.71	–
132	(R)-4,4,7a-Trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	69.872	–	–	0.61	–	–	–	–	–	–	–	–	–	–	–
133	Diethyl Phthalate	70.044	–	–	0.68	0.61	0.52	1.59	1.96	0.56	2.19	0.59	–	0.50	–	–
134	Butyl hexadecanoate	71.248	–	0.4	0.56	0.43	–	0.39	0.53	0.45	–	0.33	–	–	–	–
135	Dodecanoic acid	72.424	0.7	–	0.99	0.60	–	0.83	0.60	–	0.61	–	–	0.41		0.39
136	n-Hexadecanoic acid	73.365	–	–	5.28	–	–	1.96	–	–	–	2.56		1.97	1.67	2.78
137	Isovanillin	74.703	–	–	0.67	–	–	–	–	–	–	–	–	–	–	–
138	Vanillin	74.703	–	–	–	0.54	–	–	–	–	–	–	–	–	–	–
139	(Z,Z)-9,12-Octadecadienoic acid	77.772	–	–	–	–	–	–	–	–	–	1.26	–	–	–	–
140	Tetradecanoic acid	78.419	–	–	0.52	–	0.88	0.86	0.63	–	–	–	0.81	1.10	–	–

Note. Rice cultivars including, I; KDML 105, II; Khao Jao 15, III; Khao Mali Nin, IV; Khao Bpraa Jeen, V; Khao Bplaa Chiw, VI; Khao Gor Kor 6, VII; Khao Jao Hom Nin, VIII; Khao Pathum Thani 1, IX; Khao Sin Lek, X; Khao Mali Daeng, XI; Khao Gam, XII; Khao Gam 101, XIII; Khao Hom Surin, XIV; Khao Hom Nin. F; Fragrant rice, N; Non-fragrant rice. - ; absence of compounds

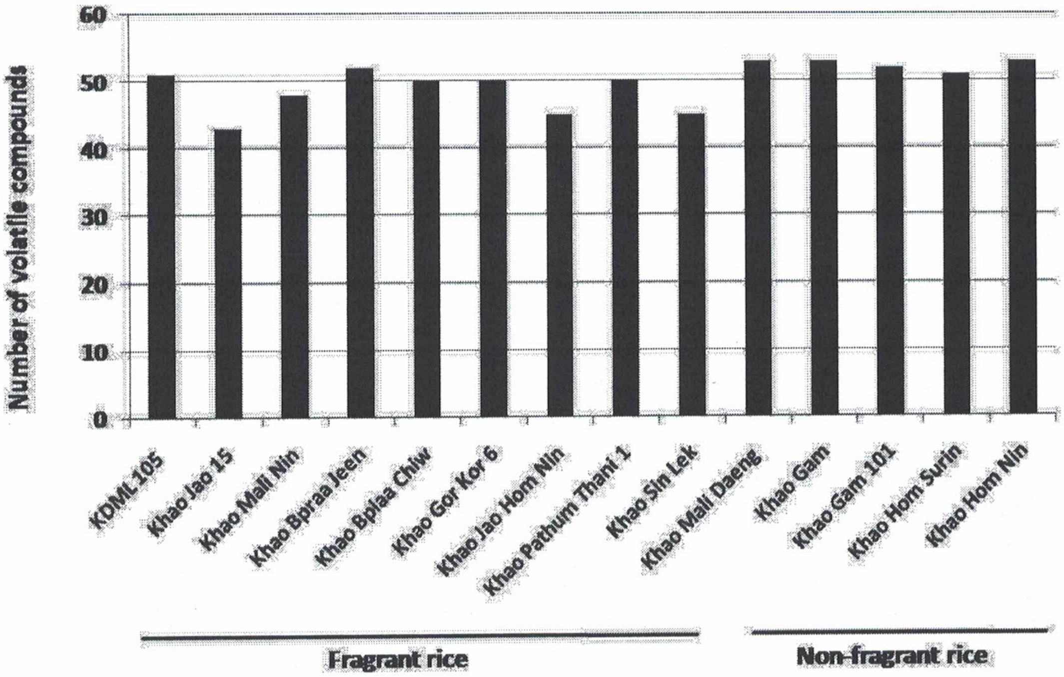

Fig. 3 The number of volatile compounds identified from 14 local rice cultivars which includes 9 fragrant rice and 5 non-fragrant rice.

that the number of volatile compounds might depend on the rice cultivars and area of rice cultivation. As was reported by Liyanaarachchi *et al.*[40], the volatile compound profiles of rice vary with the rice cultivars analyzed.

Among 140 volatile compounds, 17 compounds were observed in all rice cultivars, namely, hexanal; heptanal; dodecane; 2-pentyl-furan; styrene; octanal; tridecane; nonanal; acetic acid; 1-octen-3-ol; benzaldehyde; 1-octanol; 1-nonanol; hexanoic acid; benzyl alcohol; 6,10,14-trimethyl-2-pentadecanone and nonanoic acid. Twenty-three volatile compounds were observed only in fragrant ricecultivars, including 2-pentanol; (*R*)-(-)-2-pentanol; 1-methoxy-2-propanol; 4-methyl-2-hexanol; (*E*)-2-heptenal; 2-hexyl-furan; (*Z*)-3-hexadecene; 5-methyl-1-hexanol; 2-dodecanol; 2,3-butanediol; isobornyl acetate; sulfurous acid, octyl 2-pentyl ester; 2-butyl-2-octenal; (*Z*)-3-decen-1-ol; 2,4-dodecadienal; pentanoic acid; 3-hydroxy-2-methyl-, propyl ester; 2-methoxy-phenol; 2,2,4-trimethyl-1,3-pentanediol diisobutyrate; 2-methyl-2-pentenoic acid; (*R*)-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one; isovanillin and vanillin. In contrast, 34 volatile compounds were observed only in non-fragrant rice cultivars, namely, o-xylene; (2*R*,4*R*)-2,4-dimethylheptan-1-ol; 2,6,10-trimethyl-dodecane; 2,2,4,4,6,8,8-heptamethyl-nonane; 5-(2-methylpropyl)-nonane; 2,6,10-trimethyl-pentadecane; 2,6,8-trimethyl-decane; carbonic acid, 2-ethoxyethyl 2-methoxyethyl ester; 2-nonanone; 3-methyl-tetradecane; 6-propyl-tridecane; 5,5-dibutylnonane; 2-butoxy-ethanol; (*E*)-2-dodecenal; (*E*)-9-eicosene; 3-tetradecanol; 2-ethyl-1-decanol; nonyl-cyclopentane; 4-methyl-hexadecane; 7-methyl-pentadecane; 2-dodecenal; bornyl acetate; n-nonylcyclohexane; 2-ethyl-2-methyl-tridecanol; 6,10-dimethyl-2-undecanone; 1,2-dimethoxy-benzene; pentanoic acid; 3-tridecanone; propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester; 1,2-dimethoxy-4-chloro-benzene; octasiloxane; hexadecanoic acid, methyl ester; 2-ethylhexyl salicylate and (*Z,Z*)-9,12-octadecadienoic acid. The presence of specific volatile compounds in different rice cultivars may be related to the odor-active compound of that rice[8, 23, 39–41]. Although non-fragrant rice cultivars contain a small amount of major aroma compounds, such as 2AP, some volatile compounds, such as 2-butoxy-ethanol, are only found in non-fragrant rice and are believed to contribute to its aroma intensities[40].

3.5 Volatile compound contribution to aroma intensities in Thai local rice

Among the detected volatile compounds, 2AP is well known as a major contributor to aroma intensities[8, 21, 23, 39]. Our results showed the presence of 2AP in all rice cultivars except Khao Luang Pra-Tan and Khao Hom Nin. Moreover, a higher 2AP content was recorded in fragrant than in non-fragrant rice. Along with 2AP; hexanal; octanal; nonanal; 1-octen-3-ol; benzaldehyde and 1-nonanolwere detected in all 14 local rice cultivars and were major contributors to the aroma intensities of rice grains. Although the major aroma compounds were identified in both fragrant and non-fragrant rice, a higher content (peak area (%)[2]) of aroma compounds was measured infragrant rice compared to non-fragrant rice (**Table 2**). Mathure *et al.*[42] also reported that octanal and nonanal levels in Basmati rice are higher than in non-fragrant rice. Hexanal; octanal; nonanal; 1-octen-3-ol and benzaldehyde were recorded at higher levels in fragrant than in non-fragrant rice[23]. In addition, volatile compounds, which were responsible for aroma intensities, were present in greater amounts in colored than in non-colored rice (**Table 2**). Ajarayasiri *et al.*[43] found that the level of aroma compounds in black glutinous rice was higher than that in white glutinous rice. (*E*)-2-heptenal; isovanillin and vanillin, three other volatile compounds that contribute to aroma intensities, were only recorded in KDML 105, Khao Mali Nin, and Khao Bpraa Jeen, respectively. Previously published studies have reported that these three compounds contribute to the aroma intensities of fragrant rice[21, 34, 41]. Nine aroma compounds, namely, 1-pentanol; 6-methyl-5-hepten-2-one; 1-hexanol; 2-butoxy-ethanol; (*E*)-2-octenal; 1-tetradecene; decanal; (*E*)-2-nonenal and benzyl alcohol, were detected in fragrant and non-fragrant rice, but were more abundant in fragrant rice (**Table 2**). Previously published studies have also reported aroma compounds similar to those identified in our study; these aroma compounds are abundantly found in fragrant rice, such as Basmati rice (Indian fragrant rice), Suwandal, and Kuruluthuda rice (Sri Lanka fragrant rice)[23, 40].

4 CONCLUSION

The 2AP content of 43 Thai local rice cultivars was measured and compared with that of KDML 105. All Thai local rice cultivars were found to contain lower 2AP levels than KDML 105. The Khao Jao 15 rice cultivar had a 2AP content comparable to that of KDML 105. The 43 Thai local rice cultivars were identified as 25 fragrant and 18 non-fragrant rice cultivars, based on the 2AP content and molecular characterization. The proline content of Thai local rice grains was analyzed, and the results indicated that proline content was not correlated with 2AP content. This lack of correlation may be explained by differences in environmental stresses among the various area of rice cultivation. One hundred forty volatile compounds were recorded in Thai local rice cultivars. Among these, 18 volatile compounds were found to correlate with the aroma intensities of rice, including hexanal, 1-pentanol, octanal, (*E*)-2-heptenal, 6-methyl-5-hepten-2-one, 1-hexanol, nonanal, 2-butoxy-ethanol, (*E*)-2-octenal, 1-tetradecene, 1-octen-3-ol, decanal, benzaldehyde, (*E*)-2-nonenal, 1-nonanol, benzyl alcohol, isovanillin, and vanillin. The 18 aroma compounds were observed in both fragrant and non-fragrant rice cultivars, but were more abundant in fragrant rice. These results may inform the development of local rice cultivars that have a high potential in the rice market. Our findings can also serve as a database for fragrant and non-fragrant local rice genotype conservation.

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Conflict of interest

The authors declare no any conflict of interest.

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