ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 24, No. 1 (2023) 31 – 38

http://doi.org/10.21743/pjaec/2023.06.03

Aroma Component Analysis Using HS/SPME-FID Gas Chromatograph in Basmati Rice Varieties of Punjab, Pakistan

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Received 15September 2022, Revised 16February2023, Accepted 20February 2023

Abstract

Aroma is a promising quality factor for rice grain that impacts consumer acceptability. The principal volatile compound that adds Basmati rice fragrance is 2-acetyl-1-pyrroline (2AP). Milled White rice of 04 promising varieties i.e., Super Basmati, Basmati-515, Basmati 2000and Basmati 370 were evaluated for volatile compounds by gas chromatography (GC) coupled with Solid Phase Micro Extraction unit (SPME) using Flame Ionizing Detector (FID). Six volatile compounds (nonanal, decanal, and alcohols such as benzyl alcohol, indole) were identified in the tested varieties, among them 2-AP is only present in aromatic rice varieties. This study confirmed the occurrence of 2-AP in all studied varieties with highest concentration in Super Basmati followed by Basmati-515, Basmati 2000 and Basmati 370.

Keywords: Basmati rice, 2-acetyl-1-pyrroline, Aroma, Pakistan, Gas Chromatograph, SPME.

Introduction

Fragrant/ aromatic rice varieties demand has amplified in this decade worldwide. Scent is supreme feature in rice grain, especially where consumer approval is taken a benchmark. Currently, buyer are more conscious of the eminence of the rice consumed [1,2]. Recent world consumption data and scientific studies showed that in European countries demand for aromatic rice varieties increased, particularly Basmati due to emergent attention in Eastern ethnic cuisine. Premium quality Basmati rice only originated in Pakistan and India, and jasmine rice grown in Thailand get good market price. The tropical countries have their own scented rice varieties that are popularly consumed locally besides basmati [3, 4].

Compounds responsible for aroma in rice have been under study since previous 33 years. Several compounds were recognized in cooked and un-cooked rice. Preliminary rice selection decisions were made by eating individual grains or breathing the aroma of leaf tissue or grains either heating in water or retorting with KOH. This practice is not quantifiable and it allows classification of progeny only as scented, moderately scented, and non-scented. Presence of 2-AP has been an indicator of aroma in the selection of fragrant rice lines [5].

In 1965, ammonia, hydrogen sulfide along with acetaldehyde were found in the volatiles of heated rice. The biochemical

nature of the aroma complex remained unidentified till 1982, once Ron Buttery with his team of scientists from the US Department of Agriculture (Albany, CA) successfully recognized 2-acetyl-1-pyrroline (2AP) as the prime compound apt for the pleasing aroma in rice [6]. 2-AP [IUPAC tag: 5-acetyl-3,4-dihydro-2H-pyrrole, density 1090 kgm^{-3}, molecular weight 0.111145 kg mol^{-1}] has minute odor verge (0.1 μgkg^{-1}) so nominally detected by the human nose. It contributes popcorn like aroma in rice. In non-scented rice varieties, it is present at negligible levels reportedly 10 times lower than aromatic rice [4, 7, 8].

Rice aroma is interaction of numerous complex volatile compounds affected by pre- and post-harvest operations. The rapid advancement in the instrumentation and sampling methods for the isolation have made it possible to analyze compounds even at trivial concentrations (ppb levels). Extraction methods can be divided into traditional and modern. Traditional methods include purge and trap, steam distillation–solvent extraction, direct distillation and solvent extraction. Recent methods contain static headspace (SHS) followed by separation of aroma compounds using gas chromatography (GC) with flame ionization detector (FID) or mass spectroscopy (MS) and headspace–solid phase micro extraction (HS-SPME) [9, 10]. SPME is newest effective aroma analytical equipment combines sampling, extraction unified with liquid or gas chromatography. It has excellent repeatability and detection as compared to previous methods [11, 12]. Newly harvested aromatic rice varieties have robust smell but later successive operations i.e., milling, drying and storage decreases aroma. Lesser the milling degree, the greater the aroma absorbed in the milled rice [13].

Rice aroma component analysis is important to understand the aromatic nature of rice. Pakistan has quite a lot of traditional basmati rice cultivars retaining the fragrance. Nevertheless, their volatile profiles and the extent of impulsive compounds have thus far studied. This study may help to identity the variety and interpret the cooking quality of rice to relate it to further analysis for future rice breeding.

Methods and Materials

Rice varieties (Super basmati, Basmati-515, Basmati-2000, Basmati-370) were grown at experimental farm area of Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan under standard agronomic conditions. Paddy samples were harvested at 20% moisture content, dried to 11% moisture, de-hulled, polished with Lab polisher (SATAKE TRG058, Japan) from Milling Lab to obtain white rice [14].

Freshly milled white rice samples were grinded using UDY Cyclone mill to make fine powder. 4g of flour sample was immediately placed in 20mL glass vial. Prior analysis empty vials were heated in Hot air oven at 150^{o}C for 1 h to remove any unintended compound. After placing rice flour, vials were then instantly sealed with silicon septum/PTFE and crimp cap made with aluminum. Vial was well shaken at ambient temperature prior injection in GC [15].

The volatile compounds of rice were recognized by their different retention times from standard. Following standards were acquired from authentic manufacturer. i.e., Nonanal (Sigma Aldrich, USA), Decanal (Sigma Aldrich, USA), Vanillin (DAEJUNG, Korea), Benzyl Alcohol (Sigma Aldrich, USA), 2-acetylpyroline (Sigma Aldrich, USA), Indole (Supelco, Spain). Different dilutions of standards (0.1, 1.0, 2.0, 3.0, 4.0, 5.0 ppm and 10 ppm) were made in HPLC grade Toluene (Sigma Aldrich, USA).

Separation of volatiles mixture was achieved by Gas Chromatograph (Master GC with complete automation, DANI Instruments S.P.A, Italy) united by Flame Ionization Detector (FID), capillary column DN-WAX (30m×0.25mm×0.25µm) was used. Nitrogen gas used as carrier with flow rate of 1 mL/min. Initially 40°C column temperature was employed and then heated to 165°C @ 10°C/ min and then elevated to 250°C @ 15°C/min for 3min. The SPME fiber (57341-U) Supelco, Bellefonte, PA, USA) was desorbed in GC injector for 5 min having 250°C temperature in splitless mode. GC oven programme was hold for 1 min at 50°C to reach 100°C @ 4°C/min in increments then ramped to 240°C @ 50°C/min with ultimate hold of 2min. Identification of 2-AP and other selected volatiles was done by comparing the sample peak with standard solution peak. The vials were incubated in the auto sampler oven at 80°C, after 20 min the SPME fiber assembly was exposed to the headspace overhead the surface of the solid sample for 30 min at the same temperature. Extraction temperature set at 80°C, pre-incubation time at 10 min with adsorption period at 10 min was used for complete recovery. Area computation was used as extent of the comparing quantity during optimization.
Calibration graph for quantification of volatile compounds viz. 2-AP, decanal, nonanal, vanillin, benzyl alcohol and indole was plotted. Volatile compounds were calculated by the fraction values got from the chromatogram. Chromatogram values were subtracted from the blank solvent reading. Clarity chromatographic station software was used in this study.

Results and Discussion

The concentration of aromatic compounds evaluated by GC analysis varied among tested aromatic rice varieties. Identification of individual volatile constituents were based on comparison with standard. Six volatile compounds were separated from all tested varieties. The chromatograms are presented as Fig.1. a, b, c, d. Component detail with data is summarized in Table 1-4, as peak number, name of volatile compounds (Benzyl alcohol, Vanillin, Nonanal, Decanal, Indole and 2-AP) retention time (12.8-19.5 min), percentage peak area (548.38 mV.s) and concentration (ppm) of the identified compounds. Aroma in rice may be created by assimilated expression of many volatile compounds. The predominant compounds identified in this study were Benzyl alcohol, Vanillin, Nonanal, Decanal, Indole and 2-AP respectively in different range as discussed below.

Nonanal in food and feed is responsible for nutty flavor. Its concentration was highest in Super Basmati (14.06ppm) followed by Basmati 370 (12.52ppm) and Basmati 515. Indole exhibits musty,jasmine like flavor in food and feed.The highest concentration of Indole was present in Super Basmati and Basmati-370. Minimal traces of indole were detected in Basmati 2000. Though benzyl alcohol, responsible for the sweet floralodour, was identified in all varieties ranging 5.66 to10.67 ppm. 2-AP is the principal aromatic compound responsible for Basmati aroma, which was recognized in all studied varieties. The highest 2-APlevel was detected in Super Basmati, followed by Basmati 370 as depicted by Fig. 1. a, b, c, d.

It is apparent from Table 5, that Super Basmati contained highest quantity of all volatiles except indole and vanillin. Basmati -515 is leading aromatic cultivar of Punjab, Pakistan exhibiting notable content of 2-AP. Likewise Basmati-370, an old variety still grown in Pakistan, showed aroma volatiles in prominent quantity. Basmati-2000 contained all the scented contents with the exception of Indole content which might be a varietal character.

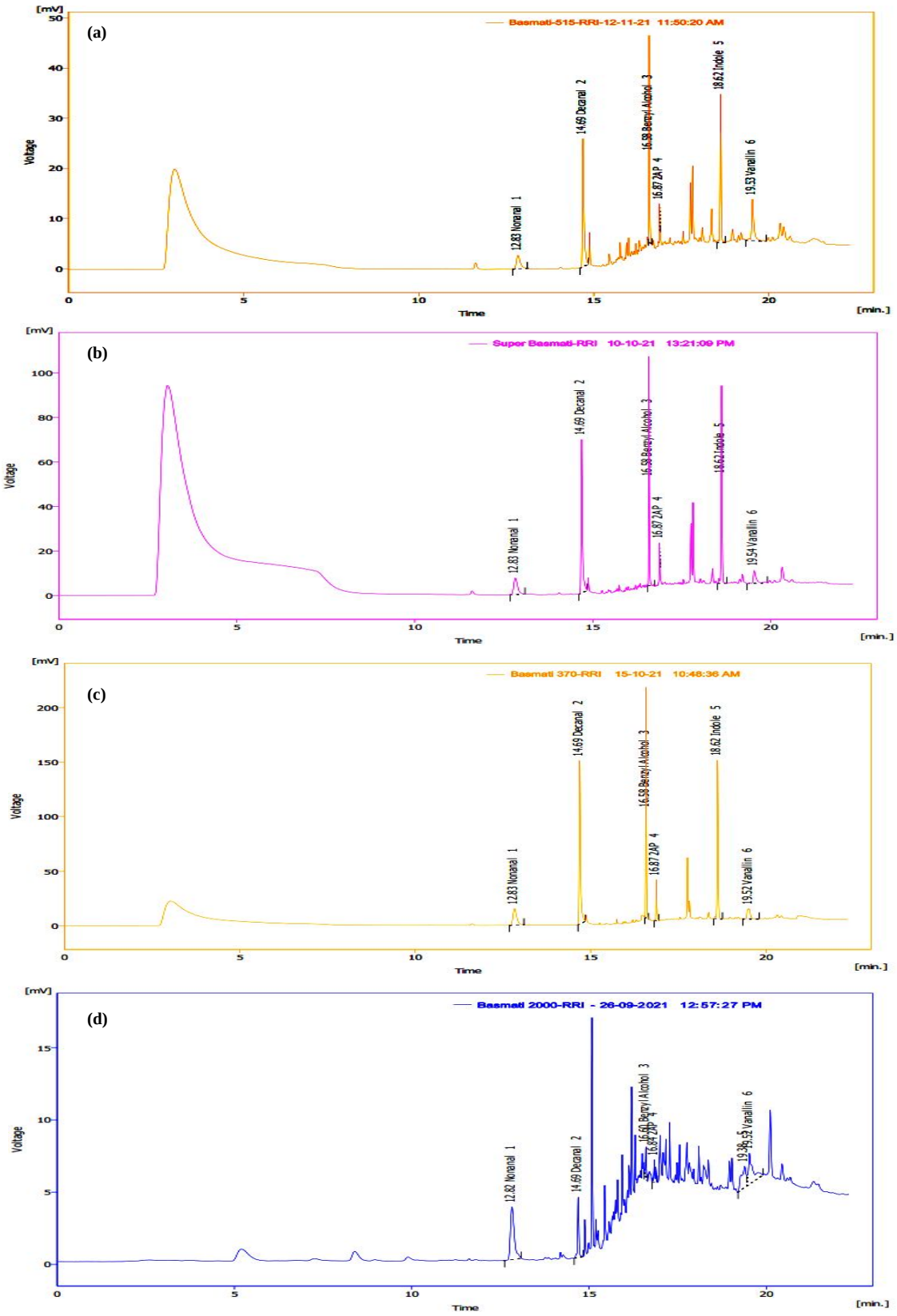

Figure 1. **a, b, c, d. Chromatogram of Basmati-515, Super Basmati, Basmati-370, Basmati-2000**

Table 1. **Basmati-515 aroma component analysis.**

	Retention Time (min)	Area (m V.s)	Concentration (ppm)	Peak type	Compound name
1	12.835	20.962	12.33	Ordnr	Nonanal
2	14.688	82.167	5.33	Ordnr	Decanal
3	16.576	60.325	9.25	Ordnr	Benzyl Alcohol
4	16.875	1.549	0.46	Ordnr	2AP
5	18.619	62.848	9.9	Ordnr	Indole
6	19.528	38.052	18.4	Ordnr	Vanillin
	Total	**265.902**	**45.67**	-	-

Table 2. **Super basmati aroma component analysis.**

	Retention Time (min)	Area (m V.s)	Concentration (ppm)	Peak type	Compound name
1	12.827	56.378	14.06	Ordnr	Nonanal
2	14.691	194.241	14.26	Ordnr	Decanal
3	16.576	150.125	16.04	Ordnr	Benzyl Alcohol
4	16.875	2.204	0.68	Ordnr	2AP
5	18.619	183.386	18.46	Ordnr	Indole
6	19.536	34.573	11.67	Ordnr	Vanillin
	Total	**620.907**	**75.17**	-	-

Table 3. **Basmati 370 aroma component analysis.**

	Retention Time (min)	Area (m V.s)	Concentration (ppm)	Peak type	Compound name
1	12.827	112.504	12.52	Ordnr	Nonanal
2	14.691	404.245	17.92	Ordnr	Decanal
3	16.576	301.525	10.67	Ordnr	Benzyl Alcohol
4	16.875	37.305	0.59	Ordnr	2AP
5	18.619	305.783	19.27	Ordnr	Indole
6	19.517	59.244	21.71	Ordnr	Vanillin
	Total	**1240.608**	**132.14**	-	-

Table4. **Basmati 2000 aroma component analysis.**

	Retention Time (min)	Area (m V.s)	Concentration (ppm)	Peak type	Compound name
1	12.824	27.839	12.33	Ordnr	Nonanal
2	14.693	11.077	5.33	Ordnr	Decanal
3	16.6	3.723	9.25	Ordnr	Benzyl Alcohol
4	16.84	3.816	0.46	Ordnr	2AP
5	18.619	0.987	9.9	Ordnr	Indole
6	19.523	18.69	18.4	Ordnr	Vanillin
	Total	**66.144**	**28.75**		

Table 5. **Aroma component analysis of basmati varieties of punjab, Pakistan.**

Sr. No.	Compound name	Basmati 515	Super Basmati	Basmati 2000	Basmati 370
1	Nonanal	12.38b	14.06a	11.60c	12.52b
2	Decanal	5.33d	14.26b	11.37c	17.92a
3	Benzyl alcohol	9.25c	16.04a	5.66d	10.67b
4	2-AP	0.46c	0.68a	0.43c	0.59b
5	Indole	9.90b	18.46a	0.00d	19.27c
6	Vanillin	18.40b	11.67d	14.69c	21.17a

Results are in line with research findings of many Indian scientists. Bryant*etal.* 2011 [16] documented hexanal (0.787 ppm), decanal (0.065 ppm), nonanal (0.242 ppm), benzyl alcohol (0.052 ppm), guaiacol (0.033 ppm) and vanillin (0.324 ppm) in Indian Basmati 370. Scented cultivars Basmati, Della Khao Dawk and Mali were evaluated for flavor quantification and 2-AP concentration in these varieties varied from 0.05 to 0.34 ppm [17]. In another study, [15] Jezussek *et al*. 2011 reported 0.122 to 0.411 ppm 2-AP concentration in 11 basmati varieties of India. Furthermore, 0.214 ppm was reported in Basmati 370 collected from market of Punjab, India in the same study. In a latest study, 152 volatile compounds were separated among the tested 12 rice cultivars using solvent extraction technique [18]. Besides about 65 volatiles identified in rice bran previously [19].

Another study [15] revealed that Basmati grown in parts of Karnataka state and Maharashtra excel in 2AP (0.122 to 0.411 ppm) on other area's Basmati. In another study slight 2AP contents were reported in black rice [20]. The majority of the volatile (heterocyclic) are prevalent in freshly harvested rice than stored [21]. The present study confirmed the prior findings that the aroma strength in rice is affected by variety selection and growing conditions mainly. Aromatic rice cultivars cannot adequately characterize just by 2-AP because other distinctive odors (e.g., earthy, nutty, roasty and green) are also demonstrating the expression [9, 22, 23]. In scented rice genotypes, 2AP can be spotted in all parts of the plant excluding roots [6] whereas in non-aromatic rice, 2AP is also found at a much lower concentration (0.0015 mg/kg) that cannot be professed easily [13].

Conclusion

Aroma profile analysis and quantification in Pakistani rice varieties was accomplished first time regarding the cultivars which was not reported earlier. We can apply it as targeted breeding approach in aromatic rice variety development as it is time-consuming and laborious to cultivate new varieties without aroma profiling. This study can be used for identification of aroma compounds of other non-basmati varieties for adulteration detection in export consignments.

Acknowledgment

Authors are thankful to all RRI scientists for execution of analysis workand field trial.

Conflict of Interest

There is no conflict of interest.

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