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RESEARCH ARTICLE

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Some factors affecting the concentration of the aroma compound 2-acetyl-1-pyrroline in two fragrant rice cultivars grown in South China

Abstract The fragrance potential of two fragrant rice (*Oryza sativa* L.) cultivars grown in South China was investigated in this study using headspace SPME and static headspace in conjunction with GC-MS. About a five-fold difference of 2-acetyl-1-pyrroline (2-AP) levels were observed among the two fragrant rice cultivars, with Guixiangzhan having the highest content ($3.86\ \mu g \cdot g^{-1}$) comparable to that obtained with Thai KDML 105 rice. Other compounds instead of 2-AP were assumed to contribute to the characteristic aroma of Peizaruanxiang. The two cultivars were subjected to two preharvest treatments (planting density and harvest date) and different storage conditions (3 to 6 months at −4, 8, 20, and 30°C). Results were discussed in terms of grain yield, milling quality, grain appearance, and amylose and protein contents of rice samples associated with differing treatments. Highest 2-AP concentrations were obtained for Guixiangzhan and Peizaruanxiang with lower planting densities, the earliest harvesting time of 10 days after heading, the shortest storage time of 3 months, and the lowest storage temperature of −4°C. These findings indicate that manipulating pre- and postharvest treatments can greatly improve the specific attributes of the domestically produced cultivars. With that in mind, China could effectively increase its share of the domestic market of fragrant rice and even tap into the international market.

Keywords fragrant rice, South China, 2-acetyl-1-pyrroline, planting density, harvest date, storage conditions

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1 Introduction

Fragrant rice is a general term used for rice cultivars that have a perfumed and nutty flavor. They are characterized by their unique popcorn-like aroma that is attributed mainly to 2-acetyl-1-pyrroline (2-AP) (Buttery et al., 1988; Widjaja et al., 1996; Champagne, 2008; Maraval et al., 2008; Yang et al., 2008a).

Despite being the largest producer of rice in the world, the pace of improvement of fragrant rice in China has been rather slow. This is partly because of the harsh situations in the early sixties when the country was facing deficit in food self-sufficiency. As a result, most rice development programs were focused mainly on increased production than grain quality improvement. The subsequent spread of hybrid cultivars with high yield (Yang, 2007) led to a sharp decline in the number of fragrant rice cultivars and their areas of cultivation. With the amelioration of living standards in mainland China, however, the fragrant rice demand is constantly increasing. Recently, some initiatives have been launched to identify and conserve whatever fragrant rice germplasm is left and to develop new cultivars suited to China's growing conditions and agronomic practices. In South China, one cultivar, namely, Guixiangzhan has dominated the domestic market for years and is believed by farmers and consumers to be highly aromatic because of the special flavor it emits when cooked. Unfortunately, its aroma chemistry has not been reported, and it is surely not the unchallenged kind of fragrant rice in the region. Many other indigenous cultivars of fragrant rice are cultivated in South China, and some new promising breeding lines like Peizaruanxiang have been developed. For a successful development of fragrant rice, research regarding factors affecting the quality of the aroma is of economic interest to rice growers and processors.

This study was conducted as a preliminary step toward the rice quality improvement of Guixiangzhan and Peizaruanxiang. First, the fragrance potential of the two rice cultivars was investigated using two related headspace sampling techniques, static headspace (SHS), and headspace solid phase microextraction (HS-SPME) coupled with GC-MS. In the second step of our study, an evaluation of the effects of some preharvest factors (planting density and harvest time) on the content of 2-AP and other grain quality attributes was considered necessary. A storage trial was also performed where the two cultivars obtained from different growing conditions underwent different storage periods at different temperatures.

2 Methods

2.1 Rice cultivars and growing conditions

Two fragrant rice cultivars were used, Guixiangzhan (medium-grained) and Peizaruanxiang (long-grained). These two fragrant rice cultivars represent the main commercial cultivars in Guangdong province (South China) and were grown in South China Agricultural University experimental farm on a sandy loam soil. Trials were performed over two seasons, the early and late seasons of 2008. Standard agronomic practices with respect to fertilization, irrigation, pest management, and weed control were similar to guidelines recommended by the Province (Tang and Wu, 2006). Transplanting was done in early April for the early season and late July for the late season.

2.2 Planting densities tested

The response of these cultivars to planting density was tested during the two seasons. Rice planting density of 20 cm × 20 cm is recommended in South China to achieve rice plant density at emergence of 28 $hill \cdot m^{-2}$ (Tang and Wu, 2006). Planting densities in this experiment were chosen to cover a range from 32% higher to 43% lower than the recommended one, which included 16 (20 cm × 35 cm), 19 (20 cm × 30 cm), 22 (20 cm × 25 cm), 28 (20 cm × 20 cm), and 37 (20 cm × 15 cm) $hill \cdot m^{-2}$ with three plants per hill. Plots were laid out in a randomized complete block design with three replications, each consisting of 1 plot of 19.60 m^2 in size. The paddy rice samples from each planting density were harvested at 30 days after heading (DAH) and randomly divided into two populations (5 kg each), which were stored for 6 months for the early season harvest and 3 months for the late season harvest. For each season samples, one population was subjected to a storage temperature of −4°C and the other at 30°C.

2.3 Harvest dates adopted

During the two seasons, rice plant samples planted at a density of 20 cm × 20 cm were taken at 10, 20, 30, 40, and 50 days after heading (DAH) in order to study the harvest date effect on the yield, quality, and aroma of the two rice cultivars. Samples were immediately brought to the laboratory and divided into two sets. One set was stored at 8°C for 6 months for the early season harvest and 3 months for the late season harvest. The second set was also stored for the same periods of time but at a temperature of 20°C. Grain moisture content was determined, and all samples were stored as whole grains in plastic bags. Storage temperatures were selected to be typical of those encountered in South China and the neighborhoods.

2.4 Authentic standards and other chemicals

Analytical standards utilized were purchased from various suppliers: 1-pentanol, 1-hexanol, heptanal, octanal, 3-methyl-1-butanol, and *d*-limonene were purchased from Fluka (Buchs, Switzerland). (*E*)-hexenal, hexanal, 1-nonanol, decanal, benzaldehyde, and benzothiazole were from Sigma (St. Louis, MO). 2,6-dimethylpyridine (2,6-DMP) used as internal standard, toluene, and 1-heptanol were obtained from BDH Chemicals (Poole, England), while 1-octanol, and nonanal were obtained from Merck (Darmstadt, Germany). The exact weight of 2,6-DMP was dissolved in a precisely measured volume of benzyl alcohol (Fisher, Loughborough, UK) to give an internal standard solution of 0.50 $mg \cdot mL^{-1}$. 2-acetyl-1-pyrroline (2-AP) was synthesized as reported elsewhere (Buttery et al., 1983) with some modifications (Sriseadka et al., 2006) using benzene and methanol from Merck (Darmstadt, Germany), 2-acetylpyrole, 5% rhodium on an activated alumina, and celite from Fluka (Buchs, Switzerland), and silver carbonate from Aldrich (Milwaukee, WI). Purification and structural confirmation of the synthetic 2-AP were done as outlined previously (Sriseadka et al., 2006). Spectroscopic data of the purified 2-AP were found to be consistent with those reported in the literature. The quantity of the synthetic 2-AP was obtained by calculating the integrated proton signal of the methyl group of 2-AP against those of tetramethylsilane (Aldirch, Steinheim, Germany). Standard solutions of 2-AP and other authentic compounds were made by dissolving a known weight of the product in a precisely measured volume of benzyl alcohol. All chemicals used for rice quality evaluation were obtained from Guanghua Chemicals (Guangzhou, China). All reagents used in the analysis were of analytical-reagent grade with a purity of higher than 98%.

2.5 Rice samples preparation

All samples were dehulled and milled with a Jing Mi machine (Guangzhou, China) to an 85% milling yield (brown rice basis) in the form of white rice. The rice samples analyzed by SPME/GC-MS were brown grains, while both brown and white grains were analyzed by SHS/GC. Ground samples were prepared by crushing of 30 g portions of rice grains in a household blender (Moulinex, Caen, France) for 30 sec and then screening the flour obtained through a 0–2 mm diameter mesh sieve (Endecotts Ltd., London, UK). Rice flour samples obtained were subjected to analysis immediately.

2.6 Headspace SPME/GC-MS analysis of overall rice volatiles

Extraction was carried out in an Autotherm heater (Walnut Creek, CA) with agitation. Optimum operating parameters for the adsorption of rice volatiles using HS-SPME were extraction temperature of 60°C, with an adsorption time of 20 min. A portion of brown rice powder weighted exactly 20.0 g was placed in a 27 mL bottle and added with 10 μL of 0.5 $mg \cdot mL^{-1}$ 2,6-DMP internal standard solution in benzyl alcohol. The sample bottles were then sealed with an aluminum cap with a hole placed on top of a PTFE/Silicone septa (Restek Corp., Bellefonte, PA). Volatile components from the headspace were collected by adsorption on an SPME fiber of 1 cm in length coated with poly (dimethylsiloxane) (PDMS) (Supelco, Bellefonte, PA), which was preconditioned in the GC injection port at 250°C for 1 h before usage. Extractions of all rice samples were performed in duplicate, with three samples per treatment. Inside the bottle, the fiber was lowered from its protective sheath and exposed to the headspace of the sample for 20 min. After that it was pulled into the needle sheath, and the syringe assembly of SPME was removed from the vial and inserted into the injection port of a GC instrument (HP6890, Agilent Technologies, DE).

The fiber was then heated at 250°C for 2 min in a flow of helium at the velocity of 1.0 $mL \cdot min^{-1}$ in order to desorb the volatiles and to pass them through the chromatographic column (a fused silica capillary, HP-5MS, 30 m × 0.25 mm i.d. × 0.25 μm film thickness, Agilent Technologies, DE). The fiber was left there for reconditioning (15 min) before it was exposed to the headspace volatiles of the next sample. The injection was done in splitless mode. The injector and the GC-MS interface temperatures were set at 250°C and 280°C, respectively. The column temperature was held initially at 40°C for 1 min and then increased at a rate of 4$°C \cdot min^{-1}$ to 150°C and further increased to 220°C at a rate of 3$°C \cdot min^{-1}$. Finally, the column temperature was maintained isothermally at 250°C for 5 min. An HP5973 mass spectrometer (Agilent Technologies, DE) equipped with an Agilent ChemStation software D.01.01.SDK for data collection was used in the electron ionization mode with the ion source temperature set at 230°C, the quadrupole temperature at 150°C, and the ionization energy at 70 eV. The scan mode was set up to monitor over m/z 35–400 with a compromise data acquisition rate of 6.35 spectra per sec and an electron multiplier voltage of 1365.

2.7 Static headspace/GC-NPD for extraction and identification of 2-acetyl-1-pyrroline

Extraction and identification of 2-AP were done as described by Sriseadka et al., 2006. SHS/GC analyses were carried out using an Agilent 6890N GC model equipped with a G1888 headspace autosampler and a nitrogen phosphorus detector (NPD), both from Agilent Technologies (Wilmington, DE). Data acquisition and evaluation were accomplished using an Agilent ChemStation software A.01.04 and B.01.03. The headspace autosampler conditions were as follows: oven temperature of 120°C, vial equilibration time of 9 min with high-speed shaking, loop filling and equilibration time of 0.01 and 0.60 min, respectively, and times for pressurizing and injection were 0.10 and 0.40 min, respectively. The sample loop and transfer line temperatures were set at 10 and 20°C higher than the oven temperature, respectively.

Rice powder weighted exactly 1.0 g was placed into a 20 mL headspace vial, followed by the addition of 1.0 μL of 0.50 $mg \cdot mL^{-1}$ 2,6-DMP in benzyl alcohol. The headspace vial was sealed immediately with a PTFE/silicone septum and aluminum crimp cap (Restek Corp., Bellefonte, PA), prior to analysis by SHS-GC. Sample headspace was collected through a 3-mL sample loop and automatically transferred to the GC via a heated transfer line. An HP-5MS fused silica capillary column with dimension of 30 m × 0.53 mm i.d. and 1.5 μm film thickness (J&W Scientific Inc., Folsom, CA), with a splitless injection at 230°C was used. The column temperature program began at 50°C and then increased at a rate of 5$°C \cdot min^{-1}$ to 125°C. The NPD temperature was set at 300°C. The carrier gas flow rate was 5 $mL \cdot min^{-1}$. Samples for each of the three plots were analyzed in duplicates. A reference mixture consisting of a known amount of the standard volatile compounds and internal standard (2,6-DMP in benzyl alcohol) was also subjected to the SHS-GC analysis.

2.8 Volatiles identification and quantification

Identification of volatile compounds was performed by comparing their corresponding mass spectra with those of the standard compounds and the reference compounds complied in both the Wiley and NIST mass spectral libraries when possible. GC retention indexes on HP-5MS columns were also calculated and compared with those reported in the literature. The chromatograms obtained from the total ion current were integrated, and the abundances of the volatiles of interest were recorded as the area under the peak assuming a recovery factor of 1. Based on an internal standard method, the quantification was performed using the following formula:

$$\text{relative concentration } (\text{ng}\cdot\text{g}^{-1}) = \frac{\text{compound area} \times \text{internal standard } (\text{ng}\cdot\mu\text{L}^{-1}) \times \text{injection volume } (\mu\text{L})}{\text{internal standard area} \times \text{rice sample weight } (\text{g})}$$

Data were expressed as the mean of duplicate measurements per three samples±standard deviation. For quantification of 2-AP, a standard curve was constructed from a concentration series of 2-AP prepared in benzyl alcohol and subjected to SHS/GC-NPD analysis under conditions identical to those described above, using a nonfragrant rice powder as a supporting material. Plot of concentrations of standard 2-AP against the corresponding peak areas divided by peak area of 2,6-DMP yielded a linear calibration curve from which the 2-AP content in the samples was derived. The average concentration of 2-AP was expressed as a weight ratio per dry matter of the rice ($\mu g \cdot g^{-1}$).

2.9 Rice yield and quality evaluation

Rough rice samples were obtained from the center of each plot at harvest, which are at least five rows from the border. Paddy yield and 1000-grain weight was determined in duplicate per plot. Samples for quality evaluation were threshed, sun-dried, and stored for 3 months at ambient temperature before processing to ensure stable milling yields. Differences in sample quality were evaluated in terms of head rice rate (sized with a JFQS-1320 testing rice grader using a 4.75-mm mesh indentation, Guangzhou, P.R. China), grain vitreosity (chalkiness on a SDE-A glassy table lit, Guangzhou, P.R. China), amylose content, and protein content using a conversion factor of 5.95 from nitrogen to protein (Juliano and Villareal, 1993).

2.10 Statistical data analysis

Analysis of variance (ANOVA) was used to find the level of significant differences due to planting density and harvest time. Duncan's multiple range test was employed with the level of significance set for $P < 0.05$. All analyses were conducted using SPSS software 15.0 (SPSS, Chicago, IL).

3 Results

3.1 Comparison of Guixiangzhan and Peizaruanxiang flavors

Two headspace extraction techniques were used in this study for analyzing 2-AP and other volatiles compounds in two fragrant rice grains from South China. By using HS-SPME, 42 compounds were identified; however, no clearly distinguishable peak was seen for 2-AP. When S-HS was applied, 2-AP could be unambiguously detected. Altogether headspace sampling allowed the determination of 10 alcohols, 3 aromatic compounds, 6 aldehydes, 2 nitrogen-containing compounds, 1 terpene, 5 ketones, and 3 esters derivatives, as it is listed in Table 1. A total of 13 peaks were assigned to hydrocarbons and have not been included in the table.

Qualitatively, the two rice cultivars had the same significant volatile aroma compounds, but there were large differences in quantity. Only three compounds were found to be unique to Guixiangzhan: benzaldehyde, 4-ethylbenzaldehyde, and benzothiazole. All compounds were substantially lower in abundance in Peizaruanxiang with the exception of nonanal and *d*-limonene. Interestingly, there was the high number of ketones detected. The presence of high amounts of 2-propanol (405.30 and 246.45 $ng \cdot g^{-1}$) and 2-ethyl-1-dodecanol (378.15 and 245.74 $ng \cdot g^{-1}$) in the two rice cultivars must also be referred. The most important difference noted was that Guixiangzhan had approximately five times more 2-AP (3.86 $\mu g \cdot g^{-1}$) than Peizaruanxiang (0.64 $\mu g \cdot g^{-1}$).

3.2 Effect of planting density on 2-acetyl-1-pyrroline content

During the early and late seasons of 2008, 2-AP content significantly ($P < 0.05$) decreases with an increase in planting density. In Fig. 1, reporting the content of 2-AP in rice grains obtained during the early season and stored for 6 months, it is observed that the highest concentration of 2-AP was obtained with grains cultivated at 19 $hills \cdot m^{-2}$ for Guixiangzhan (3.86 $\mu g \cdot g^{-1}$) and 16 $hills \cdot m^{-2}$ for Peizaruanxiang (0.69 $\mu g \cdot g^{-1}$), while the lowest concentrations were obtained with 37 $hills \cdot m^{-2}$, 3.28 and 0.43 $\mu g \cdot g^{-1}$ for Guixiangzhan and Peizaruanxiang, respectively.

Data from our current research over two seasons indicated that several yield and quality attributes of Guixiangzhan and Peizaruanxiang rice, such as average paddy yield, 1000-grain weight, amylose content, and protein content were not significantly ($P < 0.05$) affected by planting density. It was also demonstrated that higher values for head rice yield (48.48%; 60.75%) and grain vitreosity (90.67%; 87.33%) were obtained with lower seeding rates (Table 2).

3.3 Effect of harvesting time on 2-acetyl-1-pyrroline content

Irrespective of cultivars, marginal reduction of 2-AP was observed with increasing harvest date during the early season (data not shown). During the late season, however, the concentration of 2-AP in Guixiangzhan gradually decreased from 10 DAH (5.24 $\mu g \cdot g^{-1}$) and seemed to stabilize at 40 DAH (2.12 $\mu g \cdot g^{-1}$), a reduction rate of 60% (Fig. 2a). Similarly, 2-AP content in Peizaruanxiang showed the same trend but went through maxima at 20 DAH (0.83 $\mu g \cdot g^{-1}$) before significantly ($P < 0.05$) dropping below the initial concentration and continuing to

Table 1 Volatile components identified in the headspace of Guixiangzhan and Peizaruanxiang rice and their relative concentrations

RI[a]	compound	identification[b]	relative concentration[c]/($ng \cdot g^{-1}$)	
			Guixiangzhan	Peizaruanxiang
aliphatic alcohols				
536	2-propanol	MS, RI	405.30±92.01	246.45±15.27
744	3-methyl-1-butanol	MS, RI, STD	3.69±0.59	0.99±0.01
787	1-pentanol	MS, RI, STD	12.65±0.41	2.83±0.14
870	1-hexanol	MS, RI, STD	10.74±0.90	1.84±0.16
969	1-heptanol	MS, RI, STD	9.69±1.34	0.85±0.01
1075	1-octanol	MS, RI, STD	16.39±1.81	3.47±0.17
1175	1-nonanol	MS, RI, STD	30.70±5.22	2.73±0.03
1502	2-butyl-1-octanol	MS, RI	31.37±0.72	20.62±2.45
1562	(*S*)-2-methyl-1-dodecanol	MS, RI	56.95±1.39	36.84±5.12
1580	2-ethyl-1-dodecanol	MS, RI	378.15±18.14	245.74±37.08
aromatics				
760	toluene	MS, RI, STD	6.88±1.11	3.08±0.24
952	benzaldehyde	MS, RI, STD	4.22±0.04	nd[d]
1171	4-ethylbenzaldehyde	MS, RI	2.03±0.10	nd
aliphatic aldehydes				
803	hexanal	MS, RI, STD	10.68±1.15	5.60±0.78
865	(*E*)-2-hexenal	MS, RI, STD	8.19±0.40	3.62±0.38
903	heptanal	MS, RI, STD	3.82±0.15	0.49±0.05
1005	octanal	MS, RI, STD	4.15±0.48	1.07±0.05
1106	nonanal	MS, RI, STD	40.76±2.73	44.56±0.86
1206	decanal	MS, RI, STD	11.04±1.08	0.66±0.02
N-containing compounds				
918	2-acetyl-1-pyrroline	RI, STD[e]	3.86±0.09	0.64±0.02
1213	benzothiazole	MS, RI, STD	3.08±0.29	nd
terpenoids				
1022	*d*-dimonene	MS, RI, STD	11.91±2.43	13.90±0.42
aliphatic ketones				
1093	2-nonanone	MS, RI	4.15±0.03	3.02±0.16
1393	2-dodecanone	MS, RI	5.14±0.38	2.81±0.46
1584	2-tetradecanone	MS, RI	11.39±0.01	5.38±1.41
1689	2-pentadecanone	MS, RI	20.28±0.94	12.87±2.93
1845	6,10,14-trimethyl pentadecanone	MS, RI	7.05±1.49	10.62±1.60
fatty acids and esters				
1780	tetradecanoic acid	MS, RI	12.02±1.66	7.65±1.74
1824	isopropylmyristate	MS, RI	25.36±0.16	14.53±2.22
1970	hexadecanoic acid	MS, RI	14.10±2.40	1.81±0.36

Note: a) Retention index is based on a series of n-hydrocarbons using a HP-5MS column. b) identification proposal: MS represents tentative comparison of the EI-MS with the NIST and Wiley mass spectral library; RI represents tentative identification by retention indexes with literature data; STD represents comparison of retention time and spectrum of an identified compound with those of an authentic compound. c) Values are expressed as 2,6-dimethylpyridine (RI = 904) equivalent (2,6-DMP $ng \cdot g^{-1}$ brown rice) and given as average ± standard deviation. d) nd represents not detected. e) represents concentration of 2-AP obtained after static headspace-GC/NPD and expressed as a weight ratio per dry matter of the rice ($\mu g \cdot g^{-1}$).

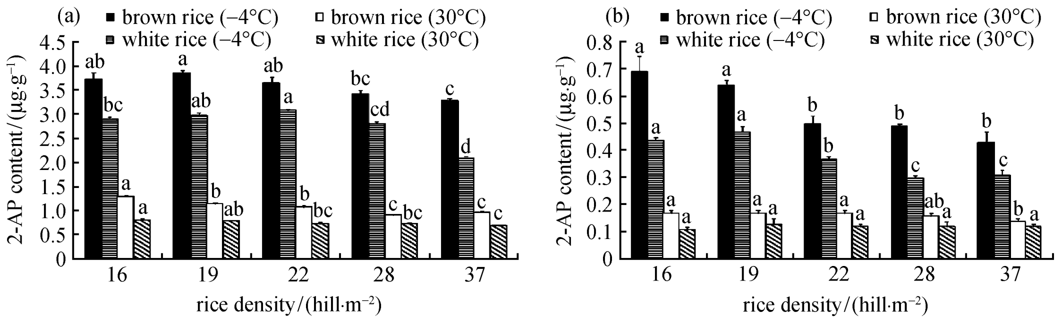

Fig. 1 Concentration of 2-acetyl-1-pyrroline (2-AP) in (a) Guixiangzhan and (b) Peizaruanxiang fragrant rice cultivars as affected by planting density after a storage period of 6 months at –4 and 30°C during the early season of 2008

Note: All samples were stored in paddy form. Data points charted are the mean values of three replicate experiments with the corresponding standard deviations as error bars. Bars sharing a common lower case letter above are different at the $P < 0.05$ level of significance by Duncan's multiple range test.

Table 2 Main effects of planting density on Guixiangzhan and Peizaruanxiang fragrant rice yield, 1000-grain weight, head rice rate, grain vitreosity, amylose content, and protein content during the early season of 2008

rice density /(hill·m^{-2})	grain yield/(t·hm^{-2})	1000-grain weight/g	head rice yield/%	grain vitreosity/%	amylose content/%	protein content/%
Guixiangzhan						
16	5.02±0.12 a	25.04±0.96 a	45.79±0.61 ab	88.33±0.33 b	16.38±0.26 a	8.93±0.20 a
19	4.65±0.07 a	25.45±0.36 a	48.46±1.52 a	90.67±0.88 a	16.29±0.36 a	8.64±0.05 a
22	4.68±0.28 a	26.07±0.16 a	48.44±1.13 a	88.00±0.58 bc	15.98±0.60 a	8.77±0.14 a
28	5.18±0.47 a	26.30±0.13 a	48.38±1.78 a	88.33±0.33 b	14.56±0.86 a	8.79±0.27 a
37	5.18±0.67 a	24.55±0.95 a	44.07±0.96 b	86.33±0.33 c	15.98±0.64 a	8.66±0.14 a
Peizaruanxiang						
16	5.06±0.27 a	19.48±0.45 a	59.15±1.00 ab	84.00±0.58 b	28.31±0.42 ab	9.04±0.18 a
19	5.43±0.41 a	19.51±0.47 a	60.75±0.87 a	82.33±0.88 b	28.36±0.62 ab	9.06±0.33 a
22	5.58±0.17 a	19.82±0.47 a	59.77±2.26 ab	87.33±0.88 a	27.80±0.64 b	9.16±0.14 a
28	4.77±0.41 a	20.08±0.48 a	55.54±1.39 b	80.00±0.58 c	27.29±0.81 b	9.28±0.16 a
37	5.29±0.35 a	19.63±0.52 a	55.48±1.38 b	78.33±0.33 c	30.13±0.44 a	9.34±0.12 a

Notes: results are expressed as mean±standard deviation. Mean for each attribute in the same column followed by the same letter are not significantly different at $P < 0.05$ by Duncan's multiple range test.

decrease before the end of the experiment with a content of 0.34 μg·g^{-1} at 50 DAH (Fig. 2b).

In Guixiangzhan, paddy yield (5.17 t·hm^{-2}) and head rice yield (61.77%) were higher when harvested at 20 DAH. With Peizaruanxiang, optimum paddy yield (6.97 t·hm^{-2}) and head rice yield (66.64%) were reached at 30 DAH (Table 3).

3.4 Effect of storage time and temperature on 2-acetyl-1-pyrroline content

Fragrant rice harvested in June and kept for 6 months at −4°C contained up to four times 2-AP in all forms (brown and white), compared to those kept at 30°C. High losses of 2-AP occurred under a very warm condition of 30°C, from 3.73 to 1.30 μg·g^{-1} for Guixaingzhan and from 0.69 to 0.17 μg·g^{-1} for Peizaruanxiang (Fig. 1). There were also significant differences in the concentration of 2-AP between samples collected in November with losses of 25 to 35 % occurring after storage of 3 months at 20°C compared to 8°C (Fig. 2).

4 Discussion

4.1 Comparison of Guixiangzhan and Peizaruanxiang flavors

We sought in our study to clarify the aroma quality of a potential fragrant cultivar of South China (Guixiangzhan) and to compare it with a new breeding line (Peizaruanxiang). Although under optimum conditions, HS-SPME did not enable the detection of 2-AP. The absence of 2-AP could only be attributed to the selectivity of the fiber used.

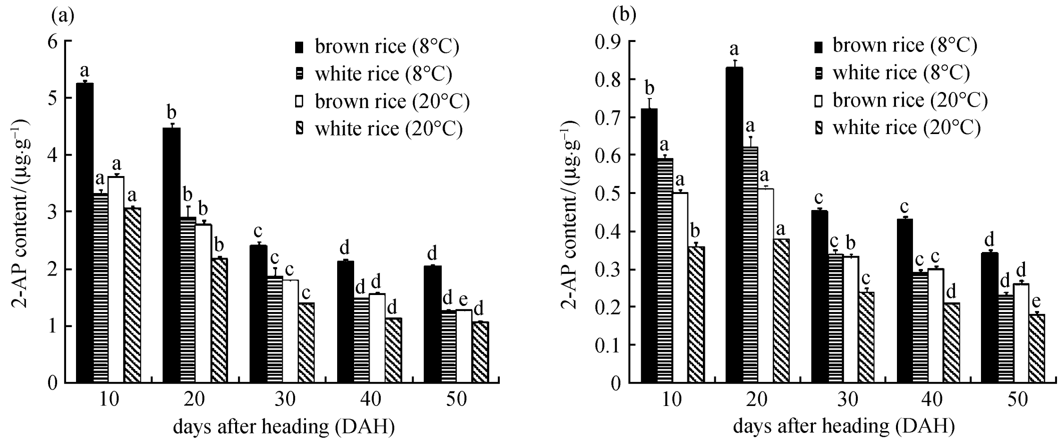

Fig. 2 Change in 2-acetyl-1-pyrroline (2-AP) concentration in (a) Guixiangzhan and (b) Peizaruanxiang fragrant rice cultivars harvested at different dates after heading (DAH) during the late season of 2008 and subjected for 3 months to different storage temperatures (8 and 20°C)
Note: All samples were stored in paddy form. Each vertical bar is the mean of triplicate data with bars representing the standard deviation. Vertical bars with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 3 Grain yield and weight and four quality traits of Guixiangzhan and Peizaruanxiang fragrant rice cultivars harvested at different dates after heading (DAH) during the late season of 2008

harvest date/DAH	grain yield/($t \cdot hm^{-2}$)	1000-grain weight/g	head rice yield/%	grain vitreosity/%	amylose content/%	protein content/%
Guixiangzhan						
10	3.88±0.26 b	25.10±0.21 b	36.18±0.33 c	91.67±0.33 a	20.43±0.10 ab	8.86±0.02 a
20	5.17±0.25 a	25.33±0.03 b	61.77±0.45 a	91.00±1.00 ab	20.60±0.03 a	9.17±0.24 a
30	5.14±0.06 a	26.37±0.13 a	61.26±0.12 ab	89.67±0.67 bc	20.57±0.16 a	9.27±0.17 a
40	5.15±0.04 a	26.30±0.10 a	60.12±0.29 b	90.00±0.58 abc	20.20±0.09 b	9.25±0.22 a
50	5.16±0.25 a	26.30±0.17 a	60.31±0.17 b	83.33±0.33 c	20.22±0.04 b	9.03±0.22 a
Peizaruanxiang						
10	4.29±0.21 c	20.30±0.21 b	40.79±0.51 d	89.33±0.88 a	28.37±0.10 a	9.91±0.13 a
20	6.01±0.03 b	22.17±0.15 a	60.08±0.33 b	84.67±0.67 b	29.02±0.27 a	9.69±0.28 a
30	6.97±0.13 a	21.83±0.23 a	66.64±0.31 a	81.67±0.88 bc	28.95±0.27 a	9.90±0.07 a
40	6.49±0.27 ab	21.80±0.26 a	60.48±0.64 b	80.00±1.53 c	28.84±0.19 a	9.50±0.24 a
50	6.10±0.20 b	21.73±0.20 a	51.60±0.19 c	78.67±1.45 c	28.88±0.18 a	9.57±0.29 a

Notes: results are mean±standard deviation. Different letters following data in a column for each parameter indicate significant differences ($P < 0.05$ using Duncan's multiple range test).

The actual SPME extraction and concentration of the rice sample headspace volatiles were performed using a PDMS fiber, or that kind of fiber because of its nonpolar nature is known to perform very effectively for a wide range of mostly nonpolar and rather high molecular weight analytes (Zeng et al., 2008). It also appeared that compounds reported to be formed predominantly in the rice core endosperm like hexanal, benzaldehyde, 1-hexanol, and nonanal (Yang et al., 2008b) were those effectively adsorbed by the PDMS fiber. When S-HS was applied, 2-AP could be unambiguously detected. NPD has the particularity to have a specific selectivity to nitrogen containing compounds (Sriseadka et al., 2006). Hence, its use provided higher detection sensitivity for 2-AP. Of all the identified compounds in the two rice cultivars, 17 have been reported elsewhere as odor-active in diverse rice cultivars, which include 3-methyl-1-butanol, toluene, 1-pentanol, hexanal, (E)-2-hexenal, 1-hexanol, heptanal, 2-acetyl-1-pyrroline, benzaldehyde, 1-heptanol, octanal, 1-octanol, nonanal, 2-nonanone, 1-nonanol, decanal, and benzothiazole (Buttery et al., 1988; Widjaja et al., 1996; Jezussek et al., 2002; Maraval et al., 2008; Yang et al., 2008a). It is likely that other compounds often cited in rice, such as 1-octen-3-ol, 2-pentylfuran, 3-octen-2-one, (E)-2-octenal, (E,E)-2-decadienal, and hexanoic acid also occur, but the conditions of the isolation method did not allow their isolation, or their mass spectra were of poor quality. This demonstrates, however, that Guixiangzhan and Peizaruanxiang have the potential of further flavor development under appropriate conditions.

The most important difference noted was that Guixiangzhan had approximately five times more 2-AP. The aroma level difference between the two fragrant kinds of rice was expected since Guixiangzhan when cooked has more desirable aroma quality than Peizaruanxiang. However, the low level of 2-AP measured for Peizaruanxiang could indicate that the cultivar is not very scented, which will be controversial. Peizaruanxiang was developed by the breeding group of South China Agricultural University in 2003 and rapidly emerged as a superior rice cultivar worthy of release as pure-line under the category "scented rice," hence, its name, which etymologically means "soft aroma." It has a relatively intense flavor that is distinctly different from nonfragrant rice, and its pleasant aroma is obviously acceptable to a great number of consumers in South China. It is unlikely that 2-AP is the main compound that contributes to the unique aroma of Peizaruanxiang. A complementary hypothesis is that no single compound is responsible for its aroma, but a combined aroma effects of many components mixed in the correct proportions. During the last decade, other examples have been reported where 2-AP was not found to be the main compound in fragrant rice. For example, in the south of Jiangsu in China, not 2-AP but 2-acetylpyridine was established as the characteristic aroma compound of the scented rice Xiangjing-8618 (Gu, 2002). Interestingly, 2-AP was not detected in most fragrant rice s from Afghanistan and Myanmar (Hien et al., 2006). In black rice grown in Korea, not only 2-AP but also guaiacol were reported to be major contributors to aroma (Yang et al., 2008a). Therefore, further investigation in Peizaruanxiang rice composition may be necessary to pinpoint those essential components responsible of its specific aroma.

For domestically produced fragrant rice s to be competitive, their aromas need to match those of imported Basmati and Jasmine rices found in the local market since they are seen as the benchmark for aroma quality. The results of our research indicate that Guixiangzhan meets this criterion with a 2-AP content of $3.86\ \mathrm{ng \cdot g^{-1}}$ comparable to $3.72\ \mathrm{ng \cdot g^{-1}}$ obtained with Jasmine rice using the same methodology (Sriseadka et al., 2006), which is promising for the future development of South China scented cultivars. It should be noted, however, that Guixiangzhan samples were analyzed after a storage period of 6 months at $-4°C$, while Jasmine had undergone storage of 1 month at ambient temperature, a condition that can result in many changes, especially the overall volatile composition, and the decrease in the content of 2-AP.

4.2 Effect of planting density on 2-acetyl-1-pyrroline content

Data was collected for the concentration of 2-AP present in rice grain planted at different densities during the early and late seasons of 2008. During both seasons, 2-AP content significantly ($P < 0.05$) decreased with an increase in planting density. However, other seed quality attributes at the exception of head rice yield and grain vitreosity were not significantly ($P < 0.05$) affected by planting density. Taken together, our results indicated that planting density can be reduced from the currently recommended 28 $\mathrm{hills \cdot m^{-2}}$ to $19 - 16\ \mathrm{hills \cdot m^{-2}}$ while maintaining optimum yield and quality, particularly in relation to aroma and flavor attributes. Also, this will be particularly important for farmers with respect to production costs since fragrant rice seeds are more expensive than those of the conventional cultivars.

4.3 Effect of harvesting time on 2-acetyl-1-pyrroline content

In our study, 2-AP content in fragrant rice decreased with harvesting time in both Guixiangzhan and Peizaruanxiang. Our results suggest that early harvesting, 10 DAH has the greatest chance to recover aroma. However, when choosing the optimum harvesting time for rice, aroma content is not the only criteria involved, but also optimum paddy yield and grain qualities have to be taken into consideration. Based on the data presented in Fig. 2 and Table 3, we propose that for the two cultivars, the most appropriate time for harvesting transplanted rice is between 18 and 24 DAH during the late season and between 26 and 32 DAH during the early season. Early harvesting at 20 DAH for Peizaruanxiang might slightly reduce the chance of grain and head rice yields recovery. However, it is well compensated for by the high level of 2-AP in both brown and white rices, which remains significant even after a storage period of 3 months at ambient temperature.

Because rice samples for the two seasons were stored for different periods of time after harvest, we could not evaluate the effect of planting season on the content of 2-AP. The flavor of rice was reported to correlate with protein and amylose content in some American rice cultivars (Champagne, 2008). In our study, we did not find a relationship between the two quality attributes and 2-AP. Protein and amylose contents remained relatively constants ($P < 0.05$) with planting density and harvesting time, while 2-AP content varied considerably.

4.4 Effect of storage time and temperature on 2-acetyl-1-pyrroline content

During storage of the two rice cultivars, 2-AP content decreased with time and appeared to be significantly affected by the temperature. It was demonstrated that higher 2-AP concentrations were obtained with the shortest storage time of 3 months and the lowest storage temperature of $-4°C$. It is important to note that for all the experiments conducted, 2-AP content was always 1.2 to 1.6 times higher in brown rice compared to white rice (Figs. 1 and 2), which was in disagreement with a previous study (Bergman et al., 2000), which showed little difference in the 2-AP contents of brown and milled rice. Similar losses of 2-AP with increasing storage duration and at high temperatures have been reported for many systems with aroma and flavor attributes of rice typically following a degradation curve over time. It was particularly found that 2-AP content displayed the highest rate of decrease at the beginning of storage (Wongpornchai et al., 2004) and was inversely correlated with fat acidity at an early stage of storage (Yoshihashi et al., 2005). This highlights the necessity to focus on storage protocols for a successful development of Guixiangzhan and Peizaruanxiang cultivars.

In most cases, fragrant rice tastes best when it is fresh. Therefore, we assume that keeping it under refrigeration and using it within six months would be a practical way to preserve its desirable character as monitored by changes in the levels of 2-AP. Results from our investigation also showed that planting at low density and early harvesting could improve aroma content and other seed qualities. Despite these, however, variations in weather conditions, availability of adapted storage facilities, and market economics may still be of concern.

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