

## RESEARCH ARTICLE

# Volatile profiles of traditional aromatic rice varieties in Sri Lanka

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**Abstract:** The fragrance of rice grain and the flavour of cooked rice are important quality factors that influence consumer acceptability. The principal volatile compound that contributes the fragrance in rice is 2-acetyl-1-pyrroline (2AP). Brown rice samples of six Sri Lankan rice varieties, including some traditional aromatic ones were analyzed for fragrant volatile compounds by gas chromatography (GC). Simultaneous steam distillation and solvent extraction methods were compared in the process of analysis. Leaf extracts of *Pandanus latifolius*, a major source for naturally occurring 2AP, was used in GC peak enrichment technique to identify the GC peaks of the tested rice varieties. In addition to 2AP, other fragrant volatile compounds were also identified in the tested varieties. They were aldehydes such as hexanal, benzaldehyde and octanal, and alcohols such as 2-butoxy ethanol, octanol, benzyl alcohol, 1-pentanol, phenol, alpha terpineol and hexanol. The volatile profiles varied among the varieties and the majority of volatiles were detected in Lanka-Samurdhi and Suwandal. However, the highest 2AP was detected in Kuruluwee. The volatile compounds detected in rice bran and husk were not detected in the polished rice of Suwandal and *vice versa*. This study reveals that volatile profiling information of potential donor parents can be employed in aromatic rice breeding programmes and in quality assurance studies.

**Keywords:** 2-acetyl - 1-pyrroline, Sri Lanka, traditional aromatic rice, volatile compounds.

## INTRODUCTION

The demand for fragrant rice varieties has increased in the recent years in countries that consume rice as a major food, as consumers are willing to pay a premium price for it. In order to increase the varietal choice of rice, the rice breeders in Sri Lanka have been requested to develop

fragrant rice varieties suited to the local environment to minimize importation of the popular fragrant rice type, Basmati. Sri Lanka imports high quality Basmati type rice from Asian countries especially from Pakistan and India.

The volatile compounds in fragrant rice, which provide the characteristic aroma and flavour have been studied by a number of researchers and more than 100 volatile components have been identified in cooked rice (Yajima *et al.*, 1979; Tsugitha *et al.*, 1980; Tsugitha *et al.*, 1983). Yasumatsu *et al.* (1996) had found volatile carbonyl compounds such as acetaldehyde, propanol, 2-butanone, pentanal and hexanol in fragrant rice varieties and Buttery *et al.* (1986) had identified 2-acetyl-1-pyrroline (2AP) as the principal fragrant compound associated with aromatic rice. It contributes to an aroma similar to popcorn due to its much higher odour potency than the other volatile compounds in rice. The compound 2AP is present in many folds in *Pandanus* species and sometimes the rice is cooked with a piece of *Pandanus latifolius* (Rampe) leaf. The concentration of 2AP in the *Pandanus* leaves is 10 times greater than milled aromatic rice and 100 times greater than milled non-aromatic rice (Buttery *et al.*, 1986). Basmati and Thai Jasmine are the two major rice types containing 2AP in the world market. Sri Lanka has several traditional rice varieties possessing the fragrant gene responsible for the 2AP production (Kottearachchi *et al.*, 2010). However, their volatile profiles and the extent of 2AP production have yet to be studied.

The volatile profile of a rice variety is important not only to use in rice breeding programmes, but also

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to assure the quality of whole grain or grain products in the market. Most of the volatiles that are produced through metabolic pathways are dependent on the variety, agronomic practices, storage conditions and post harvest handling etc. Therefore, volatile profiles may have the potential to mark the identity of the variety and to interpret the quality of rice.

Exotic rice varieties such as Basmati and Thai-Jasmine are less productive in the Sri Lankan environment due to poor adaptability while the traditional varieties are better adapted to the local environment. Therefore, it is important to analyze the volatile profiles of Sri Lankan traditional rice varieties so that they be used in breeding programmes for grain quality improvement and also in quality assurance studies.

## METHODS AND MATERIALS

### Rice samples

Rough rice samples were obtained from the 2011 harvest of commercially grown traditional varieties, Suwandal (CIC Agri Business Pvt. Ltd., Colombo), Kuruluthuda (Bio Foods Pvt. Ltd., Kandy), Kaluheenati (Bio Foods Pvt. Ltd., Kandy), a recently identified fragrant variety, Kuruluwee (Acc. No: 04903 of Plant Genetic Resources Center, Sri Lanka; grown in the Wayamba University of Sri Lanka) and an improved fragrant variety, Lanka-Samurdi (CIC Agri Business Pvt. Ltd., Colombo). Non-fragrant rice variety Bg352 (Seed Certification Service, Bathalagoda) was used as the negative control and an imported rice sample of Basmati purchased from a Sri Lankan supermarket was used for the comparison of volatile compounds. All rough rice samples were de-hulled to obtain brown rice. Suwandal brown rice was further milled to obtain bran. Suwandal polished rice, husk and bran were separately analyzed for rice volatiles.

### Authentic compounds

The volatile compounds of rice were identified by comparing the retention times of authentic standard compounds, [Hexanal (Sigma Aldrich, USA), limonene (BDH Chemical Ltd., England), 1-pentanol (BDH Chemical Ltd., England), hexanol (BDH Chemical Ltd., England), benzyl alcohol (Gupta & Company Ltd., India), benzaldehyde (Sigma Aldrich, USA), octanal (Sigma Aldrich, USA), indole (BDH Chemical Ltd., England), n-octanol (Sigma Aldrich, USA), 2-butoxy ethanol (BDH Chemical Ltd., England), linalool (BDH Chemical Ltd., England),  $\alpha$ -terpeneol (BDH Chemical Ltd., England)

and 2,4,6-trimethyl-pyridine (collidine; Sigma Aldrich, USA), which were selected from previous studies and they were provided in analytical grade by the Industrial Technology Institute (ITI), Sri Lanka.

### Extraction of volatile compounds

A rice sample of 250 g was steam distilled with 100 mL methylene chloride ( $\text{CH}_2\text{Cl}_2$ ) using the Likens and Nickerson (LN) apparatus (Schultz *et al.*, 1977). The distillation was carried out for 2 h, which was dried over anhydrous sodium sulfate.  $\text{CH}_2\text{Cl}_2$  extract was concentrated at 40 °C using rotary evaporator at 7.5 rpm.

Solvent extraction (SE) method was also performed using 1 g of ground rice to compare and detect the efficient extraction method. Rice samples were extracted using 2 mL of  $\text{CH}_2\text{Cl}_2$ , which was kept in a water bath at 80 °C for 2.5 h as reported by Bergman *et al.* (2000). The samples were extracted 3 times and rinsed with  $\text{CH}_2\text{Cl}_2$  before re-extraction. The  $\text{CH}_2\text{Cl}_2$  extract was concentrated by passing nitrogen gas.

### Gas chromatographic (GC) analysis

The composition of essential volatile compounds was determined by Shimadzu 14B gas chromatography equipped with the SUPELCOWAXTM10 fused silica capillary column with dimensions of 30 m (length)  $\times$  0.25 mm (internal diameter)  $\times$  0.25  $\mu\text{m}$  (film thickness) and flame ionization detector (FID). Samples were injected in split less mode (1  $\mu\text{L}$ ) with the starting temperature of 40 °C. Temperature was ramped up to 225 °C at the rate of 10 °C/min in an overall separation time of 40 min. GC carrier gas was argon and the flow rate was 1 ml/min. GC analysis was performed three times for each rice sample to check the presence of peaks.

### Identification of 2AP using *Pandanus latifolius* leaf extract

In order to identify 2AP in rice samples, *P. latifolius* leaf extract was used as a positive source as it contains many folds of 2AP (Buttery *et al.*, 1988). The extraction was performed using 1 g of ground fresh *P. latifolius* leaves by solvent extraction method with the same conditions stated above. The concentrated extract was analyzed using GC maintaining the same conditions as in the rice sample analysis. 2AP was identified in the samples by comparing the common peaks of rice and *P. latifolius* leaves of the GC chromatogram with the help of GC peak enrichment technique, using collidine as an internal standard as mentioned in literature (Bergman *et al.*, 2000; Wakte *et al.*, 2010).

## Quantification of volatile compounds

Volatile compounds were approximately calculated by the percentage values obtained from the chromatogram with the assumption that the extraction obtained from CH<sub>2</sub>Cl<sub>2</sub> contained total volatiles equivalent to 100. Chromatogram values obtained from the blank solvent were reduced when the volatiles were calculated.

## RESULTS

### Comparison of volatile extraction protocols

The two extraction methods, steam distillation using the Likens and Nickerson (LN) apparatus and solvent extraction, were initially optimized to detect the best extraction protocol for rice volatiles. The extracts of Basmati and Suwandal rice samples were examined by both methods. It was assured that all the volatiles extracted by the LN apparatus were also extracted by the solvent extraction (SE) protocol (Table 1).

### Comparison of volatile profiles of traditional rice varieties

The volatiles that contribute to rice aroma are aldehydes, ketones, alcohols, and heterocyclic compounds. All listed compounds in Table 2, which were identified previously in exotic rice cultivars (Buttery *et al.*, 1988;

Tava & Bocchi, 1999; Bryant & McClung, 2010), were also identified in the tested traditional rice varieties.

The volatile profiles of seven rice samples were different in their composition. Among the thirteen tested compounds most of the volatiles were detected in Lanka-Samurdhi and Suwandal. The compounds found in Suwandal were 2-butoxy ethanol, octanol, benzyl alcohol, hexanal, limonene, 1-pentanol and 2AP. Five compounds; indole, limonene, benzaldehyde, benzyl alcohol and phenol were detected in Basmati. Bg352, the non-aromatic rice variety used as the negative control only contained phenol among the list of identified volatile compounds (Table 2).

Among the tested heterocyclic compounds, 2AP, the principal aromatic compound was recognized in rice using peak enrichment technique coupled with *Pandanus latifolius* extract (Figure 1). The highest 2AP level was detected in Kuruluwee (Table 2). Varieties such as Suwandal, Kuruluthuda and Lanka-Samurdhi showed 2AP contents of 4.5, 2.5 and 0.96 %, respectively. There was no 2AP present in Bg 352, which was a non-fragrant rice variety. A quantifiable amount of 2AP was not detected in Basmati that was obtained from the supermarket.

Among the tested aldehydes, hexanal, which is responsible for rancidity and off - flavour was present only in Suwandal (3.5 %) and Lanka - Samurdhi

**Table 1:** Comparison of extraction protocols of LN and SE methods

| Compound             | Retention time | *Odour description            | Basmati    |                 | Suwandal   |                 |
|----------------------|----------------|-------------------------------|------------|-----------------|------------|-----------------|
|                      |                |                               | LN extract | Solvent extract | LN extract | Solvent extract |
| Hexanal              | 4.1            | Green grass like              | n.d.       | n.d.            | +          | +               |
| Limonene             | 6.16           |                               | +          | +               | +          | +               |
| 1-Pentanol           | 7.71           | Sweet, heavy, strong balsamic | n.d.       | n.d.            | +          | +               |
| 2AP                  | 8.43           | Popcorn like aroma            | n.d.       | n.d.            | +          | +               |
| Hexanol              | 10.23          | Herbaceous                    | n.d.       | n.d.            | n.d.       | n.d.            |
| Benzaldehyde         | 14.63          | Nutty, bitter                 | n.d.       | n.d.            | +          | n.d.            |
| Linalool             | 15.51          |                               | n.d.       | n.d.            | n.d.       | n.d.            |
| Octanal              | 15.64          | Green citrus like             | n.d.       | n.d.            | n.d.       | n.d.            |
| n-Octanol            | 16.78          | Fruity, floral                | +          | +               | +          | +               |
| $\alpha$ - Terpineol | 19.28          |                               | n.d.       | n.d.            | n.d.       | n.d.            |
| Benzyl alcohol       | 23.33          | Sweet floral                  | +          | +               | +          | +               |
| Phenol               | 25.83          |                               | n.d.       | n.d.            | n.d.       | n.d.            |
| Indole               | 34.59          |                               | +          | +               | n.d.       | n.d.            |

+ : detected, n.d. : not detected

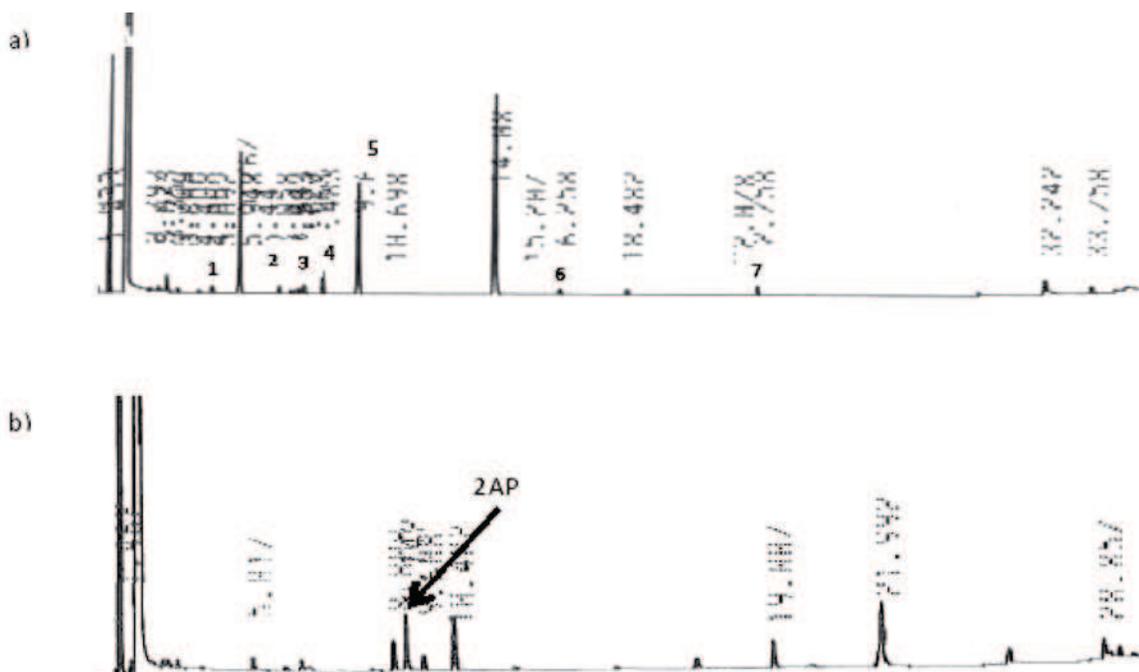
\* Source: Widjaja *et al.* (1996) ; Maga (1984)

**Table 2:** Percentages of volatile compounds detected in traditional rice varieties in comparison to that of aromatic and non - aromatic improved rice varieties

| Compound         | Peak no <sup>1</sup> | Variety <sup>2</sup> |      |      |      |      |      |      |
|------------------|----------------------|----------------------|------|------|------|------|------|------|
|                  |                      | Bas                  | Kuw  | Lan  | Kalu | Kur  | Bg   | Suw  |
| Hexanal          | 1                    | n.d.                 | n.d. | 0.46 | n.d. | n.d. | n.d. | 3.5  |
| Limonene         | 2                    | 1.9                  | n.d. | 10.6 | n.d. | n.d. | n.d. | 1.6  |
| 1-pentanol       | 3                    | n.d.                 | n.d. | 1.85 | n.d. | n.d. | n.d. | 1.4  |
| 2AP              | 4                    | n.d.                 | 9.6  | 0.96 | n.d. | 2.5  | n.d. | 4.5  |
| Hexanol          |                      | n.d.                 | n.d. | n.d. | 1.64 | n.d. | n.d. | n.d. |
| 2-butoxy ethanol | 5                    | n.d.                 | n.d. | n.d. | n.d. | n.d. | n.d. | 43.1 |
| Benzaldehyde     |                      | 3.3                  | 2.8  | n.d. | n.d. | n.d. | n.d. | n.d. |
| Linalool         |                      | n.d.                 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Octanal          |                      | n.d.                 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| n-octanol        | 6                    | n.d.                 | 5.4  | n.d. | 1.8  | 2.1  | n.d. | 1.8  |
| á-terpeneol      |                      | n.d.                 | 2.1  | 0.2  | n.d. | n.d. | n.d. | n.d. |
| Benzyl alcohol   | 7                    | 2.3                  | n.d. | 3.6  | 2.02 | n.d. | n.d. | 2.0  |
| Phenol           |                      | 1.4                  | 3.03 | 0.64 | n.d. | n.d. | 3.2  | n.d. |
| Indole           |                      | 3.6                  | n.d. | 0.3  | n.d. | n.d. | n.d. | n.d. |

<sup>1</sup> Peak numbers that represent peaks of chromatogram are shown in Figure 1

<sup>2</sup> Bas: Basmati, Kuw: Kuruluwee, Lan: Lanka-Samurdhi, Kalu: Kaluheenati, Kur: Kuruluthuda, Bg: Bg352, Suw: Suwandal  
n.d.: Not detected in quantifiable amount from the stated GC column

**Figure 1:** Chromatograms of a) Suwandal rice and b) *Pandanus latifolius*.

Note : Chemical compound corresponding to the peak number is indicated in Table 2.

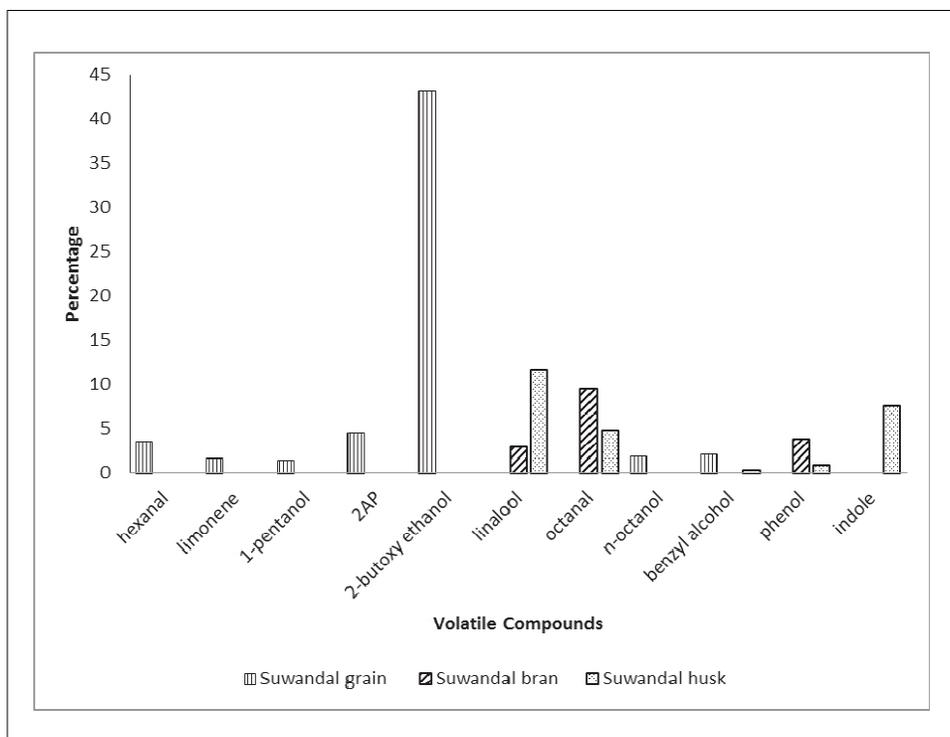


Figure 2. Percentages of volatile compounds detected in grain, bran and husk of Suwandal

(0.46 %) (Table 2). All the tested alcohols; 2 - butoxy ethanol (43.1 %), 1 - pentanol (1.4 %), octanol (1.8 %), and benzyl alcohol (2.0 %) except hexanol were detected only in Suwandal. Among the alcohols 2-butoxyethanol was the major alcohol detected in Suwandal; it was not detected in other tested varieties. The volatile compound 1-pentanol, which is responsible for the sweet heavy balsamic odour was detected in Suwandal rice and Lanka-Samurdhi while benzyl alcohol, responsible for the sweet floral odour, was detected in Suwandal (2.0 %), Basmati (2.3 %), Kaluheenati (2.02 %) and Lanka-Samurdhi (3.6 %). Octanol, which is responsible for sweet floral odour, was detected in all aromatic rice samples except Basmati and it was the highest in the cultivar Kuruluwee.

Among the tested terpenes, Lanka-Samurdhi contained the highest amount of limonene and it was 10.6 % of the total volatiles. Furthermore, Suwandal and Basmati also contained 1.5 and 1.9 % of limonene, respectively.

#### Comparison of volatile profiles of bran, husk and rice grain of Suwandal

Linalool, octanal and phenol were detected only in the Suwandal bran and husk in different percentages, but not in the grain. Indole and benzyl alcohol were present only

in Suwandal husk. However, all the other compounds including 2AP were present only in the rice grain (Figure 2). Suwandal husk contained a high amount of linalool (11.6 %) while the bran contained only 2.9 %. There was no linalool detected in Suwandal grain (Figure 2). Octanal was present in Suwandal bran and husk at the rates of 9.4 % and 4.8 %, respectively (Figure 2).

#### DISCUSSION AND CONCLUSION

Owing to the volatile nature of most of the aroma compounds, different protocols tend to show different recoveries over total yield. Due to the fact that all the volatiles extracted by LN apparatus were also extracted by solvent extraction (SE), the SE protocol was proven to be more efficient than LN due to comparatively smaller sample requirement and less time consumption. Hence, SE was selected for further analysis with all traditional rice varieties.

Suwandal and Kuruluthuda are popular fragrant traditional rice varieties among commercial growers of the country and they are sold at a higher price in local and international markets. They are mostly grown with organic farming techniques without the application of chemical pesticides and fertilizers. However, the highest 2AP level was detected in Kuruluwee, which has been an unexploited variety conserved at the Plant Genetic

Resources Center, Sri Lanka. Kuruluwee has not been commercially grown by Sri Lankan farmers to date. It was also recognized as a highly fragrant variety by a sensory evaluation study conducted by Wijerathna *et al.* (2012). According to this study, Kuruluwee contains two folds of 2AP as compared to Suwandal. As 2AP is considered as the principal volatile compound contributing to rice fragrance, Kuruluwee can be considered as a newly identified fragrant variety.

Basmati cultivars such as PusaBasmati, Basmati 370, Sugandha etc., are popular fragrant rice cultivars in the world market. However, the Basmati rice samples obtained from the supermarket did not show quantifiable amounts of 2AP. The reason would be that it has been milled and stored for a long period. It is very likely that milled rice lose volatiles as the grain is exposed to the environmental factors (Bergman *et al.*, 2000). It has been reported that some sellers adulterate Basmati by mixing long - grain low quality or non-aromatic rice (Steele *et al.*, 2008) and although sellers label the rice with the name of Basmati, the actual origin of the product is unknown. Therefore, this study suggests that volatile profiles are not only important to rice breeders for parental selection but also to mark the identity of a particular variety. Therefore, further studies on rice volatile profiles might be useful for the detection of market adulteration and rice quality.

Traceable amounts of linalool and octanal were not detected in any of the Sri Lankan rice varieties tested in this study. However, these volatiles were previously identified in exotic rice varieties by many researchers (Tsugita *et al.*, 1980; Buttery *et al.*, 1988). The presence of 2-butoxy ethanol, 1-pentanol, n-octanol, and benzyl alcohol in addition to 2AP may be the reason for the popularity of Suwandal among consumers since it is considered as the ideal for festive occasions. These compounds were mainly distributed in rice grain but not in bran and husk of Suwandal rice. However, the presence of some volatile compounds in the bran and husk reveal their prospective use in value addition processes of food items.

The present study reveals the potential use of local traditional rice varieties in breeding programmes in addition to the popular donor parent Basmati. Also this study provides information for the potential use of volatile profiles in instances where market adulteration of rice needs to be investigated.

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