

[Short Report]

## Sensory Test for Aroma and Quantitative Analysis of 2-Acetyl-1-Pyrroline in Asian Aromatic Rice Varieties

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**Key words** : 2-acetyl-1-pyrroline (2-AP), Aromatic rice, Quantitative analysis, Sensory test.

Aromatic rice (*Oryza sativa* L.) that emits a special flavor when cooked is one of important characteristics of rice. At present, the aromatic rice cultivars grown commonly in Southeast Asia have being attached special importance for satisfying the diverse demands of rice consumers and gained wider acceptance in the Middle East, Europe, Australia and the United State of America. Due to the aroma and other characteristics such as grain appearance and eating quality, aromatic rice varieties are highly favored and command higher prices in markets domestically and internationally.

Aroma of rice is caused by a number of different compounds (Widjaja et al., 1996). However, Buttery et al. (1983) showed that 2-acetyl-1-pyrroline (2-AP), a high volatile compound, is a key compound responsible for the aroma of rice. 2-AP is most closely associated with the aroma flavor and presents in all parts of the plant (stems, leaves and grains) except roots (Buttery et al., 1983; Lorieux et al., 1996; Yoshihashi et al., 2002a, b). Yoshihashi et al. (2002b) also reported that 2-AP does not form during post-harvest or cooking processes of aromatic rice varieties, but it is synthesized in aerial parts of plants during growing in paddy fields. Therefore, aroma can be detected in both grains and leaf tissue.

Several methods were developed for the detection of aroma in different rice genotypes both quantitatively and qualitatively (Sood and Siddiq, 1978; Buttery et al., 1983; Berner and Hoff, 1986). At present, the sensory test for seeds and leaf tissue after reacting with solution of KOH or I<sub>2</sub>-KI (Tragoonrung et al., 1996) is still used as a convenient and appropriate method to evaluation of aroma (Lorieux et al., 1996; Garland et al., 2000). The aromatic character of rice in sensory test showed a strong correlation with the concentration of 2-AP (Ishitani and Fushimi, 1994). The concentration of 2-AP was analyzed by gas chromatography - flame

ionization Detector (GC-FID) (Buttery et al., 1986) or gas chromatography - mass spectrometry (GC-MS) (Tanchotikul et al., 1991). Recently, Yoshihashi et al. (2002a and b) also used gas chromatography - mass spectrometry - selected ion monitoring (GC-MS-SIM) with stable isotope dilution technique for quantifying 2-AP. The isotope-labeled compounds have almost exactly the same chemical properties as the original compound or naturally occurring isotopes, and differ only in mass.

The evaluation of aroma and quantitative analysis of 2-AP are important to understand the aromatic nature of rice. The present study was carried out to obtain more information on the aromatic nature of rice and to apply it to further analysis of aroma for aromatic rice breeding program. Sensory test and quantitative analysis of 2-AP concentration using the GC-MS-SIM method were applied to aromatic rice varieties.

### Materials and Methods

#### 1. Plant materials

Forty rice varieties collected from seven Asian countries (Table 1) were grown at Honmachi farm and greenhouse of Tokyo University of Agriculture and Technology (Japan). Leaf materials for the sensory test were collected at heading stage. For quantitative analysis of 2-AP compounds in rice grains, and for the sensory test of leaves, samples were collected from the plants grown under the same growing conditions but for the analysis of the grains of Myanmar varieties, the grains from the introduced source were directly used. The rough rice samples were stored at 5°C in sealed polypropylene bag before 2-AP analysis. Rice grains were hulled by using a rice huller (TR-200, Kett, Tokyo, Japan) and milled for 30 seconds using a friction type grain testing mill (Pearlest; Kett, Tokyo, Japan). Milled rice samples were ground with a 0.5-mm-mesh screen

Received 24 November 2005. Accepted 18 January 2006. Corresponding author: Y. Hirata (yhirata@cc.tuat.ac.jp, fax: +81-42-367-5625).

**Abbreviations** : 2-AP, 2-acetyl-1-pyrroline; GC-MS-SIM, gas chromatography - mass spectrometry - selected ion monitoring.

Table 1. Sensory test and quantitative analysis of 2-acetyl-1-pyrroline (2-AP) for aroma in aromatic rice varieties.

Variety	Origin	Growing and sampling condition	Sensory test		2-AP conc. (ppb) <sup>e</sup>
			Tasting <sup>c</sup>	Smelling <sup>d</sup>	
Bala Baghlan	Afghanistan	Honmachi Farm	Aroma	Aroma	0.0
Bala Doshi	Afghanistan	Honmachi Farm	Aroma	Aroma	0.0
Luke Andrab	Afghanistan	Honmachi Farm	Aroma	Aroma	0.0
Monda Laghman	Afghanistan	Honmachi Farm	Aroma	Aroma	0.0
Pashadi Laghman	Afghanistan	Honmachi Farm	Aroma	Aroma	95.8
Phkarum Doul	Cambodia	Greenhouse	Aroma	Aroma	254.4
Phkarum Check	Cambodia	Greenhouse	Aroma	Aroma	300.0
Phkarum Chang	Cambodia	Greenhouse	Aroma	Aroma	278.5
Senpidao	Cambodia	Greenhouse	Aroma	Aroma	69.4
Basmati 370-1 <sup>a</sup>	India	Honmachi Farm	Aroma	Aroma	118.4
Basmati 370-2 <sup>a</sup>	India	Honmachi Farm	Aroma	Aroma	148.1
Surjamkhi	India	Honmachi Farm	Aroma	Aroma	187.0
Izayoi	Japan	Honmachi Farm	Aroma	Aroma	94.7
Jakouine	Japan	Honmachi Farm	Aroma	Aroma	324.2
Oitakoutou	Japan	Honmachi Farm	Aroma	Aroma	387.2
Nga Kywe Taung Pyan	Myanmar	Greenhouse <sup>b</sup>	Aroma	Aroma	0.0
Nga Kywe Yin	Myanmar	Greenhouse <sup>b</sup>	Aroma	Aroma	7.8
Mee Done Hmwe	Myanmar	Greenhouse <sup>b</sup>	Aroma	Aroma	0.0
Mee Done Taung	Myanmar	Greenhouse <sup>b</sup>	Aroma	Aroma	0.0
Paw San Shwe War	Myanmar	Greenhouse <sup>b</sup>	Aroma	Aroma	31.3
Dawdam	Thailand	Honmachi Farm	Aroma	Aroma	231.6
Jasmine 85	Thailand	Honmachi Farm	Aroma	Aroma	212.0
Khao Dawk Mali 105	Thailand	Greenhouse	Aroma	Aroma	332.2
Gie Vang 2	Vietnam	Greenhouse	Aroma	Aroma	81.4
Hoa Lai	Vietnam	Greenhouse	Aroma	Aroma	0.0
Me Huong 2	Vietnam	Green House	Aroma	Aroma	156.0
MTL250-1 <sup>a</sup>	Vietnam	Honmachi Farm	Aroma	Aroma	132.1
MTL250-2 <sup>a</sup>	Vietnam	Honmachi Farm	Aroma	Aroma	0.0
Nang Huong 2	Vietnam	Greenhouse	Aroma	Aroma	0.0
Nang Tet 2	Vietnam	Greenhouse	Aroma	Aroma	48.5
Nang Thom Cho Dao2	Vietnam	Greenhouse	Aroma	Aroma	83.6
Nang Thom Duc	Vietnam	Greenhouse	Aroma	Aroma	37.3
Nang Thom Som	Vietnam	Greenhouse	Aroma	Aroma	12.9
Tau Huong 2	Vietnam	Greenhouse	Aroma	Aroma	1.5
Thanh Tra	Vietnam	Greenhouse	Aroma	Aroma	37.8
VD20	Vietnam	Honmachi Farm	Aroma	Aroma	430.7
IR64	Vietnam	Honmachi Farm	Non-aroma	Non-aroma	0.0
Tep Hanh mutant	Vietnam	Honmachi Farm	Non-aroma	Non-aroma	0.0
Koshihikari	Japan	Honmachi Farm	Non-aroma	Non-aroma	0.0
Nipponbare	Japan	Honmachi Farm	Non-aroma	Non-aroma	0.0

<sup>a</sup> Different samples obtained from one variety.

<sup>b</sup> Leaf samples were collected from greenhouse but grain samples were introduced grains themselves.

<sup>c</sup> Tasting of grains and/or cooked rice.

<sup>d</sup> Smelling of leaf tissue was enhanced by 1.7% KOH solution.

<sup>e</sup> 2-AP concentration (ppb) in milled rice.

(ZM-100; Retsch, Haan, Germany). Moisture content of ground rice samples was calculated after oven drying at 120°C for 2 hours.

## 2. Sensory test

Aromatic characteristic of the rice varieties were identified by tasting individual grains or cooked rice.

For the sensory test of leaf tissue, 1.7% KOH solution was applied to the tissue (Sood and Siddiq, 1978). One gram of the first green leaf blade at heading stage was cut into small pieces and put into Petri dishes with 5 mL of 1.7% KOH solution at room temperature. After 30 minutes, the dishes were opened and immediately smelled. The presence or absence of aroma was scored. Each individual sample was evaluated by five persons.

### 3. Extraction of 2-acetyl-1-pyrroline

The extraction vials used were 12×32 mm and closed with a PTFE septa and screw caps. 2-acetyl-1-pyrroline was extracted from 100 mg of ground rice samples using 400 mL 100% ethanol containing 200 ppb 2-acetyl-(methyl-<sup>13</sup>C)-1-pyrroline as an internal standard for 2h at 70°C. After centrifuging at 12,000 rpm, for 15 minutes at 4°C, 150 μL of supernatant was subjected to GC-MS-SIM analysis.

### 4. Gas chromatography - mass spectrometry - selected ion monitoring (GC-MS-SIM)

The concentration of 2-AP was quantified by the GC-MS-SIM method with a stable isotope dilute technique according to the method of Yoshihashi et al. (2002b). The extract (2 μL) was injected into a fused silica capillary column (DB-WAX; 60 m×0.25 mm I.D.×0.25 μm film thickness; J&W Scientific, Folsom, CA) installed in Hewlett-Packard (HP) 5980 series 2 gas chromatograph (Palo Alto, CA). Helium gas (purity 99.9999%, passed through a molecular sieve and an oxygen trap) with a head pressure set as 40p.s.i was used as the GC carrier gas. The injector and the GC-MS interface temperatures were set at 150°C and 250°C, respectively. The column temperature was maintained isothermally at 40°C for 2 min, and then temperature was increased at a rate of 10°C min<sup>-1</sup> to 100°C and was increased further to 140°C at a rate of 5°C min<sup>-1</sup>. After this program, the column temperature was maintained isothermally at 250°C for 10 minutes. An HP 5989A mass spectrometer was used in the electron ionization mode with the ion source temperature set at 250°C, the analyzer temperature was set at 100°C, and ionization energy at 70 eV. SIM was set up to monitor m/z 111 for 2-acetyl-1-pyrroline and m/z 112 for 2-acetyl-(methyl-<sup>13</sup>C)-1-pyrroline. MS detection dwell time was 100 ms for each ion. Under these conditions, the retention times of these compounds were 12.47 and 12.46 minutes, respectively. Quantification was performed by measuring the area ratios between ions at m/z 111 and 112, corresponding to 2-acetyl-1-pyrroline and 2-acetyl-(methyl-<sup>13</sup>C)-1-pyrroline, respectively (Fig. 1). The amount in samples was calculated from a calibration curve of various concentrations of synthetic 2-acetyl-1-pyrroline against the internal standard 2-acetyl-(methyl-<sup>13</sup>C)-1-pyrroline.

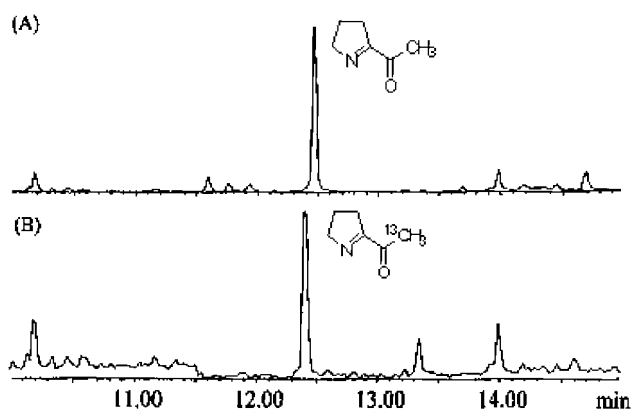


Fig. 1. Typical GC-MS-SIM chromatograms of the extract obtained from aromatic milled rice. SIM was set up to monitor m/z 111 for 2-acetyl-1-pyrroline (A) and m/z 112 for 2-acetyl-(methyl-<sup>13</sup>C)-1-pyrroline (B). MS detection dwell time was 100ms for each ion. Quantification was performed by measuring the area ratios between ions at m/z 111 and 112. The amount of 2-acetyl-1-pyrroline in samples was calculated from a calibration curve for various concentrations of 2-acetyl-1-pyrroline against 2-acetyl-(methyl-<sup>13</sup>C)-1-pyrroline. Since (A) and (B) shows same profile, more sensitive and accurate measurement can be made by using isotope 2-acetyl-(methyl-<sup>13</sup>C)-1-pyrroline on a small scale.

## Results and Discussion

Thirty-six out of 40 rice varieties studied were aromatic rice varieties screened at collected areas by tasting the individual grain or cooked rice (Table 1). Then, the classification was confirmed by a sensory test using rice leaf tissues in a 1.7% KOH solution although there was a distinct difference in strength of aroma recognized among aromatic rice varieties. This indicated a close relationship between the evaluation of aroma with the grains and that in the leaves.

The concentration of 2-AP evaluated by GC-MS-SIM analysis varied among aromatic rice varieties (Table 1). All four non-aromatic rice varieties, IR64, Tep Hanh mutant, Koshihikari and Nipponbare did not contain 2-AP, and 2-AP concentration of 36 aromatic rice varieties ranging from 0 to 430.7 ppb. The concentration of 2-AP was 0 ppb in 9 aromatic varieties Bala Baghlan, Bala Doshi, Luke Andrab, Monda Laghman, Nga Kywe Taung Pyan, Mee Done Hmwe, Mee Done Taung, Hoa Lai, MTL250-2 and Nang Huong 2. However, some other varieties such as Phkarum Doul, Phkarum Check, Phkarum Chang, Jakouine, Oitakoutou, Daw Dam, Jasmine 85, Khao Dawk Mali 105 and VD20 showed extremely high concentration of 2-AP (212.0 – 430.7 ppb). Thus, the concentration of 2-AP determined by GC-MS SIM analysis did not correlate with evaluation by sensory test.

Twelve aromatic rice varieties contained less than

10 ppb or no 2-AP (0-10 ppb) suggested that the same factors other than 2-AP influenced the strength of aroma in rice. There are several possibilities: (1) applied quantification method of GC-MS-SIM is limited, (2) some varieties classified as aromatic rice by mistake, (3) storage conditions after harvesting were different, (4) cultivation conditions were not the same and (5) there are different genetic factors controlling aroma production or unfavorable combination of these factors to 2-AP. The first possibility is eliminated because quantitative analysis of 2-AP in aromatic rice varieties by GC-MS-SIM method was applied successfully in some earlier researches (Yoshihashi et al., 2002a, b; Itani et al., 2004). Moreover, 2-AP concentrations of the aromatic rice varieties Khao Dawk Mali105, Jasmine85 and Basmati 370, which are typical aromatic rice varieties were reasonably expressed. The second possibility is eliminated because the aromatic characteristic of the analyzed aromatic rice varieties were confirmed by two estimation methods of tasting by individual grains and/or cooked rice and smelling by leaf tissue. The third possibility is also eliminated because the rice varieties were harvested at the same time and the post-harvest processes such as drying and storage were also the same for all varieties. The difference in 2-AP concentration between MTL250-1 (13.21 ppb) and MTL250-2 (0.0ppb), which belong to the same variety, may be ascribed to the difference in the lines. It is probable that they are genetically different types of the same variety, caused by segregation or genetic modification or mutation of aroma trait.

The strength of aroma might be influenced by cultivation conditions since some aromatic rice varieties that are sensitive to photoperiod were grown in a greenhouse instead in paddy field. The concentration of 2-AP in rice is influenced by environmental conditions (Yoshihashi et al., 2002a, 2004; Itani et al., 2004). Therefore, aroma strength in some aromatic rice cultivars might be decreased and/or absent because they were grown outside the original cultivation area.

Another possibility is that the key compound forming aroma in rice is not 2-AP alone. 'Aroma' flavor in rice may be produced by integrated expression of more than one volatile compound, among which 2-AP is a major component. In some cases, 2-AP may be interfered or inhibited by some compounds in its biosynthesis process. The expression of the aroma was influenced by the substrates and precursors of 2-AP and/or the modifying influence of other secondary metabolism resulting in the accumulation of 2-AP. Due to the technical difficulty of small-scale measurement, the function of the gene(s) related to 2-AP synthesis and the biosynthesis metabolism or pathway of this volatile compound have not been studied in detail.

The present study confirmed the previous findings that the aroma strength in rice is influenced by both genetic factors and the environmental conditions (Pinson et al. 1994; Yoshihashi et al., 2004; Itani et al., 2004). Thus, it is important to elucidate both genetic and environmental factors that may influence aroma nature for production and breeding of aromatic rice varieties.

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