分类号 S511 学校代码 10564
UDC 学 号 2007100011
密 级

学 位 论 文

华南农业大学

收获前后调控香稻香气和米质的研究

GOUFO PIEBIEP

指导教师姓名 唐湘如 教授

申请学位级别 博 士 **专业名称** 作物栽培学与耕作学

论文提交日期 2010 年 4 月 **论文答辩日期** 2010 年 5 月

学院名称 农 学 院 **学位授予日期** **PhD**

答辩委员会主席 官春云 院士

评阅人 刘向东 教授

谭中文 教授

梁计南 教授

钟旭华 研究员

Department of Crop Science and Technology
College of Agriculture
South China Agricultural University
(Guangzhou, P. R. China)

Appropriate Set of Pre- and Post-harvest Treatments for Obtaining High Levels of Aromas and Quality in Fragrant Rice (*Oryza sativa* L.)

By

GOUFO PIEBIEP

A dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy (Ph.D.) in CROP SCIENCE (first-level discipline), CROP CULTIVATION AND FARMING SYSTEM (second-level discipline)

Supervisor: **Prof. Xiangru Tang (唐湘如)**

Chairman of the Defense Committee: **Prof. Guan Chunyun (官春云)**

Members of the Defense Committee: **Prof. Liu Xiangdong (刘向东)**

Prof. Tan Zhongwen (谭中文)

Dr. Liang Jinan (梁计南)

Dr. Zhong Xuhua (钟旭华)

Date of thesis submission: 26 April 2010
Date of thesis defense: 25 May 2010

Dedication

To

MY MOTHER

Appropriate Set of Pre- and Post-harvest Treatments for Obtaining High Levels of Aromas and Quality in Fragrant Rice (O*ryza sativa* L.)

Piebiep Goufo

(College of Agriculture, South China Agricultural University, Guangzhou 510642, China)

Abstract: Aromatic rices (*Oryza sativa* L.) constitute a special group of rice accessions well known for their aroma and/or superfine grain quality. For a successful development of aromatic rices, research regarding factors affecting the quality of the aroma is of economic interest to rice growers and processors. Therefore, this study was conducted as a preliminary step towards the rice quality improvement of two aromatic rice cultivars growing in South China, namely Guixiangzhan and Peizaruanxiang.

The fragrance potential of two rice cultivars was investigated using headspace SPME and static headspace in conjunction with GC-MS, and under optimal conditions for the identification and quantification of the intensely popcorn-like smelling compound 2-acetyl-1-pyrroline (2-AP). About a 5-fold difference of 2-AP levels were observed among the two rices with Guixiangzhan having the highest content (3.86 μg/g) 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 four pre-harvest treatments (planting densities of 16, 19, 22, 28, and 37 hills/m^2, harvesting times of 10, 20, 30, 40, and 50 days after heading, ripening temperatures during the early and the late seasons, application of growth regulators consisting of gibberellic acid, paclobutrazol, 3-indole acetic acid and a mixture of paclobutrazol, proline, and zinc chloride) and three postharvest treatments (storage times of 3 and 6 months, storage temperatures of -4, 8, 20, and 30 ℃, milling degree of 85%). Results were also discussed in terms of antioxidants enzymes activities (peroxidase, superoxide dismutase, proline oxidase), yield attributes (number of panicles per hill, number of spikelets per panicle, grain-filling percentage, paddy yield, 1000-grain weight), milling quality (milled rice rate, whole rice rate, head rice rate), grain appearance (% area with chalkiness, grain vitreosity), malondialdehyde, proline, total soluble proteins, amylose and protein contents of rice samples associated with differing pre-harvest regimes.

Highest 2-AP concentrations were obtained for Guixiangzhan and Peizaruanxiang with the lowest planting density of 16 hills/m^2 (3.73; 0.69 μg/g), the earliest harvesting time of 10 days after heading (5.24; 0.72 μg/g), a low ripening temperature of 25 ℃ (7.12;

2.42 ng/g), the shortest storage time of 3 months (2.40; 0.45 μg/g) and the coolest temperature of -4 ℃ (3.42; 0.49 μg/g). After milling, 2-AP content decreased up to 1.5-fold in both Guixiangzhan and Peizaruanxiang. All treatments with growth regulators, although improved grain yield and quality, and enhanced the capacity of Peizaruanxiang and Guixiangzhan to scavenge and control the production of damaging species of active oxygen, resulted in reduced aroma content that negatively affected overall flavor in a smelling evaluation. Decreases ranged from 9 to 24% compared to the control (2.40; 0.41 μg/g).

These findings indicate that manipulating pre and postharvest treatments can greatly improve the specific attributes of the domestically produced aromatic rices. It is assumed from the study that keeping aromatic rice under refrigeration, milling it at a low degree and consuming 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 show that altering sowing dates to allow the critical stages of seed maturation to coincide with favorable field environments (cool and dry segment of the year) as well as planting at low density and early harvesting could improve aroma content and other seed qualities. It is also recommended that the aroma quality should be carefully monitored if growth regulators are to be used. With that in mind, China could effectively increase its share of the domestic market of fragrant rices and even tap into the international market.

Keywords: aromatic rices, 2-acetyl-1-pyrroline, quality, planting density, harvest date, growth regulators, storage conditions

摘　要

香稻是特种稻，以其芳香的气味和超优的米质被人们所熟知。随着香稻的不断发展，探明影响香稻香气成分含量和米质的有关因素，对香稻种植者和加工者的经济利益提高具有重要的作用。因此，本论文以华南地区两个香稻品种香稻桂香占和培杂软香为材料，研究了收获前后调控香稻香气和米质的作用，主要结果如下：

利用了顶空固相微萃取技术和静态顶部空间气质联用技术，在最佳的条件下定性及定量测定香稻桂香占和培杂软香的具有强烈爆米花气味的化合物 2 -乙酰- 1 -吡咯啉（2 -AP）含量，以桂香占 2-AP 含量为最高(3.86 μg/g)，高出泰国米 KDML105 5 倍；其他非 2-AP 化合物对促进培杂软香特有的香气形成具有一定的作用。

对桂香占和培杂软香两个品种进行了 4 个收获前处理：种植密度处理分别为 16、19、22、28 和 37 穴/m^2；收获时期处理分别为抽穗后 10d、20d、30d、40d 和 50d 收割；种植季节处理分别为早季和晚季；植物生长调节剂处理分别为在抽穗期施用赤霉素、多效唑、 3 -吲哚乙酸和多效唑、脯氨酸、和氯化锌的混配剂等和 3 个收获后处理：贮藏时间处理分别为贮藏 3 个月和 6 个月；贮藏温度处理分别为-4℃、8℃、20℃和 30℃及碾磨程度处理分别为和常规碾磨和 85％的常规碾磨程度。测定了不同收获前处理的香稻样品的过氧化物酶、超氧化物歧化酶和脯氨酸氧化酶活性，有效穗、每穗总粒数、结实率、千粒重和产量等性状，糙米率、精米率、整精米率、垩白粒率、垩白度和直链淀粉含量等品质性状，以及丙二醛、脯氨酸和蛋白质含量。结果表明，桂香占和培杂软香的 2-AP 含量最高值分别出现在种植密度最稀的 16 穴/m^2 处理分别为 3.73μg/g 和 0.69 μg/g；收获时间最早的处理即抽穗后 10d 收获的 2-AP 含量最高，桂香占为 5.24μg/g 和培杂软香为 0.72 μg/g；成熟期温度最低的 25℃处理的 2-AP 含量最高，桂香占为 7.12μg/g 和培杂软香为 2.42 μg/g；储藏时间最短的 3 个月处理的 2-AP 含量最高，桂香占为 2.40μg/g 和培杂软香为 0.45 μg/g；储存温度最低的-4℃处理 2-AP 含量最高，桂香占为 3.42μg/g 和培杂软香为 0.49 μg/g；碾磨后，桂香占和培杂软香的 2-AP 含量低出研磨前含量 1.5 倍；相对于对照，所植物生长调节剂处理，虽然提高了桂香占和培杂软香的谷粒产量和质量，增强了其清除和控制其产品中活性氧危害的能力，但是减少了香气物质的含量，降幅介于 9％至 24％，从而导致了在气味评估方面的消极影响。

上述结果表明，利用收获前后处理，可大大改善中国国产香米的具体特征。通

过冷藏香米、低的碾磨程度和储存时间低于 6 个月是有利于维持香米优良特征可行办法，有利于防止香米 2-AP 水平的下降；利用改变播期使种子成熟的关键阶段与良好的田间环境相适应，即，使香稻灌浆熟期处于温度相对较低和干燥的时间段，以及在低密度和适期早收的种植方式等可以提高香稻香气含量和米质；香稻生产上应注意使用植物生长调节剂对香气含量的不利影响。

因此，通过应用收获前后对香稻香气和米质有利的调控措施可以在不同程度上改良香稻香气和品质性状，增加香米国内市场份额，甚至打入国际市场。

关键词：香稻　2 -乙酰- 1 -吡咯啉 品质 密度 生长调节剂 收获期 储存

Contents

1 General introduction

1.1 Overview of the topic

Aromatic rices constitute an important subgroup of rice that is becoming increasingly popular in China and the whole world due to their distinct flavor (Glaszmann, 1987; Singh et al. 2000; Smith and Dilday, 2003; Sweeney and McCouch, 2007). They are a bit more expensive than plain white rice, but their qualities are well worth the price. Main aromatic cultivars grown in the world are Basmati from India and Pakistan (Singh et al. 2000; Bhattacharjee et al. 2002, 2003; George et al. 2005), and Jasmine from Thailand (Singh et al. 2000; Mahatheeranont et al. 2001). Therefore, most of the trade in aromatic rice is from India, Pakistan and Thailand and is exported mainly to Saudi Arabia, UAE, Kuwait, Oman, Russia, UK, and USA (Singh et al. 2000). In addition to Basmati and Jasmine, however, a diverse cross-section of specialty rice types are produced or have been developed in other countries that have unique flavour, nutritional, textural, aesthetic, or other properties that often garner higher prices in the market place.

Although accounting for about 30% of total world production, China has not been able to play a leading role in the aromatic rice production. With the amelioration of living standards in China in recent years, however, the aromatic rice demand is constantly increasing. There has been a steady growth in the export of Jasmine rices from Thailand to China during the past 10 years. In 1995, China imported 261, 553 tonnes of Jasmine rice compared to 5, 250 in 1988 (Singh et al. 2000). Therefore, the trend of increased aromatic rice production and consumption is promising for the rice industry in the country, with an opportunity to tap into the international rice market. By developing successful aromatic rice cultivars, China can take advantage of this highly priced, growing rice market. In most part of China, the development of new aromatic rice cultivars has become a high priority within several rice-breeding programs. Thus, efforts have been undertaken for the last decades to promote the production of aromatic cultivars in Guangdong, a traditional rice cultivation area in the south of China. Some old scented cultivars have been revalorized (e.g. Guixiangzhan) and new ones adapted to the tropical climate have been developed (e.g. Peizaruanxiang) (Tang and Wu, 2007; Duan et al. 2009).

The economic value of aromatic rice crop depends mainly on the aroma. In breeding new rice cultivars, however, there is the danger of changing that subtle desirable feature. Numerous studies performed in the past 50 years on the composition of volatile rice

constituents have led to the identification of close to 300 compounds up to now. This number is still increasing due to rapid development in analytical techniques and the release of new cultivars. The volatiles identified vary with the degree of milling, isolation technique, cooking method, and storage duration. Important aroma compounds have been detected in rice using advanced methods involving direct solvent extraction for collection/concentration (SE) (Bergman et al. 2000; Mahatheeranont et al. 2001; Itani et al. 2004), stable isotope dilution (SID) (Yoshihashi, 2002; Yoshihashi et al. 2002), simultaneous steam distillation/extraction (SSDE) (Lin et al. 1990; Tanchotikul and Hsieh 1991; Widjaja et al. 1996b; Tava and Bocchi 1999; Mahatheeranont et al. 2001), supercritical fluid extraction (SFE) (Bhattacharjee et al. 2003), static headspace (SHS) (Sriseadka et al. 2006), dynamic headspace also called "purge and trap" (DHS) (Yang et al. 2008a, 2008b, 2008c), headspace solid-phase micro-extraction (HS-SPME) (Grimm et al. 2001; Ghiasvand et al. 2007).

Among the volatiles identified, there are a relatively small number of odor-active compounds. It is assumed that the good smell of aromatic rices stem primarily from its 2-acetyl-1-pyrroline (2-AP) content (Buttery et al. 1988). Although most aromatic types assessed to date contain 2-AP, they have very different aromas. It is, however, not yet clear whether differences in 2-AP concentrations, or the presence of a different set of odorants, are responsible for such aroma differences.

In addition, the aroma quality is reported to depend on cultivation conditions and postharvest practices. There is no agreement, however, with regard to factors controlling that aroma, and this could probably be related to the lack of extensive and objective work on the issue. Knowledge of the changes in volatiles induced by pre-harvest treatments, processing and storage is therefore of great importance.

1.2 Aims of the work

This project was initiated in order to assist with the development of South China aromatic rices by investigating the aroma, yield and quality of a new breeding line (Peizaruanxiang) and compare this with an existing cultivar (Guixiangzhan). The two rices are grown in Guangdong Province and possess a strong characteristic aroma, which differed greatly from the aroma of non-aromatic rices. However, chemical characterization of aromatic compounds in the two rices has not been attempted.

As a preliminary step towards that objective, volatile compounds emanating from Guixiangzhan and Peizaruanxiang headspaces were identified and quantified using GC-MS,

with an emphasis placed on 2-AP considered the single most important volatile in rice.

In order to assist in the development of aromatic rice cultivars suited to a particular local environmental conditions, rice breeders have an interest in gaining access to an appropriate method for extracting volatile compounds. Two of such methods were investigated in the study: static headspace and headspace solid phase micro-extraction. To establish the fragrant potential of South China cultivars, samples of imported Thai aromatic rice (KDML 105) were also analyzed for attribute comparisons.

Of the pre-harvest factors that can affect rice yield and quality, a producer can only control few of them such as seeding date, planting density, drain time and grain moisture at harvest. In a second step of our study, the two cultivars were subjected to two different pre-harvest treatments: planting density and harvest date. Analyses performed on the data also measured seasonal variation of 2-AP over two planting seasons.

A third experiment was conducted to assess the effects of application of plant growth regulators at 25% panicle emergence on 2-AP, lipid oxidation volatiles and other odor-active compounds in the two rices. Efforts were made to correlate the special flavors of rice grains to specific volatile compounds or groups of compounds.

A storage trial was also performed where a selection of the two rice cultivars underwent a 3 to 6 month storage period at 4 different temperatures (-4 ℃, 12 ℃, 20 ℃, and 30 ℃).

For all the experiment conducted, results were also discussed in terms of antioxidants enzymes activities (peroxidase, superoxide dismutase, proline oxidase), yield attributes (number of panicles per hill, number of spikelets per panicle, grain-filling percentage, paddy yield, 1000-grain weight), milling quality (milled rice rate, whole rice rate, head rice rate), grain appearance (% area with chalkiness, grain vitreosity), malondialdehyde, proline, total soluble proteins, amylose and protein contents of rice samples associated with differing pre-harvest regimes.

Finally, the possible involvement of proline in the biosynthetic pathway of 2-AP was investigated. Rice leaves and grains were harvested at different days after heading to determine if there was any relationship between proline oxidase activity, proline content and aroma content in rice plants and grains.

1.3 Importance of the study

An understanding of how genetic, pre, and post-harvest factors affect the aroma and quality characteristics of rice will help producers and processors meet the needs of specific

customers and foster the development of a diversified rice market.

Results of this analysis will be helpful in several respects:

1. Extracting and quantifying volatiles components will be useful to infer on the type of compounds most significant in improving the scented character of aromatic rices and to know the possible involvement of new compounds associated with aroma formation with the final objective to aid the breeding of rice cultivars with specific aroma traits.

2. Addressing questions about the density can contribute to know the appropriate seeding rate to adopt for an optimum production of aromatic rices and to reduce risks associated with lodging, strong shedding and susceptibility to pests and diseases.

3. According to experts, improper harvest can lead up to 15% yield losses in rice. Accordingly determining the appropriate harvesting time is important and can aid growers in makingdecisions based on expected grain aroma content.

4. Identifying and targeting the suitable temperature is necessary because other management strategies, such as application of aroma improvers may not be feasible in certain situations.

5. Application of plant growth regulators is widely practiced to improve yield. However there may well be some impact of hormone levels on the aroma of aromatic rices. Therefore investigation of the effects of growth hormones on the aroma volatiles in rice plants and grain will help clarify requirements for aroma development. This can also help to predict the durability of the effectiveness of fertilizers and aroma improvers' application.

6. Correlation of data obtained on rice storage time and temperature, and milling degree could define an appropriate set of post-harvest treatments for obtaining aromatic rice products with low substantial loss or reduction in quality.

Understanding these relationships will have an economic impact on the aromatic rice industry in the region, with much of the information being relevant to aromatic rices grown in other countries. This could also help the Chinese rice industry to obtain a sizable portion of this fast growing, high value aromatic rice market, both domestically and internationally.

2 Materials and methods

2.1 Rice cultivars used in the study and experimental site

Rice cultivars

Two Indica fragrant rice cultivars were used throughout the study, namely Guixiangzhan and Peizaruanxiang. The two rices represent the two major types of fragrant cultivars in Guangzhou. Guixiangzhan (medium-grained) has been cultivated in South China since the sixties while Peizaruanxiang is a hybrid long-grained cultivar released recently by the breeding group of the college of Agriculture of South China Agricultural University (SCAU) with better attributes in terms of yield and grain size **(Figure 2.1, Table 2.1)**.

Figure 2.1 Rough, brown, parboiled and white grains Guixiangzhan rice.

Table 2.1 Main characteristics of Guixiangzhan and Peizaruanxiang rice cultivars

Characteristics	Guixiangzhan	Peizaruanxiang
Cultivar classification	Conventional	Hybrid
Growth duration early season(days)	110-120	115-125
Growth duration late season (days)	90-100	95-105
Foliage color	bright green	green-yellow
Plant height (cm)	90-110	110-115
Grain length/width ratio	2.98 (slender)	3.18 (medium)
Apparent amylose content (%)	20.43 (intermediate)	28.37 (high)
Average protein content (%)	8.17	9.06
Gel consistency (mm)	65 (soft)	58 (flaky)
Grain vitreosity (%)	90.67	80.33

Seeds of Guixiangzhan were collected from a previous harvest while those of Peizaruanxiang were purchased from a local shop situated inside the University. Samples of imported Jasmine Khao Dawk Mali 105 (KDML 105) were also analyzed along with Guixiangzhan and Peizaruanxiang samples. KDML samples were cultivated in a paddy of a local farm in the Surin Province in northeastern Thailand during November 2008 and kept for more than one month at an ambient temperature of ±20 ℃.

Experimental site

The two rice cultivars were grown in SCAU experimental farm **(Figure 2.2)**, located in Tianhe district (3°08'N and 113°12'E) in Guangzhou.

Figure 2.2 Guixiangzhan and Peizaruanxiang growing vigorously in SCAU experimental field.

Guangzhou is the capital of the most populous province in China, Guangdong province. It has a land area of 7,434 km^2 and it is located at 112°57'E to 114°3'E and 22°26'N to 23°56'N. The city is part of the Pearl River Delta. Neighboring areas are Hunan, Jiangxi, Fujian, and Hainan provinces; Guangxi Zhuang Autonomous Region, Hong Kong and Macao. Guangzhou is a sub-provincial city. It has direct jurisdiction over ten districts (Yuexiu, Liwan, Haizhu, Tianhe, Baiyun, Huangpu, Huadu, Panyu, Nansha, and Luogang) and two county-level cities (Conghua and Zengcheng). Guangzhou has a humid subtropical climate influenced by the Asian monsoon, with an annual average temperature of 21.8 ℃, rainfall of 1,694 mm, and a frost-free period of 345 days.

Trials were performed over two seasons, the early season of 2008 (from March 8th to July 12th) and the late season of 2008 (from July 16th to November 1st). The same field was used for the experiments in both seasons of the study and consisted of a sandy loam soil. The experimental site was left fallow during the winter. The first season was wet with high temperatures and a high humidity index, while the second was mild, dry and sunny as illustrated in **Figure 2.3**.

Early season of 2008: 5 March - 12 July

Late season of 2008: 12 July - 1 November

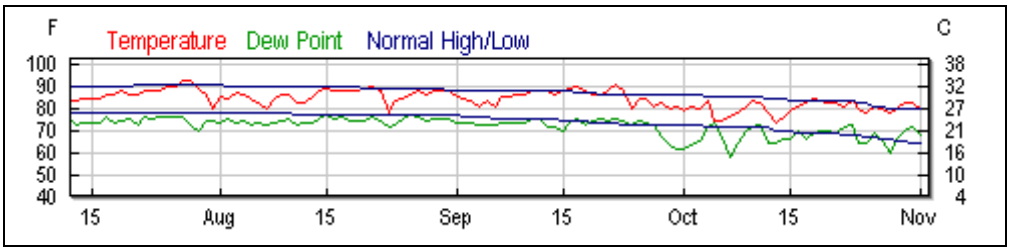


Figure 2.3 Temperature evolution during the early and late seasons of 2008 at SCAU experimental farm.

2.2 Chemicals used and their sources

Standard compounds

Authentic compounds used for identification in the headspace analysis were (*E*)-hexenal, hexanal, 1-nonanol, decanal, benzaldehyde and benzothiazole (Sigma, St. Louis, MO), heptanal, octanal, 3-methyl-1-butanol, 1-pentanol, 1-hexanol (Fluka, Buchs, Switzerland), 2,6-dimethylpyridine (2,6-DMP), 2,4,6-trimethylpyridine (2,4,6-TMP), toluene, 1-heptanol (BDH Chemicals, Poole, England),vanillin, guaiacol, 1-octanol, and nonanal (Merck, Darmstadt, Germany).

Stock solutions of standards were prepared with benzyl alcohol (Fisher, Loughborough, UK) and stored at -80 ℃ until use.

Synthesis of 2-acetyl-1-pyrroline

For 2-acetyl-1-pyrroline (2-AP) synthesis, 2-acetylpyrrole (Fluka, Buchs, Switzerland) was hydrogenated in methanol solution (Merck, Darmstadt, Germany) using

5% rhodium on an activated alumina catalyst (Fluka, Buchs, Switzerland) at room temperature under 10 psi of H_2 pressure (Buterry et al. 1983). The main product obtained, 2-(1-hydroxyethyl)pyrrolidine, was isolated, weighed and subjected to oxidation by refluxing with a stirred suspension of silver carbonate (Aldrich (Milwaukee, WI) on Celite (Fluka, Buchs, Switzerland) in toluene solution under a nitrogen atmosphere (Buttery et al. 1983). 2-AP was purified from the resulting mixture with the aid of a packed column (3% dimethylpolysiloxane coated on 80/100 mesh solid supports) of a Varian gas chromatograph (GC), model 2000 (Walnut Creek, CA). The principal peak for 2-AP emerging from the GC detector outlet was collected in a 3-mm-o.d. Pyrex tubes which was sealed under a N_2 atmosphere and stored at -20 ℃. Chemical structure of the purified 2-AP was first examined by capillary GC-MS. The major mass spectrum data (MS/El) at *m/z* 111 (27%), 83 (43%), 69 (21%), 68 (27%), 43 (100%), 42 (23%), 41 (60%) were the same as reported in the literature. Second, the chemical structure was examined by infrared (IR) at the end of which IR were also found to be consistent with what reported in the literature. The structure of 2-AP was latter confirmed by nuclear magnetic resonance (^{1}H NMR) spectroscopy by diluting an exact weight of the purified 2-AP in 1.0 mL of deuterated benzene (Merck, Darmstadt, Germany) spiked with a known quantity of tetramethylsilane (TMS) (Aldrich, Steinheim, Germany) used as an internal standard. 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 the TMS.

Plant growth regulators

Gibberellic acid 90%, paclobutrazol 95%, 3-indole acetic acid 99%, proline, and zinc chloride were purchased from Xiamen Topusing Chemical Co., Ltd (Fujian, P.R. China).

Other reagents

Reagents used for quality evaluation and enzyme activities were all procured from four different sources: Guanghua Chemicals (Guangzhou, P.R. China), Kermel (Tianjin, P.R China), Sinopharm Chemical (Shanghai, P.R. China), and Biocaro International (Hong Kong, P.R China). Pesticides and fertilizers were obtained from the same sources.

Water was purified through an Aquapro AWL-6001-P distillator (EverYoung, Guangzhou, P.R. China).

2.3 Techniques used for extraction and quantification of volatiles

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

Principle

Solid phase micro-extraction (SPME) involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (including both volatile and non-volatile) from different kinds of media, that can be in liquid or gas phase. The quantity of analyte extracted by the fiber is proportional to its concentration in the sample so long as equilibrium is reached or, in case of short time pre-equilibrium, with help of convection or agitation. After extraction, the SPME fiber is transferred to the injection port of separating instruments, such as a GC, where desorption of the analyte takes place and analysis is carried out.

The attraction of SPME is that the extraction is fast and simple and can be done without solvents, and detection limits can reach parts per trillion levels for certain compounds. The method also provides the advantage to incorporate extraction, concentration, and sample introduction in a single step, and coupled to a mass spectrometer **(Figure 2.4)** as detector, can easily help identify the compounds of interest (Pawliszyn, 1997).

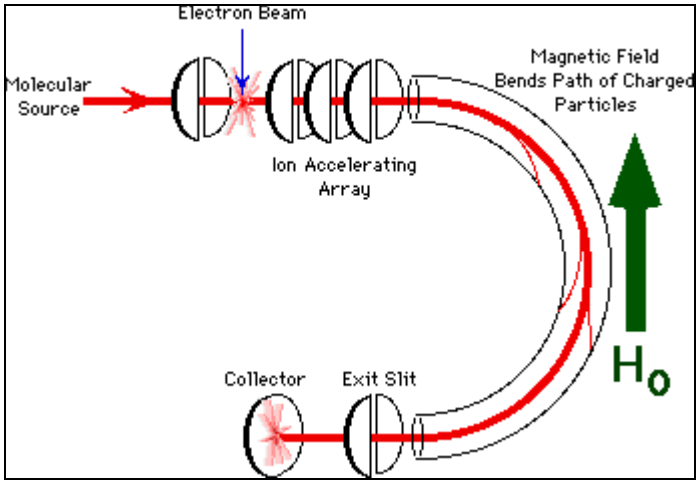


Figure 2.4 A schematic representation of a mass spectrometer.

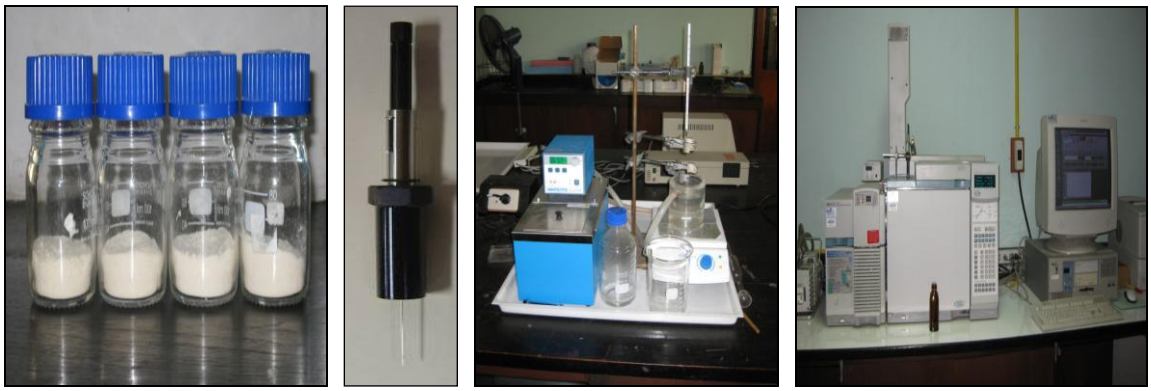
Rice samples preparation

All samples were dehulled and milled with a Jing Mi machine (Guangzhou, P.R China) to a 85% milling yield, brown rice basis. Only brown grains were analyzed by SPME/GC-MS. Ground samples were prepared by crushing of 30 g portions of rice grains in a household blender (Moulinex, Caen, France) for 30 s, 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.

Extraction and collection of volatiles

Extraction was carried out in an Autotherm heater (Walnut Creek, CA) with agitation **(Figure 2.5)**.



Brown rice in a 27-mL bottle | PDMS fiber | Extraction of volatiles in an Autotherm heater | GC/MS analysis

Figure 2.5 Procedure for headspace SPME/GC-MS analysis of rice volatiles.

Optimum operating parameters for the adsorption of rice volatiles using HS-SPME were taken as follow:

SPME	Optimum conditions
Brown rice powder size	20.0 g
Bottle size	27 mL
Extraction temperature	60 ℃
Extraction time	20 min
Internal standard (2,6-DMP 0.5 mg/L)	10.0 μL
Fiber type	PDMS
Fiber length	1 cm

A portion of brown rice powder weighted exactly 20.0 g was placed in a 27-mL bottle, and added with 10.0 μL of 2,6-DMP. The sample bottles were then sealed with an aluminum cap with hole placed on top of a PTFE/Silicone septa (Restek Corp., Bellefonte, PA). Volatile components from the headspace were collected by adsorption on the SPME fiber (Supelco, Bellefonte, PA), which was preconditioned in the GC injection port at 250 ℃ for 1 h before use. Extractions of all rice samples were performed in triplicate. Inside the bottle, the fiber was lowered from its protective sheath and exposed to the headspace of the sample for 20 min.

Gas chromatography analysis

After concentration of the volatiles, the fiber 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) with the following characteristics:

Oven parameters

OVEN	Optimum conditions
Maximum temperature	325 ℃
Equilibration time	0.50 min
Initial temperature	40 ℃ for 0 min
First ramp	4 ℃/min to 150 ℃
Second ramp	3 ℃/min to 220 ℃
Final temperature	250 ℃ for 5 min
Run time	50.83 min

Inlet parameters

INLET	Optimum conditions
Mode	Splitless
Equilibration time	0.50 min
Initial temperature	250 ℃
Pressure	7.00 psi
Purge flow	99.4 mL/min
Purge time	0.00 min
Total flow	103.5 mL/min
Saver flow	20.0 mL/min
Saver time	2.00 min
Gas type	Helium ON

Column parameters

COLUMN	Optimum conditions
Column type	Capillary AT-5MS
Maximum temperature	325 ℃
Nominal length	30.0 m
Nominal diameter	250.00 μm
Nominal film thickness	0.25 μm
Flow mode	Constant
Initial flow	1.0 mL/min
Nominal init pressure	7.00 psi
Average velocity	36 cm/s

The fiber was left there for reconditioning (15 min) before it was exposed to the headspace volatiles of the next sample.

Mass spectrometer parameters

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 following parameters:

MS parameters	Optimum conditions
Injection port temperature	250 ℃
MSD transfer line	280 ℃
Acquisition mode	Scan
EM absolute	False
EM offset	1365.0
Resulting EM voltage	2729.4
Low mass	35
High mass	400
Threshold	150
Acquisition rate	6.35 spectra/s
Turbo speed temperature	100 ℃
Quadrupole tremperature	150 ℃
Ion source temperature	230 ℃
Ionization energy	70 eV

Volatiles identification

Identification of most volatile compounds was performed tentatively according to their corresponding mass spectra, comparing them with the spectra of reference compounds in both the Wiley mass spectral library (6th Ed) and the NIST mass spectral library (v. 1.5a), and verified on the basis of mass spectra, and GC retention time values reported in the literature when available. Other compounds were positively identified by comparing their mass spectra and RI values with those of authentic compounds using the standard addition technique.

Retention time of each volatile was converted to the Kovats retention index (RI) using an alkane mixture (Fluka, Buchs, Switzerland) consisting of C_8–C_{20} alkanes (concentration of 40 mg/mL in hexane) as the references. Loading the alkane mixture onto the fiber was carried out by 5 min headspace extraction from a 27-mL SPME vial, including 1.0 mL HPLC-grade water spiked with 10 μL of the above-mentioned mixture. The Kovats index was calculated by the equation:

$$RI = 100 \times \left[n + (N - n) \frac{\log(t_{r(unknown)}) - \log(t_{r(n)})}{\log(t_{r(N)}) - \log(t_{r(n)})} \right]$$

where RI = Kovats retention index; *n* = the number of carbon atoms in the smaller alkane; N = the number of carbon atoms in the larger alkane; t_r = the adjusted retention time.

Expression of the relative concentration

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. Quantification was performed based on an internal standard method using the formula:

$$\text{Concentration (ng/g)} = \frac{\text{Compound area} \times \text{2,6-DMP concentration (ng/}\mu\text{L)} \times \text{Injection volume (}\mu\text{L)}}{\text{2,6-DMP area} \times \text{Rice sample weight (g)}}$$

The results were expressed as the average of three replicates of each rice cultivar.

2.3.2 Static headspace (SHS-GC/NPD) for quantitation of 2-acetyl-1-pyrroline

Principle

In static headspace, the sample is sealed into a vessel, warmed, and then a sample of the atmosphere surrounding the sample is withdrawn and injected into the injection port of the GC. For quantitative analysis of 2-AP, static headspace coupled to a nitrogen phosphorous detector (NPD) was validated to extract, both efficiently and conveniently, micro-volatile organic nitrogen-containing heterocyclic compounds from rice plants (Sriseadka et al. 2006). NPD is based on the flame ionization detector (FID) but differs in that it contains a rubidium or cesium silicate (glass) bead situated in a heater coil, a little distance from the hydrogen flame. The heated bead emits electrons by thermionic emission, hence the name thermionic detector. These electrons are collected under a potential of a few volts by an appropriately placed anode, and provides a background current. When a solute containing nitrogen or phosphorous is eluted from the column, the partially combusted nitrogen and phosphorous materials are adsorbed on the surface of the bead. The adsorbed material reduces the work function of the surface and, as consequence, the emission of electrons is increased which raises the current collected at the electrode **(Figure 2.6)**. The sensitivity of the detector to phosphorous is about 10-12 g/mL and for nitrogen about 10-11 g/mL at a signal to nose ratio of two.

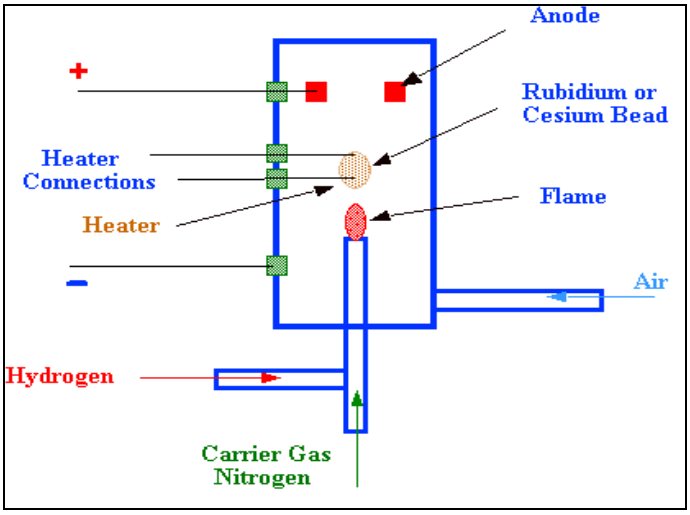


Figure 2.6 Schematic of a Nitrogen Phosphorus Detector (NPD).

Rice samples preparation

Both brown and white rice sample were ground (Moulinex, Caen, France) into a powder (less than 0.3 mm in diameter) and used immediately. Rice powder weighted exactly 1.0 g was placed into a 20 mL headspace vial, followed by the addition of 1.0 μL of 2,6-DMP in benzyl alcohol.

Sample preparation	Optimum conditions
Brown rice powder size	1.0 g
Vial size	20 mL
Internal standard (2,6-DMP 0.5 mg/L)	1.0 μL

Rice powder in a 20 mL vial | Static headspace autosampler | GC analysis

Figure 2.7 Procedure for Static headspace/GC analysis of rice volatiles

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/NPD as shown in **Figure 2.7.**

Headspace autosampler conditions

SHS-GC/NPD analyses were carried out using an Agilent 6890N GC model equipped with a G1888 headspace autosampler, 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:

Headspace parameters	Optimum conditions
Extractions per vial	1
Oven stabilization time	1.0 min
GC cycle time	30.0 min
Injection time	0.40 min
Injection volume	1.00 μL
Syringe size	10.0 μL
Loop equilibration time	0.60 min
Loop filling time	0.01 min
Loop temperature	130 ℃
Oven temperature	120 ℃
Shake	High shaking
Transfer line temperature	140 ℃
Vial equilibration time	9.0 min
Vial pressurization time	0.10 min
Sample loop	3.00 mL

Gas chromatography analysis

Sample headspace was collected through a 3 mL sample loop and automatically transferred to the GC via a heated transfer line with the following parameters:

Oven parameters

OVEN	Optimum conditions
Maximum temperature	280 ℃
Equilibration time	0.10 min
Initial temperature	50 ℃ for 0 min
Ramp	5 ℃/min to 125 ℃
Final temperature	225 ℃ for 8 min
Run time	15.0 min

Inlet parameters

INLET	Optimum conditions
Mode	Splitless
Equilibration time	0.00 min
Initial temperature	230 ℃
Pressure	3.78 psi
Purge flow	24.8 mL/min
Purge time	0.50 min
Total flow	32.9 mL/min
Saver flow	20.0 mL/min
Saver time	2.00 min
Gas type	Helium OFF

Column parameters

COLUMN	Optimum conditions
Column type	Capillary HP-5MS
Column characteristics	Agilent 19095J-323
Maximum temperature	300 ℃
Nominal length	30.0 m
Nominal diameter	530.00 μm
Nominal film thickness	1.50 μm
Flow mode	Constant
Initial flow	5.0 mL/min
Nominal init pressure	3.78 psi
Average velocity	37 cm/s
Outlet pressure	Ambient

An HP-5MS (5% phenylmethylsiloxane) fused silica capillary column (J&W Scientific Inc., Folsom, CA) was used for the GC when using NPD as detector.

NPD parameters

DETECTOR	Optimum conditions
Nature	NPD
Temperature	300 ℃
Hydrogen flow	3.0 mL/min
Air flow	60.0 mL/min
Flow mode	Constant
Makeup flow	15.0 mL/min
Makeup gas type	Nitrogen
Adjust offset	30.00
Electrometer	ON
Bead	ON
Equilibration time	5.00 min
Signal data rate	20 Hz

Identification and quantification of 2-AP

GC retention indexes on HP-5MS columns were calculated and compared with those of the synthetic 2-AP, and enabled us to positively identify 2-AP. Each sample was analyzed in triplicates.

For quantification of 2-AP, a standard curve was constructed from a concentration series of 2-AP (0.02 to 8 μg/g) prepared in benzyl alcohol and subjected to SHS-GC/NPD analysis under conditions identical to those described above. A non-fragrant rice (Pijit) was used as supporting material in the calibration procedure. It was obtained from the Department of Agronomy of Chiang Mai University in Thailand.

Non-fragrant rice size (g)	Synthetic 2-AP (μg)	Synthetic 2-AP/rice size (μg/g)	2,6-DMP Area	Synthetic 2-AP/rice size area
1	0.02	0.02	3800.2	22.4
1	0.05	0.05	3890.9	42.0
1	0.10	0.10	3802.8	86.9
1	0.80	0.80	3913.2	566.6
1	1.60	1.60	3863.8	1091.6
1	2.40	2.40	3569.2	1408.8
1	3.20	3.20	3745.1	2085.1
1	4.00	4.00	3709.2	2505.2
1	6.00	6.00	3472.8	3580.0
1	8.00	8.00	3404.5	4616.9

Plot of concentrations of standard 2-AP (synthetic 2-AP/rice powder size in μg/g) against the corresponding peak areas (synthetic 2-AP/rice size area) divided by peak area of the internal standard (2,6-DMP area) yielded a linear calibration curve **(Appendix 1)** 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 (μg/g).

2.4 Extraction and quantification of lipid degradation volatile and non-volatiles products

2.4.1 Static headspace (SHS-GC/FID) analysis of lipid oxidation volatiles

Principle

The deterioration of rice lipids involves autoxidation of unsaturated fatty acids. These oxidative reactions result in the formation of monohydroperoxides, which eventually break down into a variety of products, some of which are volatile. The products include

aldehydes, ketones, alcohols, furanones, acids, lactones, and hydrocarbons. For extraction and semi-quantification of lipid oxidation volatiles, the headspace procedure and conditions were designed to identify a wide range of compounds of interest. Instead of a nitrogen phosphorous detector, a flame ionization detector (FID) was used **(Figure 2.8)**.

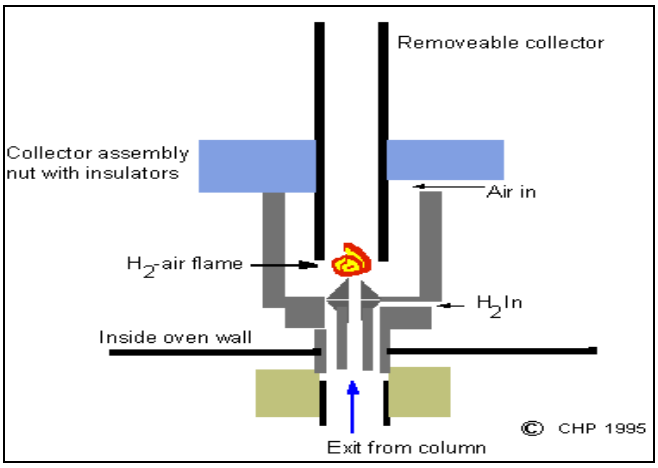


Figure 2.8 Schematic of a Flame Ionization Detector (FID).

As the name suggests, FID analysis involves the detection of ions. The source of these ions is a small hydrogen-air flame producing at high temperature positively charged ions and electrons. In order to detect these ions, two electrodes are used to provide a potential difference. The positive elementary doubles as the nozzle head where the flame is produced. The other, negative electrode is positioned above the flame. The ions thus are attracted to the collector plate (negative electrode) and upon hitting the plate, induce a current. This current is measured with a high-impedance pico-ammeter and fed into an integrator.

Rice samples preparation

Paddy grains were sun-dried, dehulled, and milled in the laboratory to remove approximately 15% bran. Only brown rice samples were used for the analysis. The rice samples were chilled at 4 ℃ for 24 h before they were ground (Moulinex, Cean, France) into a powder (less than 0.3 mm in diameter) and used immediately. Rice powder weighted exactly 3.0 g was placed into a 20 mL headspace vial, followed by the addition of 5.0 μL of 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/FID.

Sample preparation	Optimum conditions
Brown rice powder size	3.0 g
Vial size	20 mL
Internal standard (2,6-DMP 0.5 mg/L)	5 μL

Headspace autosampler conditions

SHS/GC analyses were carried out using an Agilent 6890N GC model equipped with a G1888 headspace autosampler, 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:

Headspace parameters	Optimum conditions
Extractions per vial	1
Oven stabilization time	1.0 min
GC cycle time	30.0 min
Injection time	0.40 min
Injection volume	1.00 μL
Syringe size	10.0 μL
Loop equilibration time	0.60 min
Loop filling time	0.01 min
Loop temperature	130 ℃
Oven temperature	120 ℃
Shake	High shaking
Transfer line temperature	140 ℃
Vial equilibration time	9.0 min
Vial pressurization time	0.10 min
Sample loop	3 mL

Gas chromatography analysis

Sample headspace was collected through a 3 mL sample loop and automatically transferred to the GC via a heated transfer line with the following parameters:

Oven parameters

OVEN	Optimum conditions
Maximum temperature	280 ℃
Equilibration time	0.10 min
Initial temperature	50 ℃ for 0 min
Ramp	5 ℃/min to 200 ℃
Final temperature	225 ℃ for 3 min
Run time	33.0 min

Inlet parameters

INLET	Optimum conditions
Mode	Splitless
Equilibration time	0.00 min
Initial temperature	230 ℃
Pressure	22.07 psi
Purge flow	29.6 mL/min
Purge time	0.50 min
Total flow	35.4 mL/min
Saver flow	20.0 mL/min
Saver time	2.00 min
Gas type	Helium OFF

Column parameters

COLUMN	Optimum conditions
Column type	Capillary HP-5MS
Column characteristics	Agilent 19091J-216
Maximum temperature	325 ℃
Nominal length	60.0 m
Nominal diameter	320.00 μm
Nominal film thickness	1.00 μm
Flow mode	Constant
Initial flow	3.0 mL/min
Nominal init pressure	22.05 psi
Average velocity	37 cm/s
Outlet pressure	Ambient

FID parameters

DETECTOR	Optimum conditions
Nature	FID
Temperature	250 ℃
Hydrogen flow	30.0 mL/min
Air flow	300.0 mL/min
Flow mode	Constant
Makeup flow	10.0 mL/min
Makeup gas type	Nitrogen
Lit offset	2.0
Electrometer	ON
Flame	ON
Signal data rate	20 Hz

An HP-5MS (5% phenylmethylsiloxane) fused silica capillary column (J&W Scientific Inc., Folsom, CA) was used and each sample was analyzed in triplicate.

Choice of standard lipid-derived products

A reference mixture consisting of a known amount of the standard volatile compounds and internal standard (2,6-DMP) was also subjected to the SHS-GC/FID analysis. The choice of standard compounds was based on the major lipid-derived compounds previously identified in rice and covered a wide range of alcohols and aldehydes.

Volatile identification

Rice sample volatiles were identified by their GC retention times relative to those of standards run under the same conditions, and by comparison of the retention indices (RI) with the literature data. RI of the analytes of interest were calculated based on the linear temperature programme retention index (LTPRI) method, using the mixture of alkanes C_8–C_{20} (concentration 40 mg/mL in hexane) as retention index probs (Fluka, Buchs, Switzerland). RI probes were loaded onto the headspace vial prior to the sample extraction and the Kovats index was calculated by the equation:

$$RI = 100 \times \left[n + (N - n) \frac{\log(t_{r(unknown)}) - \log(t_{r(n)})}{\log(t_{r(N)}) - \log(t_{r(n)})} \right]$$

where RI = Kovats retention index; n = the number of carbon atoms in the smaller alkane; N = the number of carbon atoms in the larger alkane; t_r = the adjusted retention time.

Expression of the relative concentration

The concentration of the volatile components was calculated using the response factor determined for each volatile compound. To obtain recovery factors for individual volatile components, a reference mixture consisting of a known amount of the standard volatile compounds and internal standard (2,6-DMP) was subjected to SHS-GC/FID analysis. The relative recovery factor of compounds of interest was determined relative to the internal standard:

$$\text{Recovery factor} = \frac{\text{Compound area (extract)} \times \text{2,6-DMP area (direct injection)}}{\text{2,6-DMP area (extract)} \times \text{Standard area (direct injection)}}$$

Peak areas were obtained with the aid of the instrument's digital integrator and the concentration expressed as:

$$\text{Concentration (ng/g)} = \frac{\text{Compound area} \times \text{2,6-DMP concentration (ng/}\mu\text{L)} \times \text{Injection volume (}\mu\text{L)}}{\text{2,6-DMP area} \times \text{Rice sample weight (g)} \times \text{Recovery factor}}$$

The results were expressed as the average of three replicates of each rice cultivar with most of the components showing satisfactory recoveries ranging from 0.9 to 1.0.

2.4.2 Determination of malondialdehyde (MDA) content

Principle

Lipid peroxidation has been established as a major mechanism of cellular injury in many biological systems of plant and animal origin. The mechanism involves a process whereby unsaturated lipids are oxidized to form additional radical species as well as toxic by-products that can be harmful to the host system. Polyunsaturated lipids are especially susceptible to this type of damage when in an oxidizing environment and they can react to form lipid peroxides. Lipid peroxides are themselves unstable, and undergo additional decomposition to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides further react to form malondialdehyde (MDA). MDA can be found in most biological samples as a result of lipid peroxidation, and has become one of the most widely reported analytes for the purpose of estimating oxidative stress effects on lipids. In our study, MDA content was measured as an additional lipid peroxidation marker in growing rice grains. The assay is based on the reaction of MDA with thiobarbituric acid (TBA), forming a MDA-TBA_2 adduct (white color for grains and orange color for leaves) that absorbs strongly at 532 nm.

Chemicals

Extraction buffer: 0.05 M potassium phosphate buffer, pH 7.8 (checked with a Mettler Toledo Delta 320 pH, Mettler Toledo Inc; Columbus, USA); 1% insoluble polyvinylpolypyrrolidone (PVPP).

Reagents: 0.5% thiobarbituric acid (TBA) (light yellow; not to be refrigerated; stable at least one week; and dissolved with heating).

Harvesting and extraction of MDA from samples

Two panicles and 10 flag leaves from the centre four rows of paddy plots were selected and transported to the laboratory under dry ice. Fresh rice grains were separated by shaking and immediately dehulled by hands. The midrid and petioles of leaves were discarded. Cooled tissues (three samples of approximately 0.2 g fresh weight per treatment) were then ground to a fine powder in a chilled mortar and pestle, in the presence of 0.3 g washed quartz sand, and then homogenized for 1 min in 3 mL of extraction buffer. All operations were carried out at 4 ℃. The homogenates were centrifuged at 20,000 × *g* for 15 min at 4 ℃ (Hema TGL-16 R refrigerated centrifuge, Shanghai, P.R China), and the white supernatant fraction kept at 4 ℃ in a Haier BCD 195K refrigerator (Haier, Qingdao, P.R China) until analysis.

MDA colorimetric assay

The method used was modified from the one proposed by Buege and Aust (1978). The total volume of reaction mixture of 3.5 mL was heated in a KW-1000 DA boiling water bath (Guangzhou Sinosource co., Ltd, Guangzhou, P.R China) for 20 min at 100 ℃ and allowed to cool rapidly in an ice bath (development of a light white color with the grains and an orange color with the leaves). The assay mixture consisted of the following:

Reagent	Assay	Blank
0.5% TBA (mL)	2.5	2.5
Supernatant (mL)	1.0	0.0
0.05 M Phosphate buffer pH 7.8 (mL)	0.0	1.0
Heating in a boiling water bath at 100 ℃ for 20 min		
Centrifugation at 10,000 × *g* for 10 min		
Spectrophotometric determination at 532, 600, and 450 nm		

After a second centrifugation at 10,000 × *g* for 10 min, and at 25 ℃ (HIMAC centrifuge, Hitachi SCR20BC, Japan), the resulting supernatant was incubated at room temperature for 5 min, and was used for spectrophotometric determination of MDA using a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). Absorbance at 532 nm (representing the maximum absorbance of the TBA-MDA complex) was recorded and corrected for non specific turbidity at 600 nm and interference generated by TBA-sugar complexes at 450 nm. MDA concentrations were calculated by means of an extinction

coefficient of 156/mM/cm and expressed in μmol/g fresh weight using the equation:

$$\text{MDA content } (\mu\text{mol/g}) = \frac{\text{Extraction volume (mL)} \times [6.45 \times (\text{OD532} - \text{OD600}) - 0.56 \times \text{OD450}]}{\text{Sample weight (g)} \times \text{Supernatant volume (mL)}}$$

2.5 Sensory evaluation protocol

For sensory analysis, 2 trained panelists and 12 untrained students were selected. The profile used by the panelists included only one attribute that described rice odor of brown rice powder and of cooked milled rice. Three samples were chosen for each treatment and each sample was exposed to the panelists twice.

2.5.1 Sniffing test for warmed brown rice powder

In the first test, a portion of brown rice powder weighted exactly 20.0 g was placed in a 27-mL bottle and sealed with an aluminum cap. Cooking without water was carried out in an Autotherm heater (Walnut Creek, CA) with agitation for 20 minutes at 80 ℃. The warm cooked rice powder was filled into sniffing bottles presented to the panelists who were required to smell and tell which of the samples has the most intense odor.

2.5.2 Hedonic text for cooked milled rice

In the second test, portions of milled rice were rinsed, and then cooked using a QLT-3651 rice cooker (Taiwan Quality Group CO., Taiwan) with a rice:water ratio of 1:2. The amount of time the red light of the cooker was on was considered as the cooking time. After cooking, samples were held for 10 min in the cooker before testing. The aroma intensities of all rice samples were rated on a hedonic scale of 5 points as shown below.

Score	Description
0	non-aromatic
1	subtle aromatic
2	clearly aromatic but not as strong as Guixiangzhan/Peizaruanxiang
3	aromatic and strong as Guixiangzhan/Peizaruanxiang
4	more aromatic than Guixiangzhan/Peizaruanxiang

The reference samples of Guixiangzhan and Peizaruanxiang grown without growth regulators were presented to the panelists before test, and the two cultivars were tested in different sessions.

2.6 Yield and yield components evaluation

Yield and yield components were estimated as outlined by Yang et al. (2007). The following attributes were taken into consideration:

2.6.1 Number of panicles per hill

Tillers were counted and separated into panicle bearing and non-bearing plants. Panicle stand counts of each hill were taken at physiological maturity by counting the number of effective tillers (main stems) in the centre two rows from an area 2.8 m in length in each plot. Twenty hills were chosen the result recorded as panicle number per hill using the formula:

$$\text{Number of panicles/Hill} = \frac{\text{Effective tillers for hill 1} + \ldots \text{Effective tillers for hill 20}}{20}$$

2.6.2 Number of spikelets per panicle

All panicles of two hills were collected and hand threshed in the laboratory. The total number of spikelets was counted and recorded as the total number of spikelets per panicle:

$$\text{Number of spikelets/Panicle} = \frac{\text{Spikelet number for hill 1} + \text{Spikelet number for hill 2}}{\text{Number of panicle per hill} \times 2}$$

Filled spikelets were later separated from unfilled spikelets by submerging them in tap water. Filled spikelets were taken to count the number of ripened spikelets per panicle.

Number of ripened spikelets/Panicle = Total spikelets per panicle – Unfilled spikelets

2.6.3 Grain-filling percentage

Grain-filling percentage was calculated according to the formula:

$$\text{Grain-filling percentage (\%)} = \frac{\text{Ripened spikelet number}}{\text{Total spikelet number}} \times 100$$

2.6.4 Weight of 1000-grains rough rice

Panicles were threshed to separate seeds. The separated seed were weighed, and the resulting 1000-seeds weight recorded using a Sartorius BT 124S scale (Sartorius scientific instruments, Beijing, P.R China).

2.6.5 Average grain yield

Grain yield was determined from the 5-m^2 area in each plot and collected by harvesting the center four rows with a small plot combine (200 hills in total). Yield samples from the 200 hills were weighed and adjusted to the standard moisture content of 12-14%. The moisture content of the paddy at maturity was determined by the standard oven method. Approximately 5 g of rice grain was accurately weighed into a moisture dish. The sample was dried in a vacuum oven (Bluepard DHG-9240A, Shanghai, P.R China) at 70 ℃ until a constant weight was reached. The dishes were taken from the oven and cooled for 10 min in a desiccator. The moisture content was calculated as a percentage with the weight lost corresponding to moisture lost using the equation:

$$\text{Moisture content (\%)} = \frac{\text{Weight sample before drying (g)} - \text{Weight sample after drying (g)}}{\text{Weight sample before drying (g)}} \times 100$$

After a constant weight was attained, the average grain yield was calculated using the following formula in which "10" represents the conversion factor from kg/m^2 to t/ha:

$$\text{Yield (t/ha)} = \frac{\text{Total hills per plot x Yield of selected hills (kg)}}{\text{Number of hills selected x Plot area } (m^2)} \times 10$$

2.7 Milling quality evaluation

The milling quality of rice may be defined as the ability of rice grain to stand milling and polishing without undue breakage so as to yield the greatest amount of total recovery and the highest proportion of head rice to broken (Juliano and Villareal, 1993). The milling process generally consists of five fundamental operations which are (1) cleaning the rough rice to remove leaves, rice stems and other foreign matter, (2) shelling or dehulling the cleaned rice to remove the hulls and form the brown rice (3) cleaning the brown rice to remove hulls not totally removed by dehulling (4) milling or polishing the brown rice to

remove the bran and produce milled rice, and (5) separating whole grains from broken kernels. Prior to milling and in order to prevent deterioration after harvest, paddy is usually dried down to a level of water activity that will enable safe storage by reducing respiration, inhibiting mould growth and preventing production of mycotoxins. This corresponds to a moisture content of about 12-14%, which is considered adequate for safe storage, milling and further storage as milled rice. In our case, samples of Guixiangzhan and Peizaruanxiang were threshed and sun-dried to about 14% moisture content for two weeks. Seeds of each cultivar for individual plots were then packaged, labelled and stored for 3 months at ambient temperature before processing to ensure stable milling yields.

2.7.1 Brown rice rate

Duplicate 80-g rough rice samples for each plot were dehulled with a SDL-A type testing husker, model JY7134 (Shanghai, P.R. China). First, the roller distance of the dehusking machine was adjusted to 0.90 mm and this for the two cultivars. The samples were then poured into the hopper with two passes per sample. The weight of the cleaned brown rice as well as the weight of the remaining rough rice (hulled samples) was recorded. Average brown rice rate was calculated from three replications of each treatment using the formula:

$$\text{Brown rice rate (\%)} = \frac{\text{Weight of brown rice (g)}}{\text{Weight of original rough rice (g)} - \text{Weight of remaining rough rice (g)}} \times 100$$

2.7.2 Milled rice rate

A representative sample (40 g) of the brown rice obtained as described above was then polished by an abrasive JNMJ-6 type polisher (Guangzhou, P.R. China) with the 680 g added weight on the pressure cover for 50 sec, followed by 20 sec without the added weight, to obtain a typical degree of polish of c. 8%. The fraction removed was considered bran in the first milling and that after the second milling, polish. All samples were replicated three times. The milled rice sample was collected in a jar or thick paper bag and sealed immediately. The weight of the cleaned polished rice was recorded. Milled rice yield was expressed as the percentage ratio of milled rice after husking and polishing, to the weight of unmilled paddy (brown rice).

$$\text{Milled rice rate (\%)} = \frac{\text{Weight of total milled rice (g)}}{\text{Weight of brown rice (g)} \times \text{Brown rice rate (\%)}} \times 100$$

2.7.3 Head rice rate

Head rice yield was determined by sizing milled rice with a JFQS-13x20 testing rice grader using a 4.75-mm mesh indentation (Guangzhou, P.R. China). Two plates of the same size were used for each run. Broken kernels were discarded and the weight of the resulting whole grain fraction recorded. Head rice yield was expressed as the percentage ratio of the weight of whole (unbroken) kernel to the weight of brown rice.

$$\text{Head rice rate (\%)} = \frac{\text{Weight of whole rice (g)}}{\text{Weight of brown rice (g)} \times \text{Brown rice rate (\%)}} \times 100$$

2.8 Estimation of grain vitreosity and percentage area with chalkiness

Grain appearance depends upon the size and shape of the kernel but also the chalkiness of the grain either on the dorsal side (white belly), the ventral side (white back) or in the centre (white centre). The starch granules in the chalky areas are less densely packed as compared to translucent areas. Therefore, the chalky areas are not as hard as the translucent areas with grains with chalkiness more prone to breakage during milling (Juliano and Villareal, 1993). Rice samples with high chalkiness and hence low vitreosity have poor appearance and low market value.

The grain vitreosity was estimated on three samples of 100 milled grains. Only mature whole rice grains were used. A magnifying glass (SDE-A glass type, Guangzhou, P.R. China) was used to identify chalky grains. Grains were placed on the glassy table lit with a 60-W electric lamp. Milled grains were visually observed and grains with short spots of pearl were considered as chalky. The traits were expressed in percent as follow:

$$\text{Grain vitreosity (\%)} = 100 - \frac{\text{Number of chalky grains}}{\text{Total number of milled grains}} \times 100$$

Milled grains were also visually scored for the percentage area with chalkiness. For that, 10 chalky grains were randomly selected and were placed horizontally under the spotlight. Endosperm chalkiness of milled samples was classified on a 0 to 9 scale according to increasing intensity, using the following scale:

Scale	% area with chalkiness
0	None
1	Less than 10%
5	10 to 20%
9	More than 20%

2.9 Determination of the apparent amylose content

Principle

Many of the cooking and eating characteristics of milled rice are influenced by the ratio of two kinds of starch: amylose and amylopectin. Amylose is a linear polymer of glucose (usually in the range of 300 to 3000 units) linked mainly by α(1→4) bonds. The common test for amylose is to mix it with a small amount of yellow iodine solution. Iodine molecules fit neatly inside the helical structure of amylose, developing a blue-black color whose intensity can be tested at 620 nm wavelengths of light. Amylose starch is less readily digested than amylopectin and at amylose concentration of more than 25%, amylopectin shows increased iodine binding instead of amylose. Therefore, apparent amylose content is usually reported.

Chemicals

Reaction solution: 95% ethanol, 1 M NaOH (40 g anhydrous NaOH in 1000 mL distilled water), 1 M acetic acid 99.5% (approximately 5.775% v/v, 57.75 mL of glacial acetic acid in 1000 mL final solution).

Iodine solution: 0.2% iodine (I_2), 2% potassium iodide (KI). The iodine solution consisted of 0.2 g I_2 and 2 g KI in 100 mL of boiled aqueous solution (dark-red in color and to be prepared daily).

Standard amylose: 0.4 % potato amylose.

Preparation of rice flour

A representative milled rice sample (20 whole grains) was ground in a SDM-A cyclone mill (Mofenji, Guangzhou, P.R. China) with 60-mesh sieve and 0.1 g of rice powder analyzed for amylose content according to the manual method described by Juliano and Villareal (1993).

Measurement of amylose

The proximate composition of 0.1 g rice powder in amylose content was determined by introducing into a 100-ml volumetric flask the powder and the necessary reagents as shown in the table below. After the addition of 95% ethanol into the volumetric flask, any sample adhering to the flask was washed down with distilled water. NaOH was then added and the content immediately immersed in a 100 ℃ KW-1000 DA water bath (Guangzhou Sinosource co., Ltd, Guangzhou, P.R China) for 10 min in order to gelatinize the starch. After cooling for 60 min, the volume was made up with distilled water and a 5-ml aliquot collected for iodine coloration at 620 nm (Shimadzu UV-2450 spectrophotometer, Tokyo, Japan).

Reagents	Assay	Blank
95% ethanol (mL)	1	1
1 M Sodium hydroxide (mL)	9	9
Heating in a boiling water at 100 ℃ for 10 min		
Cooling at room temperature for 60 min		
Distilled water (mL)	90	90
Vortex, and then levy 5 mL starch solution		
Starch solution levied (mL)	5	5
1 M glacial acetic acid (mL)	1	1
Iodine solution (mL)	2	2
Distilled water (mL)	92	92
Homogenization and incubation at room temperature for 20 min		
Spectrophotometric determination at 620 nm		

Standard curve for amylose

Two different methods were used to construct the standard curve.

Reagents	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Standard solution (mL)	0.0	1.0	2.0	3.0	4.0	5.0
1 M acetic acid (mL)	0.0	0.2	0.4	0.6	0.8	1.0
Iodine solution (mL)	2.0	2.0	2.0	2.0	2.0	2.0
Distilled water (mL)	98.0	96.8	95.6	94.4	93.2	92.0
Standard solution (mg)	0	4	8	12	16	20
Homogenization and incubation at room temperature for 20 min						
Spectrophotometric determination at 620 nm						

With the first method, 400 mg of potato amylose of known moisture content were mixed with 10 mL ethanol and 90 mL 1 M NaOH, heated for 10 min in a boiling water bath, and then cooled at room temperature for 60 min (standard solution). Different volumes (0, 1, 2, 3, 4, 5 mL) of the standard solution were introduced in 100-ml volumetric flasks. The standard solution was acidified with 1 M glacial acetic acid and treated as shown in the below table. A standard curve was made with each set of unknown samples by plotting the absorbance of check milled samples against their known amylose content.

Apparent amylose content in the samples was then determined from the calibration curve using a conversion factor including the dilution factor of 20 (initial starch solution of 100 mL divided by the aliquot of 5 mL), and the results expressed on a dry weight basis (mg/100 mg) using the formula:

$$\text{Amylose content (\%)} = \frac{\text{Value from the curve (mg)} \times \text{Initial starch solution (mL)}}{\text{Starch solution levied (mL)}}$$

For each set of samples run, waxy, very low, intermediate and high amylose standard cultivars were simultaneously included to serve as checks, with concentrations of 0.0%, 1.6%, 11.0%, 18.5%, and 26.4%, respectively. A calibration curve (see **Appendix 2**) was then constructed from which the apparent amylose content was directly determined.

Amylose content classification

Amylose content in the samples was classified as follow:

Score	Classification	Amylose content (%)
1	Waxy	0.0-5.0
2	Very low	5.1-12.0
3	Low	12.1-18.0
4	Intermediate	18.1-25.0
5	High	>25.0

2.10 Estimation of protein content by the Kjeldahl method

Principle

The method consists of mineralizing the sample with concentrated sulphuric acid and alkalizing with sodium hydroxide solution. Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonium sulfate, and other organic matter to $C0_2$ and H_20. The ammonium solution is then made alkaline by

addition of sodium hydroxide (neutralization), which converts the ammonium sulfate into ammonia gas collected by distillation and recovered in an excess boric acid solution. The low pH of the solution converts the ammonia gas into the ammonium ion, and simultaneously converts the boric acid to the borate ion. Subsequent titration with hydrochloric acid made it possible to calculate the initial amount of ammonium present in sample, using a suitable indicator to determine the end-point of the reaction (color change of the solution from green to purple).

Chemicals

Kjeldahl catalyst: Potassium sulphate (K_2SO_4), copper (II) sulphate ($CuSO_4, 5H_2O$). The copper catalyst contained 100 g K_2SO_4 and 10 g $CuSO_4, 5H_2O$.

Digestion solution: Kjeldahl catalyst, sulphuric acid 96% (d = 1.84 and to be used with a mask and gloves), distilled water.

Indicator solution: 95% ethanol, methyl red, bromocresol green. The methyl red solution was prepared by dissolving 100 mg in 100 mL boiled ethanol with stirring (red blood in color). Likewise, the bromocresol green solution was prepared by dissolving 100 mg in 100 mL boiled ethanol with stirring (red light in color).

Distillation solution: Boric acid 1% (p/v) for Kjeltec 2300/2400 and 4% (p/v) for Kjeltec 2100/2200, NaOH 40% (w/w) (Alkali Solution). To prepare the boric acid solution (Receiver Solution), 10 g of boric acid was dissolved in 600 mL boiled water, the content completed to 900 mL, stirred and cooled at room temperature. Then 10 mL of the bromocresol green solution and 7 mL of the methyl red solution were added. The volume was made up to 1000 mL with distilled water (green in color).

Titration solution: 0.1N hydrochloric acid.

Sample preparation

Twenty whole grains of milled rice were ground and homogenized in a SDM-A cyclone mill (Mofenji, Guangzhou, P.R. China) with 60-mesh sieve. Nearly 0.2 g of rice flour was weighed on a nitrogen-free paper and placed in the digestion tube (Juliano and Villareal, 1993). A blank test was also prepared, using 5 mL of distilled water instead of the sample.

Digestion, distillation and titration

Reagents were added to the digestion flask containing the flour sample. Digestion tubes containing the samples were put into a bloc-digest unit with the fume extractor on

(Digestor 2040, Foss Tecator, Shanghai, P.R. China). The digestion was carried out with a programme set at 125 ℃ for 30 min, 200 ℃ for 30 min, and 400 ℃ for 120 min.

Reagents	Assay	Blank
Rice flour (g)	0.2	0.0
Distilled water (mL)	0	5
Kjeldahl catalyst (g)	2	2
96% sulphuric acid (mL)	5	5
Digestion at 125 ℃ (20 min) 200 ℃ (30 min) 400 ℃ (120 min), and cooling		
Distilled water (mL)	30	30
40% NaOH (mL)	30	30
1% boric acid solution (mL)	30	30
Distillation for 2 min in the Kjeltec Analyzer		
Hydrochloric acid (N)	0.1	0.1
Titration until purple color		

At the end of the process, the liquid obtained was a transparent blue in color. The sample was then cooled at ambient temperature, resulting in a white color. After digestion, the samples were transferred to the Kjeltec Analyser unit (Foss 2300/2400, Shanghai, P.R. China). The distillation was carried out for 2 min. The distillate obtained was titrated with 0.1 N hydrochloric acid until the solution changed in color from green to purple.

Detected nitrogen and protein

Protein contents of the samples were obtained using the below equation, the first expression of the equation representing the nitrogen content in percentage.

$$\text{Protein content (\%)} = \frac{1.4 \times N \times (V1-V0) \times F}{m} \times 5.95$$

N being the hydrochloric acid normality, V1 the hydrochloric acid volume used in the titration (mL), V0 the hydrochloric acid volume used in the blank test (mL), F the correction factor of 0.1 N HC1, m the sample weight in g, and 5.95 the protein conversion factor for rice.

2.11 Studies on the relationship between proline and 2-acetyl-1-pyrroline biosynthesis

Ten young and fully expanded flag leaves and two panicles were randomly obtained from the centre of each plot at least four rows from the border, and immediately brought to the laboratory in boxes cooled up to 4 ℃. The panicles were gently threshed, and then impurities and unfilled grains removed by manual sorting. Fresh rice grains were dehulled by hands to obtain brown rice. Before analysis, the midrid (main vein) and petioles (leaf stalk) of the leaves were discarded, and the remaining parts deribbed and chopped into small pieces with a scissor. Grain and leaf samples were stored in plastic bags at -20 ℃ in a Sanyo medical freezer VR-L6111W (Sanyo Electric Co; Ltd, Moriguchi, Japan) until further treatment or analysis.

2.11.1 Assay for proline oxidase (POX) activity

Principe

Proline oxidase (POX, EC: 1.5.99.8) is a flavin containing bifunctional enzyme that catalyzes the first step in proline degradation (the oxidation of proline to pyrroline-5-carboxylate in the presence of oxygen and water) and thus initiates a sequence of reactions whereby proline can directly contribute its carbons to the tricarboxylic acid cycle as α-ketoglutarate. The specific activity of proline oxidase increases with age and the enzyme is a transcriptional repressor of the genes in the proline biosynthetic pathway. Proline oxidase activity is determined by the trichloroacetic acid method measuring the formation of pyrroline-5-carboxylate. With proline as the substrate, the reaction product, pyrroline-5-carboxylate reacts with α-aminobenzaldehyde, forming a dihydro-quinazolinium egg-yellow compound which is recovered by centrifugation and absorbs at 440 nm.

Chemicals

Extraction buffer: 0.1 M potassium phosphate buffer pH 7.4 (checked with a Mettler Toledo Delta 320 pH, Mettler Toledo Inc; Columbus, USA), 0. 5% (v/v) triton X-100.

Reaction solution: 0.1 M potassium phosphate pH8.0 (checked with a Mettler Toledo Delta 320 pH, Mettler Toledo Inc; Columbus, USA), 0.5% (v/v) triton X-100, 15 mM L-proline. The reaction mixture consisted of 0.5 mL triton X-100 and 0.1727 g proline in 100 mL 0.1 M potassium phosphate pH8.0.

Cytochrome C solution: 0.15 mM cytochrome C bovine heart (red in color and to be stored at -20 ℃ or on ice when in use) prepared by dissolving 100 mg cytochrome C in 2 mL 0.1 M potassium phosphate pH8.0.

Termination solution: 10% trichloroacetic acid (TCA), 0.5% α-amino-benzaldehyde dissolved in 95% ethanol (yellow in color, and to be stored at -20 ℃ or on ice when in use).

Preparation of the enzyme homogenate

The grain and leaf tissues (0.2 g) were ground in a cold pestle and mortar with quartz powder (1:1 wt/wt) and 3 mL of the extraction buffer. The resulting slurry was centrifuged at 3,000 × *g* for 10 min at 4 ℃ (Hema TGL-16 R refrigerated centrifuge, Shanghai, P.R China). The supernatant solution (white for grains and green for leaves) used for POX assay was quickly decanted and stored at -20 ℃ and used within one month.

POX activity assay

The assay mixture in a total volume of 1.4 mL was prepared as described below (Kandpal et al. 1981). A total of three replications were used for each treatment both in blank and assay conditions.

Reagent	Assay	Blank
Reaction solution (mL)	0.3	0.3
Cytochrome C solution (μL)	20.0	20.0
Supernatant (mL)	0.2	0.0
Distilled water (mL)	0.0	0.2
Incubation in a boiling water at 37 ℃ for 30 min		
10% trichloroacetic acid (TCA) (mL)	0.5	0.5
0.5% α-amino-benzaldehyde (mL)	0.4	0.4
Incubation at room temperature for 30 min		
Centrifugation at 9,000 × *g* for 10 min at 25 ℃		

The incubation was carried out at 37 ℃ for 30 min in a KW-1000 DA boiling water bath (Guangzhou Sinosource co., Ltd, Guangzhou, P. R. China) and the reaction terminated by adding 0.5 mL of 10% trichloroacetic acid. The egg-yellow color was developed by incubating the reaction mixture with 0.4 mL of 0.5% α-amino-benzaldehyde in 95% ethanol for 30 min. The denatured proteins (black color) were removed by centrifugation

and absorbance measured at 440 nm in a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). Blanks consisting of the same reaction mixture but with water substituting for the substrate were subtracted from samples before calculating the formation of pyrroline-5-carboxylate.

Specific activity of POX was expressed as micromoles of pyrroline-5-carboxylate formed per minute per mg of soluble proteins, using the equation:

$$\text{POX } (\mu\text{mol/min/mg}) = \frac{\text{OD} \times \text{Ve (mL)}}{2.71 \times 10^{-3}\ (\mu\text{mol}^{-1}) \times \text{m (g)} \times \text{t (min)} \times \text{Vs (mL)} \times \text{P (mg/g)}}$$

With POX being the proline oxidase activity, OD the optical density recorded, Ve the extraction solution volume, 2.71×10^{-3} the micromolar extinction coefficient of the pyrroline-5-carboxylate-α-amino-benzaldehyde complex, m the sample weight, t the incubation time at room temperature, Vs the supernatant volume used for the analysis, and P the amount of soluble protein in the sample.

Soluble protein estimation

The soluble proteins present in the grain and leaf extracts were estimated by the method of Bradford (1976) as described below, using bovine serum albumin as a standard.

2.11.2 Coomassie-Bradford method for total soluble protein determination

Principle

The assay is based on the observation that the maximum absorbance for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm (reddish/brown form of the dye) to 610 nm (blue form of the dye) when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change. The difference between the two forms of the dye is greatest at 595 nm, so that it is the optimal wavelength to measure the blue color from the Coomassie dye-protein complex. Development of color in Coomassie dye-based protein assays has been associated with the presence of certain basic amino acids, primarily arginine, lysine and histidine, in the protein. In general, the mass of a peptide or protein must be at least 3,000 daltons to be assayed with this reagent (Bradford, 1976).

Chemicals

Extraction buffer: the same buffer solution used to extract proline oxidase from rice grains and leaves was used and consisted of 0.1 M potassium phosphate buffer pH 7.4 (checked with a Mettler Toledo Delta 320 pH, Mettler Toledo Inc; Columbus, USA), 0.5% (v/v) triton X-100.

Bradford reagent: Coomassie brilliant blue G-250, 95% ethanol (or methanol), 85% (v/v) phosphoric acid. The Bradford reagent was prepared by dissolving 100 mg Coomassie in 50 mL 95% ethanol, added with 100 mL phosphoric acid. When the dye has completely dissolved (red dark with pH 0.01), it was diluted to 1.0 L, then filtered through a Whatman 1 paper to rid the reagent of blue components (light brown with pH 1.1, stable for weeks in a dark bottle at 4 ℃).

Protein standard: Bovine Serum Albumin (BSA).

Protein extraction

The preparation of the total soluble protein solution followed that described above for POX. Leaves and grains (0.2 g) were separated and ground to a fine powder in a chilled mortar and pestle, in the presence of washed quartz sand (0.2 g), and then homogenized in 3 mL of the appropriate extraction buffer. Homogenates were centrifuged at 3,000 $\times g$ for 10 min at 4 ℃ (Hema TGL-16 R refrigerated centrifuge, Shanghai, P.R China). The supernatant (white for grains and green for leaves) was kept at -20 ℃ until used.

Protein assay

The sample was simply added to the tube containing the Bradford reagent and distilled water and the resultant blue color measured at 595 nm in a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan), following a short room-temperature incubation. The control set of leaves and grains was also processed similarly with three replications.

Reagents	Assay	Blank
Supernatant (mL)	0.1	0.0
Distilled water (mL)	0.9	0.9
Bradford reagent (mL)	5.0	5.0
Stirring and incubation for 5 min at room temperature		
Measurement of OD_{595} absorption		

Although this did not happen in our case, if the samples are not readily soluble in the colored reagent, an equal volume of 1 M NaOH can be added to allow the solubilization of membrane proteins and reduce the protein-to-protein variation in color yield (add NaOH to standards as well if this option is used). Because Coomassie dye stains the glass or quartz cuvettes used to hold the solution in the spectrophotometer while the color intensity is being measured, cuvettes should be cleaned with a strong detergent solution or methanol.

Standard curve

By dissolving 100 mg of BSA in 10 mL 0.1 M potassium phosphate buffer pH7.4, a stock solution of soluble proteins with a concentration of 10 mg/mL was obtained. Six BSA solutions of 1.0 mL each were made: 0.00, 0.25, 0.50, 1.00, 1.50, and 2.00 mg/mL. A convenient standard curve of absorbance versus micrograms protein was plotted and the equation for the trendline calculated. A standard curve example is given in **Appendix 3**.

Reagents	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Stock protein solution (mL)	0.000	0.025	0.050	0.100	0.150	0.200
Phosphate buffer pH7.4 (mL)	1.000	0.975	0.950	0.900	0.850	0.800
Levy of 0.1 mL aliquot of the BSA solution						
BSA solution levied (mL)	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water (mL)	0.9	0.9	0.9	0.9	0.9	0.9
Bradford reagent (mL)	5	5	5	5	5	5
Standard protein content (mg)	0.000	0.025	0.050	0.100	0.150	0.200
Agitation and incubation at room temperature for 5 min						
Measurement of OD_{595} absorption						

For each original sample, the amount of protein was determined using the equation:

$$\text{Total soluble protein (mg/g)} = \frac{\text{Amount from the curve (mg)} \times \text{Extraction solution (mL)}}{\text{Sample weight (g)} \times \text{Supernatant volume (mL)}}$$

2.11.3 Colorimetric method for measuring free proline

Principe

The method proposed by Bates et al. (1973) is based on the reaction that takes place between ninhydrin and amino acids. Ninhydrin is a powerful oxidant that produces the oxidative deamination of the α-amino group, releasing ammonium, CO2, the corresponding aldehyde and ninhydrin in reduced form. Released ammonium reacts with an additional molecule of ninhydrin and with reduced ninhydrin, thus producing a purple

complex. For quantitative colorimetric amino acids determination, this purple complex has an adsorption maximum at 570 nm. However for proline which is strictly an imino acid (partially substituted amino group), the reaction with ninhydrin will be different to the rest of proteic amino acids, forming a colored complex whose maximum absorption is near to 520 nm.

Chemicals

Extraction solution: 3% anhydrous sulfosalicylic acid (SSA) (w/v).

Reagents: glacial acetic acid, 6 M phosphoric acid, ninhydrin, toluene. The Acid-ninhydrin solution was prepared by warming at 100 ℃ 1.25 g ninhydrin in 30 mL glacial acetic acid (green in color), and adding 20 mL 6 M phosphoric acid (yellow in color) with agitation, until dissolved. Acid-ninhydrin will keep stable only for 24 h, at 4 ℃ (use of a mask and gloves required).

Standard solution: purified L-proline.

Proline extraction from samples

Approximately 0.2 g of plant material was powdered in an ice cold mortar with quartz sand until pulverization and homogenized with 5 mL of 3% SSA to precipitate the proteins. Then the homogenate was filtered through a Whatman # 2 filter paper. After centrifugation at 5,000 $\times g$ for 10 min at 4 ℃ (Hema TGL-16 R refrigerated centrifuge, Shanghai, P.R China), the supernatant was used for determination of proline content.

Reaction with ninhydrin

Two mL of filtrate was reacted with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid in a test tube for 1 hour at 100 ℃ (KW-1000 DA water bath, Guangzhou Sinosource co., Ltd, P.R China) to allow the formation of the purple complex, and the reaction terminated in an ice bath. The reaction mixture was extracted with 5 mL toluene, mixed vigorously with a MRK multiple flash mixer (Mitamura Riken Koyyo, Tokyo, Japan) for 15 s to separate the organic and inorganic phases. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance of the resulting organic layer read at 520 nm (Shimadzu UV-2450 spectrophotometer, Tokyo, Japan) using toluene for a blank. For each treatment, extraction was conducted on three independent samples.

Reagents	Assay	Blank
Supernatant (mL)	2	0
3% sulphosalicylic acid (mL)	0	2
Glacial acetic acid (mL)	2	2
Acid-ninhydrin (mL)	2	2
Boiling in water bath at 100 ℃ for 60 min		
Cooling the tubes in ice bath for 5 min		
Toluene	5	5
Vortexing the tubes for 15 s		
Incubation in the dark for 30 min		
Maximum absorbance at a wavelength of 520 nm		

Preparation of the standard curve

Purified proline was used to standardize the procedure for quantifying sample values. One hundred (100) mL of 3% SSA was added to 10 mg of commercial purified L-proline and a stock proline solution of 100 μg/mL obtained.

Reagent	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
Stock proline (mL)	0.0	0.50	1.25	2.50	5.00	7.50
3% SSA (mL)	50	49.5	48.75	47.5	45	42.5
Levy of 2 mL aliquot of the standard proline solution						
Standard proline levied (mL)	2	2	2	2	2	2
Glacial acetic acid (mL)	2	2	2	2	2	2
Acid-ninhydrin (mL)	2	2	2	2	2	2
Boiling in water bath at 100 ℃ for 60 min						
Cooling the tubes in ice bath for 5 min						
Toluene (mL)	5	5	5	5	5	5
Standard proline content (μg)	0	2	5	10	20	30
Vortexing the tubes for 15 s						
Incubation in the dark for 30 min						
Maximum absorbance at a wavelength of 520 nm						

Several standard proline solutions of 50 mL each were prepared at different concentrations (0.0, 1.0, 2.5, 5.0. 10.0, 15.0 μg/mL) to cover the expected range of unknown sample concentrations. All tubes in standard curve followed the same procedure used with the samples, according to the ninhydrin reaction.

Using the data obtained from the different standard solutions, a lineal regression was done (comparing absorbance *vs.* proline concentration) and an equation allowing sample concentration calculation obtained **(Appendix 4)**. Knowing its molecular weight (115.13

μg/μmol), free proline concentration was expressed relative to fresh weight as follow:

$$\text{Free proline } (\mu\text{mol/g}) = \frac{\text{Amount from the curve } (\mu\text{g}) \times \text{Extraction solution (mL)}}{115.13 \times \text{Sample weight (g)} \times \text{Supernatant volume (mL)}}$$

2.12 Determination of anti-oxidative enzyme activities for stress studies

Ten young and fully expanded flag leaves and two panicles were randomly obtained from the centre of each plot at least four rows from the border, and immediately brought to the laboratory in boxes cooled up to 4 ℃. The panicles were gently threshed, and then impurities and unfilled grains removed by manual sorting. Fresh rice grains were dehulled by hands to obtain brown rice. Before analysis, the midrid (main vein) and petioles (leaf stalk) of the leaves were discarded, and the leaves deribbed and chopped into small pieces with a scissor. Grain and leaf samples were stored in plastic bags at -20 ℃ in a Sanyo medical freezer VR-L6111W (Sanyo Electric Co; Ltd, Moriguchi, Japan) until further treatment or analysis.

2.12.1 Assay for superoxide dismutase (SOD) activity

Principe

The enzyme superoxide dismutase (SOD, EC: 1.15.1.1) catalyzes the dismutation of two superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen, providing the first line of defense against oxygen toxicity and thereby oxidative stress in various organisms. The method employed is essentially that of Beauchamp and Fridovich (1971) and is based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium by superoxide and generate a water-insoluble blue formazan dye (max: 560 nm) by a reaction with O_2^-. One unit is defined as that amount of enzyme causing half the maximum inhibition of NBT reduction.

Chemicals

Extraction buffer: 0.05 M Potassium phosphate buffer, pH 7.8 (checked with a Mettler Toledo Delta 320 pH, Mettler Toledo Inc; Columbus, USA), 1% insoluble Polyvinylpolypyrrolidone (PVPP).

Reagents: 0.1 mM Ethylene diamine tetraacetic acid (EDTA-Na2), 0.02 mM Riboflavin (dark yellow and to be stored in a dark bottle and under cold conditions), 0.75

mM Nitroblue tetrazolium (NBT) (yellow light to be stored in the fridge), 130 mM L-Methionine.

Enzyme extraction

The frozen tissue (0.2 g) was homogenized with mortar and pestle with ¼ teaspoon of sand and 3 mL of the extraction buffer. The homogenate was centrifuged (Hema TGL-16 R refrigerated centrifuge, Shanghai, P.R China) at 15,000 × *g* for 20 min. All procedures were carried out at 0 ° C–4 ℃. The supernatant (white light for grains and leaves) was stored at –20 ℃ and used for measuring SOD activity.

SOD activity assay

Superoxide dismutase activity was measured spectrophotometrically at 560 nm using a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). The reaction mixture contained the following:

Reagents	Assay	Blank	Control
0.05 M Phosphate buffer pH 7.8 (mL)	1.5	1.8	1.5
130 mM Methionine (mL)	0.3	0.3	0.3
0.75 mM NBT (mL)	0.3	0.0	0.3
0.1 mM EDTA-Na2 (mL)	0.3	0.3	0.3
Distilled water (mL)	0.25	0.25	0.25
Incubation for 5 min at room temperature			
0.02 mM Riboflavin (mL)	0.3	0.3	0.3
Supernatant (mL)	0.05	0.0	0.0
Illumination of tubes with 40 W fluorescent lamp for 20 min at 25 ℃			
Measurement of OD_{560} absorption			

The reaction was started by illuminating the tubes with two 40W fluorescent lamps (Life Apparatus PGX-330A-12HM, Shanghai, P.R. China) for 20 min at 25 ℃, and stopped by switching off the lights and covering the tubes with black cloth. A reaction mixture without the protein extract, developing maximum dark-blue colour, served as a control, while the mixtures without the NBT and protein extract served as blanks.

Specific enzyme activity was expressed as units per g of fresh sample using the formula:

$$\text{SOD (Unit/g)} = \frac{(\text{OD Control} - \text{OD Sample}) \times \text{Extraction volume (mL)}}{\text{OD Control} \times 0.5 \times \text{Sample weight (g)} \times \text{Supernatant volume (mL)}}$$

2.12.2 Assay for peroxidase (POD) activity

Principle

Peroxidase (POD, EC: 1.11.1.7) is a hemoprotein catalyzing the oxidation by hydrogen peroxide of a number of substrates such as ascorbate, ferrocyanide, cytochrome C and the leuco form of many dyes. The method utilized here uses guaiacol as hydrogen donor (MacAdam et al. 1992). The increase in the absorption as a result of the formation of the orange oxidized product (tetraguaiacol) resulting from the decomposition of hydrogen peroxide is measured at 470 nm using the extinction coefficient of 26.6/Mm/cm. One unit results in the decomposition of one micromole of hydrogen peroxide per minute at 25℃ and pH 7.0 under the specified conditions.

Chemicals

Extraction buffer: 0.05 M Potassium phosphate buffer, pH 7.8 (checked with a Mettler Toledo Delta 320 pH, Mettler Toledo Inc; Columbus, USA), 1% insoluble Polyvinylpolypyrrolidone (PVPP).

Reagents: 0.05 M Potassium phosphate buffer pH 7.0 (checked with a Mettler Toledo Delta 320 pH, Mettler Toledo Inc; Columbus, USA), 0.3% (v/v) Hydrogen peroxide (to be used with gloves and mask), 0.2% guaiacol (v/v) (stir to homogenize). H_20_2 and guaiacol are to be prepared fresh daily and store on ice.

Enzyme extraction

Frozen leaves or grains (0.2 g) were pulverized with sand using a mortar and pestle and then resuspended in 3 mL of the extraction buffer. The suspension was centrifuged (Hema TGL-16 R refrigerated centrifuge, Shanghai, P.R China) at 15, 000 × *g* for 20 min at 4℃. The supernatant (white light for grains and leaves) was stored at –20 ℃ and used for measuring POD activity.

POD activity assay

The reaction was started by adding 0.05 mL of enzyme extract to the reaction mixture comprising of the following:

Reagent	Assay	Blank
0.05 M Phosphate buffer pH 7.0 (mL)	1.00	1.00
0.2 % Guaiacol (mL)	0.95	0.95
0.3% (v/v) Hydrogen peroxide (mL)	1.00	1.00
Incubation at 25 ℃ in the spectrophotometer for 2 min		
Supernatant (mL)	0.05	0.00
Stir and immediately put into the spectrophotometer cuvette		
Measurement of OD_{470} absorption for 2 min		

The increase in A_{470} for 2 min (development of an orange color) was recorded with a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). Total peroxidase activity was expressed in units per g of sample weight, one activity unit considered as the oxidation of 1 mg of guaiacol per minute per g of weight of sample.

$$\text{POD (Unit/g)} = \frac{(\text{ODfinal} - \text{ODinitial}) \times \text{Extraction volume (mL)}}{\text{Sample weight (g)} \times \text{time (min)} \times \text{Supernatant volume (mL)}}$$

3 Topics, results and discussion

3.1 Fragrance quality of two rice cultivars grown in South China analyzed by headspace techniques coupled to GC-MS

3.1.1 Introduction

Fragrant rice is a general term used for rice cultivars that have a perfumed and nutty flavor. They are generally medium to long-grain, and have a light, fluffy texture when cooked. Awareness about the unique grain cooking and eating quality of fragrant rices is increasing all over the world and aroma quality is now rated as the major criteria for preference among many consumers.

The high demand for fragrant rice has driven the development of methods to distinguishing fragrant and non-fragrant cultivars. A number of biochemical methods (Awasthi et al. 1997; Nadaf et al. 2006), chemical methods (Sekhar, 1982; Paule and Powers, 1989; Hien et al. 2006), sensory methods (Paule and Powers, 1989; Hori et al. 1994; Yau and Liu, 1999; Wilkie et al. 2004) and agronomic characteristics (Itani, 2002; George et al. 2005) have been described to assist breeders, each of these methodical approaches offering a number of individual advantages but also suffering from specific limitations. During recent decades, however, the quest for an understanding of the composition of fragrant rices and how it differs from non-fragrant cultivars has been mostly advanced by the comparison of volatile profiles (Petrov et al. 1996; Widjaja et al. 1996b; Laguerre et al. 2007; Maraval et al. 2008). Most volatiles recorded so far from raw or cooked non-fragrant and fragrant rices are the same except for their relative proportion. Of the over 300 volatile compounds observed in rice, only a few have been identified as affecting cooked rice aroma and flavor and 2-acetyl-1-pyrroline (2-AP) referred to as cracker or popcorn-like pointed out as the principal component of fragrant rices (Buttery et al. 1988).

It was reported that only fragrant rice cultivars possess the genetic potential for accumulating 2-AP and that fragrance is due to an eight-base pair deletion in exon 7 of a gene on chromosome 8 encoding a putative betaine aldehyde dehydrogenase 2 (Berner and Hoff, 1986; Ahn et al. 1992; Pinson, 1994; Lorieux et al. 1996; Chen et al. 2006). Knowledge of the most likely genetic cause of fragrance has therefore led to the

development of markers-assisted selection methods for fragrance genotyping and discrimination between fragrant and non-fragrant rice cultivars (Jin et al. 2003; Bradbury et al. 2005; Champagne et al. 2008). Recently however, Fitzgerald et al. (2008) discovered that some fragrant cultivars without the 8-bp deletion contained 2-AP leading to the conclusion that the 8-bp deletion in the fragrance allele is not the only cause of aroma, and that at least one other mutation drives the accumulation of 2-AP. Therefore, most markers linked with the fragrance gene cannot be used to predict the fragrant status of any one rice sample with 100% accuracy. This is added to the fact that most of them indicate the presence or absence of the gene, but not the level of expression.

After more than 30 years of research on fragrant rices, only one method has been mostly used to authenticate the aromatic characteristic of rice cultivars, which is the quantification of 2-AP. A number of research groups have reported concentrations of 2-AP in fragrant rice in the range of 0.04-0.20 mg/kg, whereas non-fragrant ones possessed much lower concentrations (Paule and Powers, 1989; Lin et al. 1990; Tanchotikul and Hsieh, 1991; Laksanalamai and Ilangantileke, 1993; Tava and Bocchi, 1999; Mahatheeranont et al. 2001; Yoshihashi, 2002; Champagne et al. 2008). The compound is synthesized chemically (Buttery et al. 1983; De Kimpe et al. 1993; Hofmann and Schieberle, 1998) and can also be used to flavor diverse food products (Buttery al. 1985; Apintanapong and Noomhorm, 2003; Laohakunjit and Kerdchoechuen, 2007).

Despite being the largest producer of rice in the world, 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 led to a sharp decline in the number of fragrant rice cultivars and their areas of cultivation (Yang, 2007). Until recently, information about fragrant rice chemistry in the region was mainly from Hong Kong (Chung et al. 2004) and Taiwan (Huang et al. 2008). With the amelioration of living standards in mainland China, however, the fragrant rice demand is constantly increasing. Nowadays, a larger section of population prefers to eat fragrant rice on a daily basis rather than cooking only on ceremonial occasions. 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 king 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.

This study was conducted as a preliminary step towards the characterization of the volatile chemistry of Guixiangzhan and Peizaruanxiang by the identification and quantification of the intensely popcorn-like smelling compound 2-AP. The fragrance potential of the two rices was investigated using two related headspace sampling techniques, static headspace (S-HS) and headspace solid phase micro-extraction (HS-SPME) coupled with a gas chromatography-mass spectrometry (GC-MS).

3.1.2 Experimental

3.1.2.1 Rice cultivars

Two fragrant rice cultivars were used, Guixiangzhan (medium-grained) and Peizaruanxiang (long-grained). These two rice cultivars represent the main commercial cultivars in Guangdong province (South China). Guixiangzhan has been cultivated in South China since the sixties. Peizaruanxiang is a new hybrid line released recently by the breeding group of the College of Agriculture of South China Agricultural University (SCAU) with better qualities in terms of yield and grain appearance. The two cultivars were grown in SCAU experimental farm on a sandy loam soil. The trend associated with the two cultivars with respect to composition and concentration of each volatile compound was based on levels in plants obtained during the early season of 2008 and stored for 6 months at -4 ℃. Samples of imported Thai Jasmine KDML 105 grown during the late season of 2008 and stored at 20 ℃ for more than one month were also analyzed for 2-AP comparison with the Chinese cultivars.

3.1.2.2 Rice samples preparation

After harvest, all samples were dehulled and milled to a 85% milling yield (brown rice basis) in the form of white rice, then grounded. Rice flour samples obtained were subjected to analysis immediately. The rice samples analyzed by SPME-GC/MS were brown grains, while both brown and white grains were analyzed by SHS-GC with a selection between two detectors, a nitrogen-phosphorus detector (NPD) for the determination of 2-AP and a flame ionization detector (FID) for other major volatiles, as described in the “Materials and Methods” section.

3.1.2.3 Statistical data analysis

All treatments were replicated three times to achieve reliability of the test results and also to obtain a measure of the experimental error. Data of volatile compounds content obtained by headspace sampling were expressed as the mean of triplicate measurement ± standard deviation. All analyses were conducted using SPSS software 15.0 (SPSS, Chicago, IL).

3.1.3 Results

3.1.3.1 Extraction method for 2-acetyl-1-pyrroline

By using HS-SPME, 37 compounds were identified, however, no clearly distinguishable peak was seen for 2-AP. **Figure 3.1.1** illustrates an example of the mass spectrum obtained as a result of HS–SPME.

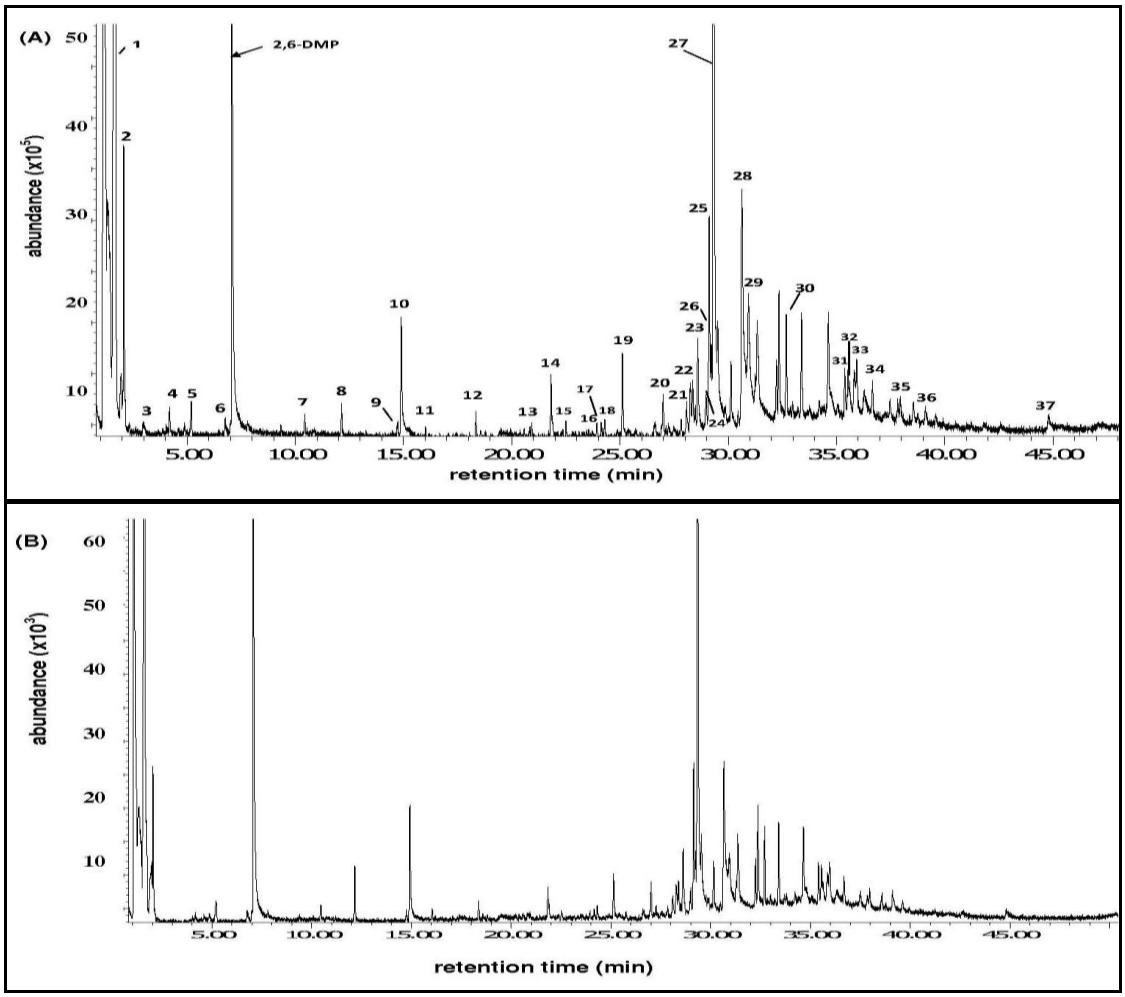
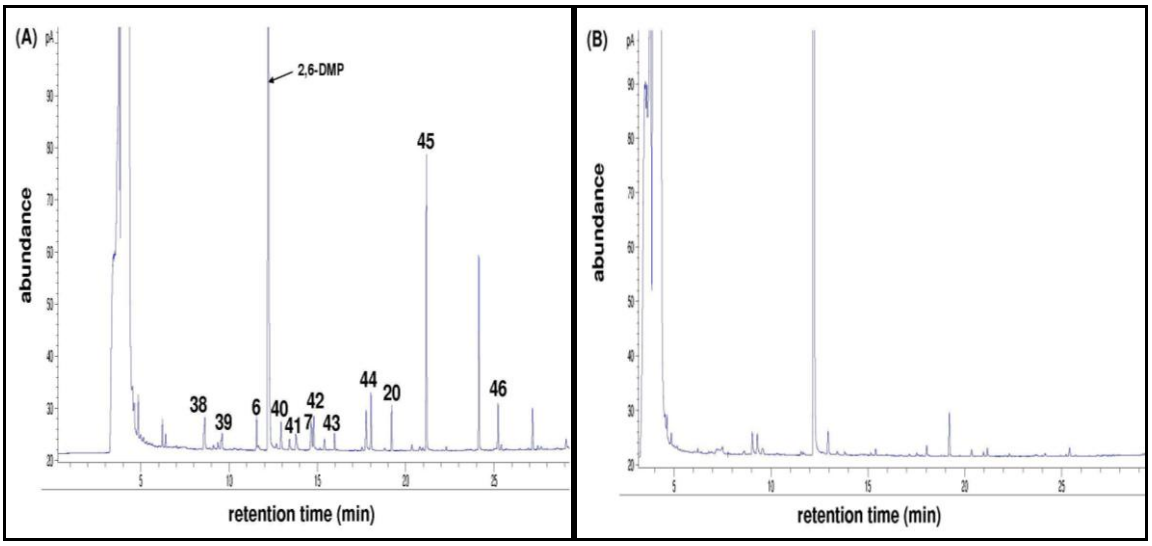
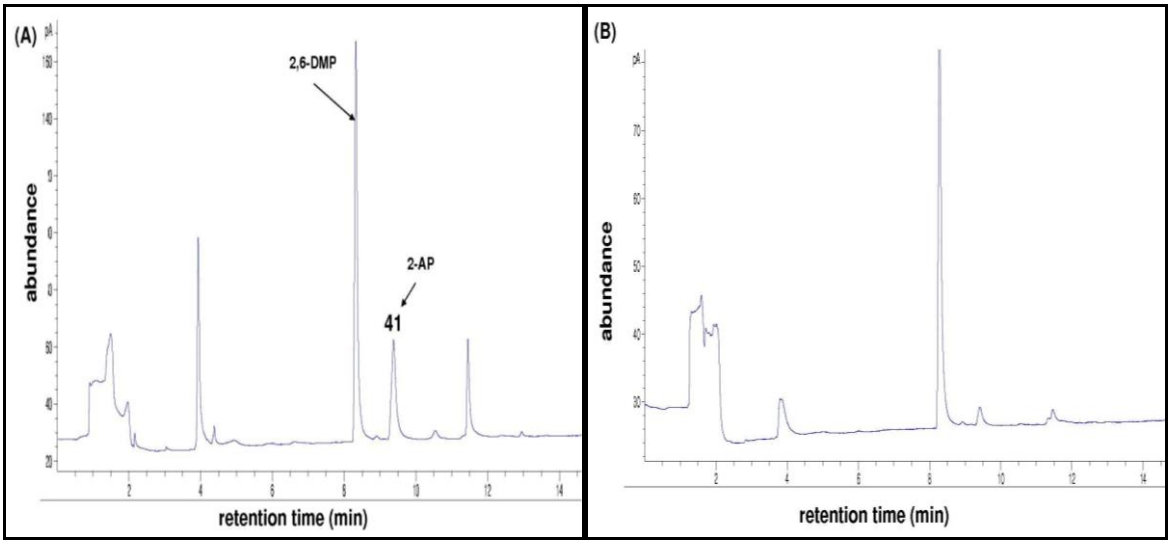


Figure 3.1.1 Reconstructed GC-MS total ion chromatogram (TIC) of (A) Guixiangzhan and (B) Peizaruanxiang brown rice volatiles extracted by headspace solid phase micro-extraction (SPME). The numbers refers the compounds listed in Table 3.1.1 to Table 3.1.6.

When S-HS was applied, 2-AP could be unambiguously detected. The chromatograms obtained using two different detectors (nitrogen-phosphorus detector, NPD and flame ionization detector, FID) can be compared in **Figure 3.1.2** and **Figure 3.1.3**. Comparison of retention times and mass spectra obtained by injection of an authentic compound also enabled the identification of some more polar constituents, most of them considered to be important contributors to the overall aroma of rice.



Figuree 3.1.2 GC-FID chromatogram of an extract from 3 g of uncooked (A) Guixiangzhan and (B) Peizaruanxiang brown rice utilizing a static headspace extraction.



Figuree 3.1.3 GC-NPD chromatogram of an extract from 1 g of uncooked (A) Guixiangzhan and (B) Peizaruanxiang brown rice utilizing a static headspace extraction.

3.1.3.2 Comparison of Guixiangzhan and Peizaruanxiang flavors

Altogether headspace sampling allowed the determination of 10 alcohols, 3 aromatic compounds, 6 aldehydes, 2 nitrogen containing compounds, 1 terpenic compound, 5 ketones, 13 hydrocarbons, and 3 esters derivatives as it is listed in **Table 3.1.1** to **Table 3.1.6**. Qualitatively, the two rices had the same significant volatile aroma compounds, but there were large differences in quantity. Only 3 compounds were found to be unique to Guixiangzhan; benzaldehyde, 4-ethylbenzaldehyde, and benzothiazole **(Table 3.1.2)**. All compounds were quantitatively substantially lower in abundance in Peizaruanxiang with the exception of nonanal **(Table 3.1.3)** and *d*-limonene **(Table 3.1.4)**. Interestingly is the high number of ketones detected **(Table 3.1.5)**. The presence of high amounts of 2-propanol (405.30 and 256.45 ng/g) and 2-ethyl-1-dodecanol (378.15 and 245.74 ng/g) in the two rices **(Table 3.1.6)** must be referred. The most important difference noted was that Guixiangzhan had approximately 5 times more 2-AP (205.98 ng/g) than Peizaruanxiang (37.39 ng/g). This corresponded to 2-AP concentrations of 3.73 and 0.69 μg/g respectively **(Table 3.1.4)**.

3.1.3.3 Fragrance potential of South China fragrant rices

Guixiangzhang and Peizaruanxinag flavors were compared to that of Jasmine rice seen as the benchmark for aroma quality. Guixiangzhan had a 2-AP content of 3.20 μg/g comparable to 3.73 μg/g obtained with Jasmine rice using the same methodology **(Figure 3.1.4)**.

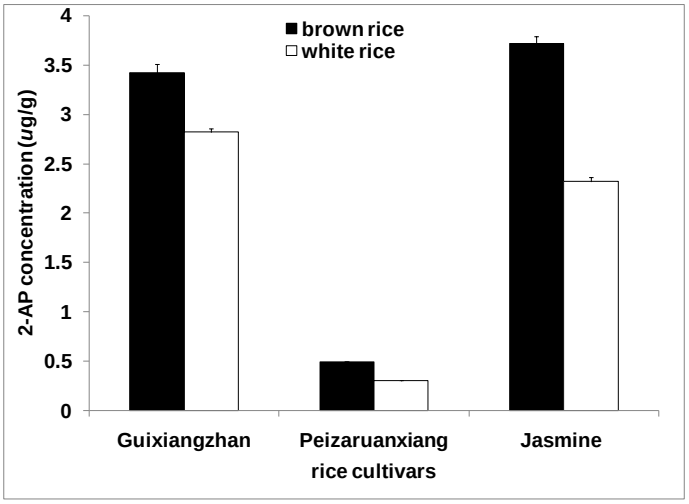


Figure 3.1.4 Comparative analysis of 2-AP content in Chinese Guixiangzhan, Chinese Peizaruanxiang and Thai Jasmine KDML 105 fragrant rice samples. Capped bars represent the standard deviations.

Table 3.1.1 Hydrocarbons identified in the headspace of Guixiangzhan and Peizaruanxiang rice cultivars and their relative concentrations

RI[b]	Compound	Peak[c]	RT[d]	MW[e]	Identification[f]	Concentration (ng/g)[a]		EI-MS fragmentation (% relative intensity)
						Guixiangzhan	Peizaruanxiang	
600	Hexane	2	2.074	86.18	MS, RI	63.95 ± 6.87	33.59 ± 4.66	86,71,57(100),43
1134	(*E*)-5-undecene	22	28.355	154.29	MS, RI	15.67 ± 0.04	11.02 ± 2.32	154,126,111,97,84(100),70,55,41
1198	Dodecane	12	18.333	170.34	MS, RI	7.64 ± 0.57	4.40 ± 0.12	170,141,126,114,98,85,71,57(100),43
1296	Tridecane	14	21.809	184.20	MS, RI	19.54 ± 0.01	14.39 ± 0.63	184,140,127,113,98,85,71,57(100),43
1337	1-tridecene	24	28.975	182.35	MS, RI	11.08 ± 0.59	8.15 ± 1.13	182,154,125,113,98,84,78,57(100),43
1359	2,5-dimethyldodecane	15	22.498	198.39	MS, RI	5.00 ± 0.10	2.56 ± 0.02	198,141,85,71,57(100)
1365	2-methyltridecane	16	23.917	198.39	MS, RI	7.01 ± 0.05	4.21 ± 0.24	199,141,99,85,71,57(100),43
1372	3-methyltridecane	17	24.131	198.39	MS, RI	7.00 ± 0.10	4.36 ± 0.09	197,140,99,86,71,57(100),43
1400	Tetradecane	19	25.101	198.39	MS, RI	30.23 ± 1.11	13.29 ± 091	198,140,99,85,71,57(100),43
1436	1-tetradecene	28	30.625	196.38	MS, RI	129.55 ± 4.66	74.10 ± 15.50	193,171,140,125,111,97,83,68,55(100),43
1454	4-methyltetradecane	20	26.991	212.42	MS, RI	17.10 ± 0.82	12.41 ± 1.49	212,197,169,155,113,99,85,71(100),57,43
1498	Pentadecane	21	28.248	212.42	MS, RI	18.99 ± 0.19	9.03 ± 0.14	212,168,154,141,128,113,97,85,71,57(100),43
1810	1-octadecene	34	37.949	252.48	MS, RI	2.81 ± 0.15	1.37 ± 0.56	252,224,182,154,140,126,111,97,85,71,57(100),43

[a] Values expressed as 2,6-DMP equivalent and given as average ± standard deviation (n = 3). [b] Retention index based on a series of n-hydrocarbons using a HP-5MS column. [c] Compound numbers correspond to those labeled on the TIC in **Figure 3.1.1.** [d] Retention time. [e] Molecular weight (g/mol). [f] Identification proposal: MS, tentatively comparison of the EI-MS with the NIST and Wiley mass spectral library; RI, tentative identification by retention indexes with literature data.

Table 3.1.2 Aromatic volatiles identified in the headspace of Guixiangzhan and Peizaruanxiang rice cultivars and their relative concentrations

RI[b]	Compound	Peak[c]	RT[d]	MW[e]	Identification[f]	Concentration (ng/g)[a]		EI-MS fragmentation (% relative intensity)
						Guixiangzhan	Peizaruanxiang	
760	toluene	4	4.185	92.14	MS, RI, STD	6.88 ± 1.11	3.08 ± 0.24	91(100),77,65,51, 39
952	benzaldehyde	7	10.436	106.12	MS, RI, STD	4.22 ± 0.04	ND[g]	105(100),78,77,51,52, 50
1171	4-ethylbenzaldehyde	11	16.013	134.18	MS, RI	2.03 ± 0.10	ND	133(100),119,105,91,7 7,63,51,39
1568	1-butylhexylbenzene[h]	26	29.208	218.38	MS, RI	21.16 ± 0.07	15.10 ± 2.51	218,161,147,105,91 (100),77,38
1597	1-propyloctylbenzene[h]	30	32.681	232.40	MS, RI	31.98 ± 0.99	20.44 ± 3.21	232,189,133,119,105, 91(100),43
1692	1-propylnonylbenzene[h]	32	35.936	246.43	MS, RI	26.02 ± 1.69	14.68 ± 3.55	246,203,133,91(100), 76,66,55,41

[a] Values expressed as 2,6-dimethylpyridine (RI = 904) equivalent (ng 2,6-DMP/g brown rice) and given as average ± standard deviation (n = 3). [b] Retention index based on a series of n-hydrocarbons using a HP-5MS column and compared to those in the Flavornet (http://www.flavornet.org) and Pherobase (http://www.pherobase.net) databases accessed March 2008. . [c] Compound numbers correspond to those labeled on the TIC in **Figure 3.1.1.** [d] Retention time. [e] Molecular weight (g/mol). [f] Identification proposal: MS, tentatively comparison of the EI-MS with the NIST and Wiley mass spectral library; RI, tentative identification by retention indexes with literature data; STD, by comparison of retention time and spectrum of an identified compound with those of an authentic compound. [g] ND = not detected. [h] Compounds identified as environmental contaminants in the headspace.

Table 3.1.3 Aliphatic aldehydes identified in the headspace of Guixiangzhan and Peizaruanxiang rice cultivars and their relative concentrations

RI[b]	Compound	Peak[c]	RT[d]	MW[e]	Identification[f]	Concentration (ng/g)[a]		EI-MS fragmentation (% relative intensity)
						Guixiangzhan	Peizaruanxiang	
803	hexanal	5	5.196[g]	100.16	MS, RI, STD	10.68 ± 1.15	5.60 ± 0.78	100,82,72,56, 44(100)
865	(*E*)-2-hexenal	39	*9.551*	98.14	RI, STD	8.19 ± 0.40	3.62 ± 0.38	97,83,69(100), 40
903	heptanal	40	*12.673*	114.18	RI, STD	3.82 ± 0.15	0.49 ± 0.05	115,98,97(100), 69,55
1005	octanal	43	*15.963*	128.21	RI, STD	4.15 ± 0.48	1.07 ± 0.05	129,111,95,84,81,70,69(100),57,56,55
1106	nonanal	10	14.892	142.24	MS, RI, STD	40.76 ± 2.73	44.56 ± 0.86	142,124,114,98,82,70, 57(100),41
1206	decanal	46	*25.223*	156.20	RI, STD	11.04 ± 1.08	0.66 ± 0.02	142,128,112,95,82,71, 57(100),43

[a] Values expressed as 2,6-dimethylpyridine (RI = 904) equivalent (ng 2,6-DMP/g brown rice) and given as average ± standard deviation (n = 3). Since odorants extraction was done by different headspace sampling methods, the values are only meant to give an idea of the order of magnitude. [b] Retention index based on a series of n-hydrocarbons using a HP-5MS column and compared to those in the Flavornet (http://www.flavornet.org) and Pherobase (http://www.pherobase.net) databases accessed March 2008. [c] Compound numbers correspond to those labeled on the total ion chromatogram in **Figure 3.1.1** and **Figure 3.1.2**. [d] Retention time. [e] Molecular weight (g/mol). [f] Identification proposal: MS, tentatively comparison of the EI-MS with the NIST and Wiley mass spectral library; RI, tentative identification by retention indexes with literature data; STD, by comparison of retention time and spectrum of an identified compound with those of an authentic compound. [g] Retention times given in italics for compounds identified using Static Headspace.

Table 3.1.4 Nitrogen-containing compounds, tepenoids and esters derivatives identified in the headspace of Guixiangzhan and Peizaruanxiang rice cultivars and their relative concentrations

RI[b]	Compound	Peak[c]	RT[d]	MW[e]	Identification[f]	Concentration (ng/g)[a]		EI-MS fragmentation (% relative intensity)
						Guixiangzhan	Peizaruanxiang	
				Nitrogen-containing compounds				
918	2-acetyl-1-pyrroline	41	*13.776*[g]	111.14	RI, STD	205.98 ± 2.65	37.39 ± 1.09	111,83,69,68,43(100),41
1213	benzothiazole	13	20.914	135.19	MS, RI, STD	3.08 ± 0.29	ND[h]	135(100),108,91,82,69 ,54,45
				Terpenoids				
1022	*d*-limonene	8	12.140	136.24	MS, RI, STD	11.91 ± 2.43	13.90 ± 0.42	136,121,107,93(100),79 ,68,53,39
				Fatty acids and esters				
1780	tetradecanoic acid	33	36.262	228.37	MS, RI	12.02 ± 1.66	7.65 ± 1.74	228,185,129,97,83,73,60, 55,43(100),41
1824	isopropylmyristate	35	38.551	270.45	MS, RI	25.36 ± 0.16	14.53 ± 2.22	270,228,211,185,129,102, 91(100),83,73,57,43
1970	hexadecanoic acid	37	44.792	256.42	MS, RI	14.10 ± 2.40	1.81 ± 0.36	256,239,213,185,171,157, 129,102,83,71,60,55(100) ,43,41

[a] Values expressed as 2,6-DMP equivalent and given as average ± standard deviation (n = 3). Since odorants extraction was done by different headspace sampling methods, the values are only meant to give an idea of the order of magnitude. [b] Retention index based on a series of n-hydrocarbons using a HP-5MS column. [c] Compound numbers correspond to those labeled on the total ion chromatogram in **Figure 3.1.1** and **Figure 3.1.3**. [d] Retention time. [e] Molecular weight (g/mol). [f] Identification proposal: MS, tentatively comparison of the EI-MS with the NIST and Wiley mass spectral library; RI, tentative identification by retention indexes with literature data; STD, by comparison of retention time and spectrum of an identified compound with those of an authentic compound. [g] Retention times given in italics for compounds identified using Static Headspace. [h] ND = not detected.

Table 3.1.5 Ketone volatiles identified in the headspace of Guixiangzhan and Peizaruanxiang rice cultivars and their relative concentrations

RI[b]	Compound	Peak[c]	RT[d]	MW[e]	Identification[f]	Concentration (ng/g)[a]		EI-MS fragmentation (% relative intensity)
						Guixiangzhan	Peizaruanxiang	
1093	2-nonanone	9	14.727	142.24	MS, RI	4.15 ± 0.03	3.02 ± 0.16	142,85,71,58,57, 43(100)
1393	2-dodecanone	18	24.290	184.32	MS, RI	5.14 ± 0.38	2.81 ± 0.46	184,163,126,110,95,85,71, 43(100)
1584	2-tetradecanone	29	31.246	212.37	MS, RI	11.39 ± 0.01	5.38 ± 1.41	212,197,169,154,127,96,8 2,71,58(100),43
1689	2-pentadecanone	31	35.822	226.40	MS, RI	20.28 ± 0.94	12.87 ± 2.93	221,208,168,166,96,85,71, 58(100),43
1845	6,10,14-trimethylpenta decanone	36	39.104	268.48	MS, RI	7.05 ± 1.49	10.62 ± 1.60	268,200,208,137,123,109, 95,85,71,58(100),43

[a] Values expressed as 2,6-dimethylpyridine (RI = 904) equivalent (ng 2,6-DMP/g brown rice) and given as average ± standard deviation (n = 3). [b] Retention index based on a series of n-hydrocarbons using a HP-5MS column and compared to those in the Flavornet (http://www.flavornet.org) and Pherobase (http://www.pherobase.net) databases accessed March 2008. [c] Compound numbers correspond to those labeled on the total ion chromatogram in **Figure 3.1.1**. [d] Retention time. [e] Molecular weight (g/mol). [f] Identification proposal: MS, tentatively comparison of the EI-MS with the NIST and Wiley mass spectral library; RI, tentative identification by retention indexes with literature data.

Table 3.1.6 Aliphatic alcohols identified in the headspace of Guixiangzhan and Peizaruanxiang rice cultivars and their relative concentrations

RI[b]	Compound	Peak[c]	RT[d]	MW[e]	Identification[f]	Concentration (ng/g)[a]		EI-MS fragmentation (% relative intensity)
						Guixiangzhan	Peizaruanxiang	
536	2-propanol	1	1.129	60.10	MS, RI	405.30 ± 92.01	246.45 ± 15.27	59,45(100),42,27
744	3-methyl-1-butanol	3	2.968	88.15	MS, RI, STD	3.69 ± 0.59	0.99 ± 0.01	88,71,70,69,59,55(100),41
787	1-pentanol	38	*8.612*[g]	88.15	RI, STD	12.65 ± 0.41	2.83 ± 0.14	70,69,58,55(100),50,42
870	1-hexanol	6	*11.548*	102.17	RI, STD	10.74 ± 0.90	1.84 ± 0.16	101,84,69,56(100),44,31
969	1-heptanol	42	*14.783*	116.20	RI, STD	9.69 ± 1.34	0.85 ± 0.01	98,70,55(100),43
1075	1-octanol	44	*18.028*	130.23	RI, STD	16.39 ± 1.81	3.47 ± 0.17	71,57(100),43,41
1175	1-nonanol	45	*21.164*	144.26	RI, STD	30.70 ± 5.22	2.73 ± 0.03	139,97,83,69,56,43,40(100)
1502	2-butyl-1-octanol	23	28.590	186.33	MS, RI	31.37 ± 0.72	20.62 ± 2.45	182,168,154,133,125,111,97, 85,71,57(100),43
1562	(*S*)-2-methyl-1-dodecanol	25	29.116	200.40	MS, RI	56.95 ± 1.39	36.84 ± 5.12	197,182,154,140,125,111,97, 83,71,57(100),43
1580	2-ethyl-1-dodecanol	27	29.312	214.33	MS, RI	378.15 ± 18.14	245.74 ± 37.08	219,192,168,139,125,111,97, 83,71,57(100),43

[a] Values expressed as 2,6-dimethylpyridine (RI = 904) equivalent (ng 2,6-DMP/g brown rice) and given as average ± standard deviation (n = 3). Since odorants extraction was done by different headspace sampling methods, the values are only meant to give an idea of the order of magnitude. [b] Retention index based on a series of n-hydrocarbons using a HP-5MS column and compared to those in the Flavornet (http://www.flavornet.org) and Pherobase (http://www.pherobase.net) databases accessed March 2008. [c] Compound numbers correspond to those labeled on the total ion chromatogram in **Figure 3.1.1** and **Figure 3.1.2**. [d] Retention time. [e] Molecular weight (g/mol). [f] Identification proposal: MS, tentatively comparison of the EI-MS with the NIST and Wiley mass spectral library; RI, tentative identification by retention indexes with literature data; STD, by comparison of retention time and spectrum of an identified compound with those of an authentic compound. [g] Retention times given in italics for compounds identified using Static Headspace.

3.1.4 Discussion

3.1.4.1 Extraction method for 2-acetyl-1-pyrroline

We sought in this study to clarify the aromatic quality of a potential cultivar of South China and to compare it with a new breeding line. That objective being set, careful attention was paid to find an extraction and identification method focusing on 2-acetyl-1-pyrroline (2-AP) described as "popcorn-like" and today well accepted as the major contributor to the aroma of cooked fragrant rice (Buttery et al. 1988). Two different extraction techniques were used for analyzing 2-AP and other volatiles compounds in the rice grains: extraction with headspace solid phase micro-extraction (HS-SPME) applied to brown samples, and static headspace (S-HS) applied to both brown and white samples.

No clearly distinguishable peak was seen for 2-AP using HS-SPME. When optimizing the conditions for HS-SPME, a factorial design of 24 treatment combinations was performed using four temperature values (50, 60, 70 and 80 ℃), three extraction times (15, 20, and 30 min), and two adsorption times (20, and 30 min). In some cases, 2-AP was observed in the library hit table but it was never the first hit and the MS signal was too weak for an unequivocal interpretation. The compound could only be identified by comparing it with the reference substance on the basis of the retention time on the stationary phase.

The absence of 2-AP could only be attributed to the selectivity of the fiber used. 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 non-polar nature is known to perform very effectively for a wide range of mostly non-polar and rather high molecular weight analytes (Grimm et al. 2001; Ghiasvand et al. 2007; Zeng et al. 2008). It also appeared that compound reported to be formed predominantly in the rice core endosperm like hexanal, benzaldehyde, 1-hexanol, nonanal (Yang et al. 2008b) were those effectively adsorbed by the PDMS fiber. On the contrary, most aldehydes and alcohols reported by several groups were not identified, probably because of their high volatility and short retention time on the GC system used for these analyses. Also, a total of as high as 13 peaks were assigned to hydrocarbons using SPME.

2-AP could be unambiguously detected when using S-HS, since the headspace procedure was designed to identify lower boiling components. NPD has the particularity to have a specific selectivity to nitrogen containing compounds. Hence, its use not only provided higher detection sensitivity for 2-AP, but also a higher chromatographic resolution.

3.1.4.2 Comparison of Guixiangzhan and Peizaruanxiang flavors

Overall, 43 compounds were detected in the two rices using headspace sampling. Of the identified compounds, 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, benzothiazole (Buttery et al. 1988; Widjaja et al. 1996b, Jezussek et al. 2002; Chung et al. 2004; Maraval et al. 2008, Yang et al. 2008a; Yang et al. 2008c). Structural identification of these compounds was based on experiments performed by using either commercially available or synthesized reference odorants. 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, hexanoic acid also occurs, 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 for further flavor development under appropriate conditions. The high number of ketones must be referred since they have been reported to contribute sweet, floral and fruity notes to rice (Widjaja et al. 1996), and and the high amount of 2-propanol and 2-ethyl-1-dodecanol although they are not necessarily directly important to flavor.

All compounds including 2-AP were quantitatively substantially lower in abundance in Peizaruanxiang. The aroma level difference between the two rices was expected since Guixiangxhan when cooked has more desirable aroma quality. The low level of 2-AP measured for Peizaruanxiang could however, indicate that the cultivar is not very scented which will be controversial. Peizaruanxiang was developed by the breeding group of South China Agricultural University recently and rapidly emerged as a superior rice variety worthy of release as pure-line under the category “scented rices” hence its name which etymologically means “soft aroma”. It has a relatively intense flavor that is distinctly different from non-aromatic rices 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 rices from Afghanistan and Myanmar (Hien et al. 2006). In black rices grown in Korea, not only 2-AP, but also guaiacol were reported to be major contributors to aroma based on odor thresholds, relative

concentrations, and olfactometry (Yang et al. 2008a). Therefore, further investigation in Peizaruanxiang rice composition may be necessary to pinpoint those essential components responsible of its specific aroma.

3.1.4.3 Fragrance potential of South China fragrant rices

For domestically produced rices 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 this research indicate that Guixiangzhan meets this criterion with a 2-AP content comparable to that obtained with Jasmine rice using the same methodology, 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 ℃, while Jasmine had undergone storage of more than one month at ambient temperature, a condition which can result in many changes, especially the overall volatile composition, and the decrease in content of 2-AP.

3.1.5 Conclusion

The fragrance potential of two rice cultivars grown in Guangzhou, namely Guixiangzhan and Peizaruanxiang was established in this study. In a first step, brown rice samples were analyzed for the identity and concentration of volatile aroma compounds present. The use of a SPME method in conjunction with GC-MS helped to identified 37 volatiles, most of them high molecular weight compounds. Some compounds highlighted from previous studies as odor-actives in rice like decanal and 2-AP could not be detected by the PDMS fiber used, but were extracted and quantified without any ambiguity using a rapid method employing static headspace coupled to GC. The results indicated that levels of 2-acetyl-1-pyrroline were highest in Guixiangzhan (3.20 μg/g) compared to Peizaruanxiang (0.69 μg/g). Other compounds instead of 2-AP were assumed to contribute to the characteristic aroma of Peizaruanxiang. Importantly, it was found that specialty characteristics of Guixiangzhan, such as aroma, flavor, and appearance, could match those of imported Jasmine rice (3.73 μg/g) analyzed under the same conditions. Findings from these analyses indicate that there is an opportunity for South China to increase its share of the domestic market of fragrant rice and even to tap into the international market.

3.2 Factors affecting concentration of 2-acetyl-1-pyrroline, and other seed quality traits in aromatic rice

3.2.1 Introduction

Of the pre-harvest factors that can affect rice yield and quality, a producer can only control few of them like sowing date, depth and uniformity of planting, drain time and grain moisture at harvest. After emergence, controlling diseases, weeds, insects, and birds, as well as having suitable growing conditions, ultimately affect the yield and quality of rice.

Studies investigating the effect of planting density on rice grain yields have been sporadically conducted since the 1930s. Previous studies, across rice producing areas in the world, have shown that for some cultivars rice grain yields decrease as planting density decrease while for others there is no effect on aboveground biomass production or rice yield (Zeng and Shannon, 2000; Bond et al. 2008). For a selected cultivar, planting density also has a notable effect on rice quality. With an increase in planting density, the unpolished and polished grain ratio, protein content and transparency of rice decreases, whereas chalkiness ratio, unfilled grain percentage, amylose content, and grain consistency increases, consequently greatly reducing the quality of rice appearance and processing (Bond et al. 2008). Despite numerous studies on planting density however, the rate of yield of aromatic rices has not been fully quantified or developed. Or as a rule, scented rices compared to the leading cultivarts are taller, have fewer panicle number, higher stem weight, lower yields, awned and of particular importance, are susceptible to lodging (Glaszmann, 1987; Singh et al. 2000; Bradbury et al. 2008). The general hypothesis is that many producers plant excessively high seeding rates and reduced seeding rates can be utilized to decrease lodging, disease pressure, and still maintain high yields. Furthermore, because aromatic rice seeds are more expensive than seeds of conventional cultivars, seeding rates or planting densities are very important to minimize production costs.

Most studies conducted in so far indicated that harvest time influences the grain yield, the grain quality and the eating quality of rice. According to rice experts, improper harvest can lead up to 15% yield losses and delay in harvesting appears to be a common stress affecting milling yield evaluations (McCauley and Way, 2002). For most studies, the early harvesting of rice causes both quantitative and qualitative losses while a harvest delay reduces whole-milled grain. Harvesting early at higher moisture contents, while improving head rice yield, may lead to problematic microbial growth with associated off-flavor metabolites if drying is delayed

(Champagne et al. 2008). Accordingly determining the appropriate grain moisture and harvest time is important and should be assessed on a cultivar basis.

Of all the environmental factors, temperature, especially in the stage of grain-filling is the most important factor affecting rice quality (Lee et al. 1996). For example, the best ripening conditions for germinability and seedling vigor of japonica cultivars are reported to be a 20 ℃ day temperature combined with strong light (12 h natural day light) and low humidity (50-60%). Temperatures as high as 30 ℃ were considered to be unfavorable, especially at the time of flowering, grain filling and maturity, and could drastically lower the head rice rate (Sheehy et al. 2006). Research analyzing the effects of temperature on aroma and flavor components of aromatic rice cultivars, however, is not well-documented.

Crop production guidelines often provide generalizations concerning the potential yield loss for seeding a crop species after using a specific seeding date, planting density or harvest date within a geographical region. However, most optimum conditions for pre-harvest treatments are chosen based on attributes like average yield, 1000-grains weight, L/B ratio, chalkiness, head rice rate, cooking and nutritional properties. Until now there has been no objective and easily applicable method to identify the optimum pre-harvest conditions of aromatic rice taking into account aroma content and flavor quality. For a successful development of fragrant rices, research regarding factors affecting the quality of the aroma is of economic interest to rice growers and processors. One possibility to expand the quality value of aromatic rice would be to sow and harvest it just on time, with the appropriate plant stand.

This experiment was designed to provide growers with flexibility in selecting pre- and postharvest practices that will maximize yield and quality without sacrificing rice flavor and texture. To achieve that objective, two aromatic rice cultivars, namely Guixiangzhan and Peizaruanxiang were chosen and their performance in terms of yield, quality and aroma evaluated under a combination of three different pre-harvest treatments (planting season, planting density, and harvest time). A storage trial was also performed where the two cultivars obtained from different growing conditions underwent different storage periods at different temperatures. For all the experiment conducted, the level of 2-AP in the brown and milled white rice was compared.

3.2.2 Experimental

3.2.2.1 Rice planting seasons

Field studies were established in 2008 in Guangzhou to evaluate grain aroma and quality of aromatic rice cultivars grown under various field conditions. One conventional

medium-grain cultivar (Guixiangzhan) and a new hybrid line with long grains (Peizaruanxiang) were evaluated.

The two rice cultivars were grown in SCAU experimental farm. Trials were performed over two seasons, the early season of 2008 and the late season of 2008. During its growth, rice completes 3 distinct phases which are vegetative (from sowing to panicle initiation), reproductive (panicle initiation to flowering), and grain filling (flowering to maturity). In this paper, rice development is divided into 6 parts: germination, tillering, booting, heading, ripening, and maturity. Panicle initiation was determined by dissecting six main stems in each plot every other day. The date was recorded as panicle initiation for the cultivar when at least 90% examined main stems had visible panicle primordia. Flowering was determined when 90% of hills had at least one stem that started anthesis. The crop reached physiological maturity when 95% of spikelets had turned from green to yellow. Daily weather records (temperature and precipitation) for each reproductive part were obtained from the weather station adjacent to the experimental farm and are summarized in **Table 3.2.1**.

Table 3.2.1 Average daily air temperature and accumulative rainfall during the main growing stages of Peizaruanxiang and Guixiangzhan rice cultivars at the experimental farm of SCAU during the early and late seasons of 2008

Growing phase	Rainfall (mm)		Temperature (℃)	
	early 2008	late 2008	early 2008	late 2008
Germination	0.03	8.87	20.68	29.03
Tillering	5.08	2.01	24.84	30.34
Booting	13.37	3.15	25.14	29.54
Heading	34.09	7.09	26.08	29.28
Ripening	22.05	9.45	28.81	25.26
Maturity	11.68	0.00	27.90	27.53

3.2.2.2 Rice growth conditions

The same field was used for the experiments in both seasons of the study and consisted of a sandy loam soil. Field preparation each site year consisted of fall disking and two passes in opposite directions with a two-way bed conditioner equipped with rolling baskets and S-tine harrows set to operate at a 6-cm depth. Each season plots received 50 kg N/ha as urea [$(NH_2)_2CO$], 75 kg P/ha as phosphoric oxide (P_2O_5) and 30 kg K/ha as potassium chloride (KCl). Due to high soil pH, 3 kg Zn/ha as zinc sulfate ($ZnSO_4$) was also applied. All Zn along with 40% N, P and K were broadcasted manually before transplanting and the remaining (60%) one week after transplanting.

Before planting, rice seeds were immersed in the water for 48 h before being drained,

then kept moist for another 24 h to sprout. The pre-sprouted seeds were evenly distributed by hand into a plot measuring 1.40 m × 4.90 m to raise uniform seedling, on March 8th for the early season and July 16th for the late season. Transplanting was done by hands at the specified hill spacing with three seedlings per hill on April 1st for the early season and on August 1st for the late season, with dates corresponding to the optimum planting period for rice in South China. Harvesting occurred on July 12th and November 1st, for the early and the late season, respectively. Experimental sites were left fallow during the winter. Standard agronomic practices with respect to pest management and weed control were similar to guidelines recommended by the Province (Tang and Wu, 2006; Duan et al. 2009). Surface irrigation occurred immediately after seeding, at the two- to three-leaf rice stage. At maturity, plots were drained approximately two weeks before harvest.

Plots were laid out in a randomized complete block design with three replications in all experiments conducted. Each replication consisted of 1 plot measuring 9.80 m × 2.00 m (19.60 m^2 area size) for the early season **(Appendix 5)**, and 9.00 m × 1.80 m (16.20 m^2 area size) for the late season **(Appendix 6)**. Each plot was surrounded by levees for irrigation and flooding.

3.2.2.3 Rice densities practiced

The response of these cultivars to planting density was tested in the early and late seasons of 2008 in separate experiments, but conducted concurrently. Rice planting density of 20 cm × 20 cm is recommended in South China to achieve rice plant density at emergence of 28 hills/m^2. Planting densities in this experiment were chosen to cover a range from 32% higher to 43% lower than the recommended one, which included 16, 19, 22, 28 and 37 hills/m2 **(Table 3.2.2)**, with three plants per hill.

Table 3.2.2 Spacing adopted to study the effect of planting density on the aroma of Guixiangzhan and Peizaruanxiang

	Plant density (cm ×cm)	Plant stand (hills/m^2)
1	20 cm × 35 cm	16
2	20 cm × 30 cm	19
3	20 cm × 25 cm	22
4	20 cm × 20 cm	28
5	20 cm × 15 cm	37

For aroma studies, the paddy rice samples from each planting density and harvested at 30 days after heading (DAH) were randomly divided into 2 populations (5 kg each) and stored for 6 months. One population was subjected to a storage temperature of -4 ℃ and the other at 30 ℃.

Rice flag leaf and grains were also harvested at 7, 14, and 21 DAH to study the effect of planting density on POX, POD, SOD activities, and MDA, proline and soluble protein contents as reported in the ¨Materials and Methods ¨section.

3.2.2.4 Harvest dates adopted

During the early and late seasons of 2008, rice plant samples planted at a density of 20 cm × 20 cm were taken at 10, 20, 30, 40, and 50 DAH in order to study the harvest date effect on the yield, quality and aroma of the two cultivars. Full heading stage occurred at 115 days after transplanting for Guixiangzhan and 120 days after transplanting for Peizaruanxiang **(Table 3.2.3)**. Samples were immediately brought to the laboratory and divided into two sets. One set was stored at 8 ℃ for 3 months. The second set was also stored for the same period, but at a temperature of 20 ℃. 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 its neigbourhoods.

Table 3.2.3 Dates adopted to study the effect of the harvesting time on the aroma of Guixiangzhan and Peizaruanxiang

	Days after heading (DAH)	Days after transplanting (DAT)	Days after sowing (DAS)
1	10	67	83
2	20	77	93
3	30	87	103
4	40	97	113
5	50	107	123

Rice leaves and grains were also harvested at 10, 20, 30, 40, and 50 days after heading to determine if there was any relationship between POX activity, proline content and aroma content in rice plants and grains. 2-AP content was determined as reported in the “Materials and Methods” section.

3.2.2.5 Yield and quality parameters measured

Plants of both cultivars were sampled at maturity and thereafter separated. After sun-drying, seed of each cultivar for individual plots were packaged, labelled and stored at room temperature for 3 months before analysis. Observations were recorded on yield components, milling quality, grain appearance, amylose content and protein content.

3.2.2.6 Statistical analysis

Analysis of variance (one-way ANOVA) was used to find the level of significant differences in the yield, quality and aroma of rice due to planting density, and harvest time. Duncan multiple range values were calculated by employing the compound mean square of error (MSE) to detect the difference among the samples, with triplicates used for all the analysis. Level of significance was set for $P<0.05$. Percentage decrease values between the concentration of 2-AP in brown and milled samples, between the concentration of 2-AP in samples stored at -4 and 30 ℃, and in samples stored at 8 and 20 ℃, were also calculated to determine the effect of milling and storage temperature on rice aroma. Standard deviations were used to know the difference between results obtained from the early and late seasons of 2008. Simple Pearson correlation coefficients were calculated and linear regressions plotted for the relationship between 2-AP content, proline content and POX activity. All the analyses were conducted using SPSS software (SPSS version 15.0, Chicago, IL).

3.2.3 Results

3.2.3.1 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. Static headspace coupled with a GC and NPD as detector was utilized during the isolation and separation process. A typical chromatogram of 2-AP obtained from brown sample harvested at 20 DAH and stored at 8 ℃ for 3 months is given in **Figure 3.2.1**. The chromatogram shows that 2-AP elutes at 9.26 min while the internal standard 2,6-DMP elutes at 8.14 min.

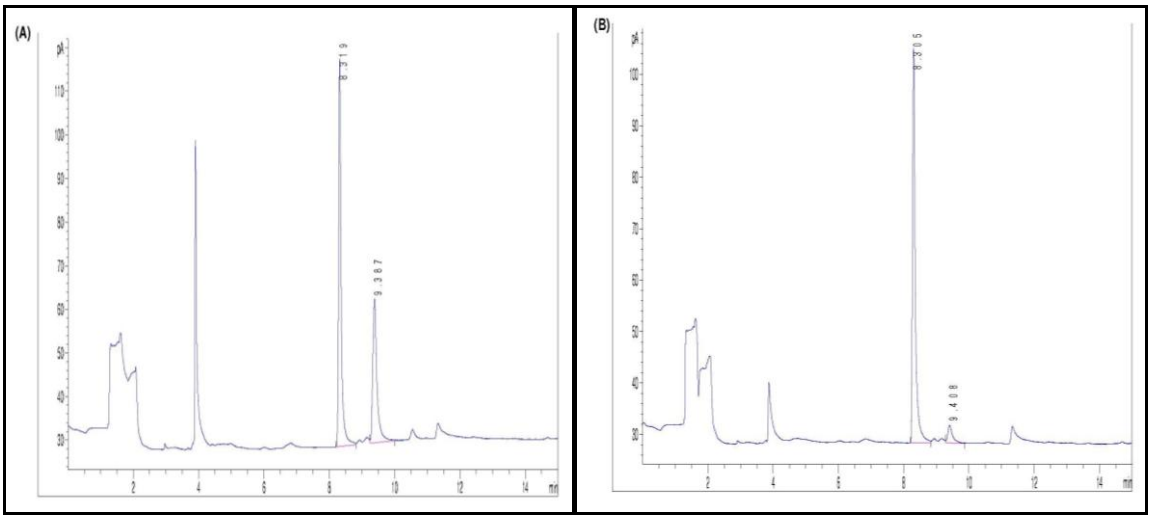


Figure 3.2.1 Gas chromatographic pattern of 2-AP and 2,6-DMP from headspace fractions extracted from (A) Guixiangzhang and (B) Peizaruanxiang using a NPD.

It is evident from **Figure 3.2.2** reporting the content of 2-AP in rice grains obtained during the early season that with an increase in planting density, 2-AP content significantly ($P<0.05$) decreases.

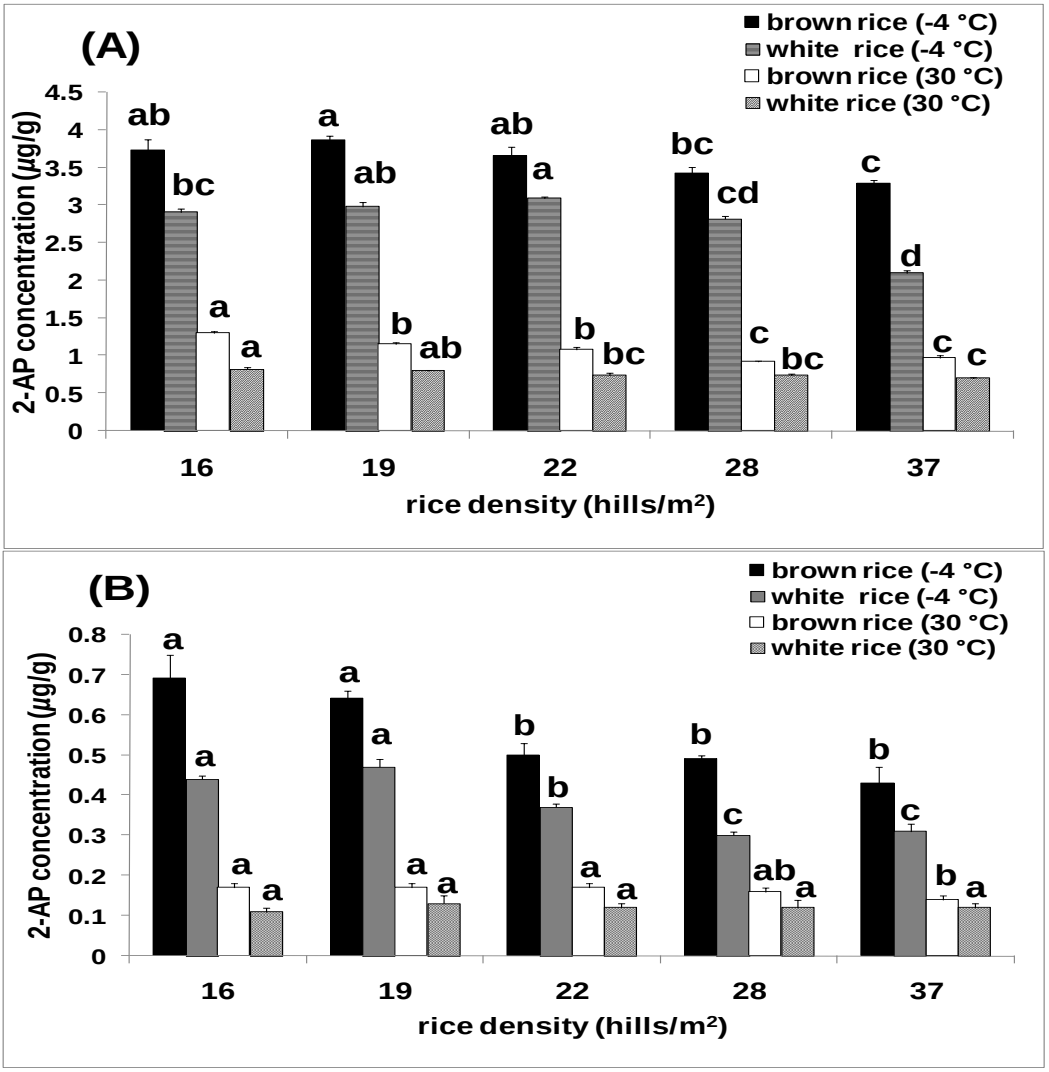


Figure 3.2.2 Concentration of 2-AP in (A) Guixiangzhan and (B) Peizaruanxiang rice cultivars as affected by planting density after a storage period of 6 months at -4 and 30 ℃. Data points charted have the corresponding standard deviations as error bars. Bars sharing a common lower case letter above are different at the $P<0.05$ level of significance by Duncan's multiple range test.

The highest concentration of 2-AP was obtained with grains cultivated at 19 hills/m^2 for Guixiangzhan (3.86 μg/g) and 16 hills/m^2 for Peizaruanxiang (0.69 μg/g) while lowest concentrations were obtained with 37 hills/m^2, 3.28 and 0.43 μg/g for Guixiangzhan and Peizaruanxiang, respectively.

Table 3.2.4 Means for grain yield and yield attributes of Guixiangzhan and Peizaruanxiang rice cultivars planted at different densities during the early season

Rice density (hills/m^2)	Panicles/Hill	Ripened spikelets/Panicle	Total spikelets/Panicles	Grain-filling percentage (%)	Grain weight (g/1,000)	Grain yield (t/ha)
			Guixiangzhan			
16	15.13 ± 0.60 a[a]	69.13 ± 2.15 a	150.33 ± 3.84 a	46.12 ± 2.60 a	25.04 ± 0.96 a	5.02 ± 0.12 a
19	14.83 ± 0.68 a	66.99 ± 3.63 a	144.67 ± 7.89 a	46.97 ± 4.87 a	25.45 ± 0.36 a	4.65 ± 0.07 a
22	11.93 ± 0.43 b	66.04 ± 1.90 a	127.00 ± 4.16 bc	52.10 ± 2.16 a	26.07 ± 0.16 a	4.68 ± 0.28 a
28	10.63 ± 0.32 b	63.49 ± 2.80 a	115.33 ± 4.91 c	55.45 ± 4.79 a	26.30 ± 0.13 a	5.18 ± 0.47 a
37	8.97 ± 0.45 c	50.24 ± 1.19 b	115.34 ± 5.93 c	43.91 ± 3.39 a	24.55 ± 0.95 a	5.17 ± 0.67 a
			Peizaruanxiang			
16	14.63 ± 0.70 a	106.61 ± 3.93 a	253.33 ± 4.06 a	42.15 ± 2.22 a	19.48 ± 0.45 a	5.00 ± 0.27 a
19	13.00 ± 0.81 ab	101.51 ± 4.96 ab	252.33 ± 8.35 a	40.31 ± 2.38 a	19.51 ± 0.47 a	5.43 ± 0.41 a
22	11.33 ± 0.18 bc	106.01 ± 2.04 a	246.00 ± 8.08 a	43.13 ± 0.66 a	19.82 ± 0.47 a	5.58 ± 0.17 a
28	9.83 ± 0.68 cd	92.50 ± 3.31 bc	221.33 ± 3.18 b	41.85 ± 2.11 a	20.08 ± 0.48 a	4.77 ± 0.41 a
37	8.87 ± 0.19 d	84.33 ± 2.27 c	187.67 ± 7.31 c	45.03 ± 1.68 a	19.63 ± 0.52 a	5.29 ± 0.35 a

[a] Results are mean ± standard deviation (n = 3). Different letters following data in a column for each parameter indicate significant differences ($P<0.05$ using Duncan's multiple range test).

3.2.3.2 Effect of planting density on rice yield and quality

Data from this current research over two seasons indicated that several yield and quality attributes of rice were not significantly (P<0.05) affected by planting density. Average grain yield (eg 5.18 and 4.77 t/ha for 28 hills/m^2) and 1000-grain weight (eg 26.30 and 20.08 g hills/m^2) remained constant across the 5 planting densities for Guixiangzhan and Peizaruanxiang, respectively, as was grain-filling rate and panicle density **(Table 3.2.4)**.

Table 3.2.5 Main effects of planting density on the milling quality of Guixiangzhan and Peizaruanxiang rice cultivars

Rice density (hills/m^2)	Brown rice yield (%)	Milled rice yield (%)	Head rice yield (%)
	Guixiangzhan		
16	80.11 ± 0.68 a[a]	65.82 ± 1.49 a	45.79 ± 0.61 ab
19	80.46 ± 0.38 a	68.30 ± 0.42 a	48.46 ± 1.52 a
22	80.63 ± 0.20 a	68.29 ± 0.50 a	48.44 ± 1.13 a
28	80.51 ± 0.35 a	68.02 ± 0.29 a	48.38 ± 1.78 a
37	79.78 ± 0.43 a	67.24 ± 0.67 a	44.07 ± 0.96 b
	Peizaruanxiang		
16	81.47 ± 0.37 a	69.36 ± 0.51 a	59.15 ± 1.00 ab
19	82.03 ± 0.35 a	70.65 ± 0.07 a	60.75 ± 0.87 a
22	81.83 ± 0.69 a	69.77 ± 0.96 a	59.77 ± 2.26 ab
28	83.20 ± 1.59 a	70.66 ± 1.06 a	55.54 ± 1.39 b
37	82.38 ± 0.57 a	70.51 ± 0.67 a	55.48 ± 1.39 b

[a] Results are expressed as mean ± standard deviation (n = 3). 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.

Table 3.2.6 Variation in grain vitreosity, amylose content and protein content of Guixiangzhan and Peizaruanxiang rice cultivars planted at different densities

Rice density (hills/m^2)	Grain vitreosity (%)	% area with chalkiness	Amylose content (%)	Protein content (%)
		Guixiangzhan		
16	88.33 ± 0.33 b[a]	31.00 ± 1.53 a	16.38 ± 0.26 a[c]	7.93 ± 0.20 a
19	90.67 ± 0.88 a	32.67 ± 1.45 a	16.29 ± 0.36 a	7.64 ± 0.05 a
22	88.00 ± 0.58 bc	25.33 ± 0.88 b	15.98 ± 0.60 a	7.77 ± 0.14 a
28	88.33 ± 0.33 b	23.00 ± 1.15 b	14.56 ± 0.86 a	7.79 ± 0.27 a
37	86.33 ± 0.33 c	32.67 ± 0.67 a	15.98 ± 0.64 a	7.66 ± 0.14 a
		Peizaruanxiang		
16	84.00 ± 0.58 b	27.00 ± 1.15 c	28.31 ± 0.42 ab	9.04 ± 0.18 a
19	82.33 ± 0.88 b	25.00 ± 1.53 c	28.36 ± 0.62 ab	9.06 ± 0.33 a
22	87.33 ± 0.88 a	27.33 ± 0.33 bc	27.80 ± 0.64 b	9.16 ± 0.14 a
28	80.00 ± 0.58 c	31.33 ± 0.88 a	27.29 ± 0.81 b	9.28 ± 0.16 a
37	78.33 ± 0.33 c	30.67 ± 1.20 ab	30.13 ± 0.44 a	9.34 ± 0.12 a

[a] Means (n = 3) ± standard deviation in the same column (for each rice cultivar) with different letters are significantly different (P<0.05) according to Duncan's multiple range test.

It was also demonstrated that higher values for head rice yield (48.46% and 60.75%) **(Table 3.2.5)** and grain vitreosity (70.07% and 88.69%) **(Table 3.2.6)** were obtained with lower seeding rates. Planting density brought about a non-significant ($P<0.05$) difference in the protein content in Guixiangzhan and Peizaruanxiang, 7.79 and 9.28%, respectively. The results of amylose and protein contents determination as a function of planting density treatments are shown in **Table 3.2.6**. The amylose content seemed to increase with increasing planting densities for Peizaruanxiang (30.13 to 28.31%) while Guixiangzhan densities did not differ significantly ($P<0.05$) with regard to amylose content (14.56%).

3.2.3.3 Effect of planting density on proline content, lipid peroxidation and antioxydative systems of rice

Highest POX, POD, SOD activities **(Table 3.2.7.)**, proline, and soluble protein content **(Table 3.2.8.)** in brown rice grains and flag leaves were obtained with plant grown at reduced seeding rates. MDA content **(Table 3.2.8.)** on the other hand appears to be low in plants grown at reduced seeding rate

Table 3.2.7 Effect of planting density on SOD, POD, POX activities in brown rice grains and flag leaves harvested at different days after heading (DAH)

SOD activity in brown rice grains (Unit/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	235.47 ± 3.42 a B[a]	221.12 ± 2.64 b C	249.61 ± 2.13 b A
19	206.79 ± 1.96 c B	245.46 ± 3.56 a A	237.97 ± 2.82 c A
22	207.99 ± 4.33 bc C	221.47 ± 1.55 b B	261.70 ± 2.20 a A
28	209.01 ± 2.62 bc C	222.26 ± 5.12 b B	245.97 ± 3.03 bc A
37	217.08 ± 3.34 b A	213.11 ± 3.50 b A	179.69 ± 3.09 d B
	Peizaruanxiang		
16	184.31 ± 7.90 bc B	248.65 ± 2.89 a A	249.12 ± 7.82 ab A
19	171.71 ± 0.64 c B	246.66 ± 6.46 ab A	238.64 ± 6.52 ab A
22	189.04 ± 1.25 ab C	237.66 ± 4.73 ab B	254.20 ± 4.88 a A
28	152.06 ± 4.61 d B	223.29 ± 3.37 c A	234.36 ± 2.85 b A
37	198.80 ± 4.03 a B	235.11 ± 0.06 bc A	196.31 ± 0.74 c B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

SOD activity in rice flag leaf (Unit/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	295.85 ± 18.44 a A[a]	271.73 ± 13.79 ab A	123.57 ± 11.33 b B
19	236.46 ± 12.09 b A	235.73 ± 17.44 b A	147.67 ± 08.60 b B
22	180.49 ± 15.19 c B	286.04 ± 12.08 a A	210.65 ± 10.46 a B
28	185.38 ± 13.74 c B	247.64 ± 14.19 ab A	151.28 ± 07.52 b B
37	264.85 ± 14.58 ab A	237.01 ± 12.22 b A	134.87 ± 16.00 b B
	Peizaruanxiang		
16	252.84 ± 15.09 c B	320.29 ± 17.78 a A	216.03 ± 14.85 a B
19	279.33 ± 16.73 bc A	313.57 ± 06.26 a A	114.62 ± 03.61 b B
22	343.84 ± 17.88 a A	283.27 ± 18.58 ab B	184.45 ± 14.97 a C
28	315.70 ± 12.81 ab A	286.91 ± 16.48 ab A	197.52 ± 15.62 a B
37	254.72 ± 05.60 c A	265.78 ± 13.12 b A	110.04 ± 06.26 b B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

POD activity in brown rice grain (Unit/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	16.57 ± 1.68 a A[a]	31.07 ± 2.16 a A	20.50 ± 1.53 a A
19	19.64 ± 1.18 a B	25.62 ± 0.42 ab A	21.38 ± 2.00 a A
22	17.70 ± 1.75 a C	23.96 ± 1.30 bc A	11.12 ± 1.90 b B
28	2.85 ± 0.80 c AB	13.95 ± 2.01 d A	10.61 ± 0.86 b B
37	9.70 ± 0.07 b C	19.58 ± 2.23 c A	12.37 ± 0.75 b B
	Peizaruanxiang		
16	8.46 ± 0.72 a A	10.00 ± 1.48 b A	6.58 ± 0.44 abc A
19	8.99 ± 0.81 a A	13.10 ± 0.19 a B	5.24 ± 0.28 bc A
22	5.15 ± 1.01 b B	8.88 ± 0.74 b AB	4.97 ± 0.78 c A
28	6.28 ± 1.24 ab A	3.82 ± 0.24 c A	7.49 ± 0.97 ab A
37	6.45 ± 0.65 ab A	5.25 ± 1.23 c A	7.83 ± 0.88 a A

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

POD activity in rice flag leaf (Unit/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	308.02 ± 12.55 a A[a]	242.93 ± 6.71 a B	203.15 ± 8.56 a C
19	251.39 ± 18.73 bc A	201.39 ± 8.34 ab B	204.48 ± 9.24 a B
22	280.43 ± 16.19 ab A	183.64 ± 15.25 bc B	186.41 ± 4.85 a B
28	206.87 ± 15.78 c A	220.03 ± 13.35 ab A	196.93 ± 12.54 a A
37	257.42 ± 17.91 abc A	152.61 ± 18.94 c B	198.42 ± 16.20 a AB
	Peizaruanxiang		
16	218.37 ± 11.77 a A	175.52 ± 11.81 ab A	190.69 ± 17.51 ab A
19	218.45 ± 15.57 a A	159.46 ± 8.18 b B	185.15 ± 13.64 ab AB
22	185.21 ± 15.18 a AB	151.33 ± 15.55 b B	221.73 ± 15.67 a A
28	216.94 ± 15.02 a A	207.09 ± 8.60 a A	194.88 ± 9.90 ab A
37	227.06 ± 10.65 a A	172.46 ± 4.22 b B	154.10 ± 17.62 b B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

POX activity in brown rice grains (μmol/min/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	108.08 ± 6.40 a A[a]	101.88 ± 5.89 a A	91.79 ± 7.47 a A
19	106.81 ± 2.62 a A	78.11 ± 4.31 b B	98.55 ± 5.96 a A
22	105.55 ± 8.45 a A	88.26 ± 5.69 ab AB	69.91 ± 3.64 b B
28	22.45 ± 8.09 b B	22.79 ± 7.59 c B	66.83 ± 5.23 b A
37	6.15 ± 1.07 b B	7.54 ± 0.86 c B	66.82 ± 7.63 b A
	Peizaruanxiang		
16	102.04 ± 9.40 abc A	100.24 ± 5.94 a A	90.66 ± 5.44 a A
19	103.79 ± 5.79 ab A	78.19 ± 8.88 b A	84.10 ± 7.38 ab A
22	123.82 ± 6.71 a A	94.66 ± 6.17 ab B	81.43 ± 9.14 ab B
28	79.54 ± 4.14 c A	80.11 ± 6.95 ab A	66.63 ± 8.27 b A
37	93.89 ± 8.72 bc A	90.41 ± 3.20 ab A	78.11 ± 6.15 ab A

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

POX activity in rice flag leaf (μmol/min/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	164.52 ± 6.40 a A[a]	101.78 ± 5.98 a B	46.74 ± 7.85 a C
19	87.33 ± 7.58 b A	31.67 ± 7.80 b B	35.36 ± 1.71 ab C
22	144.22 ± 6.87 a A	102.09 ± 5.44 a B	30.44 ± 6.48 ab C
28	44.90 ± 7.35 c AB	47.66 ± 4.34 b A	28.60 ± 2.77 b B
37	98.71 ± 6.05 b A	40.90 ± 6.66 b B	19.68 ± 5.25 b C
	Peizaruanxiang		
16	68.88 ± 7.91 a A	41.21 ± 8.29 a B	24.91 ± 2.74 a C
19	49.20 ± 3.07 ab A	28.29 ± 1.11 ab B	12.91 ± 0.92 a C
22	44.90 ± 6.85 bc A	32.90 ± 5.86 ab AB	24.60 ± 7.58 a B
28	28.60 ± 5.76 c A	18.76 ± 4.92 b B	15.99 ± 4.59 a B
37	44.59 ± 6.66 bc A	22.14 ± 3.24 b B	11.38 ± 3.42 a C

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

Table 3.2.8 Effect of planting density on MDA, soluble protein, protein content in brown rice grains and flag leaves harvested at different days after heading (DAH)

MDA content in brown rice grains (μmol/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	0.69 ± 0.02 b A[a]	0.66 ± 0.05 b A	0.57 ± 0.01 b A
19	0.62 ± 0.04 b AB	0.80 ± 0.04 ab A	0.51 ± 0.02 b B
22	0.57 ± 0.07 b A	0.59 ± 0.09 b A	0.59 ± 0.04 b A
28	0.74 ± 0.03 b A	0.66 ± 0.02 b A	0.75 ± 0.03 a A
37	1.37 ± 0.06 a A	0.93 ± 0.06 a B	0.62 ± 0.05 ab C
	Peizaruanxiang		
16	0.47 ± 0.04 b A	0.86 ± 0.06 ab A	0.50 ± 0.01 b A
19	0.57 ± 0.05 ab A	0.68 ± 0.07 bc A	0.74 ± 0.05 a A
22	0.75 ± 0.04 a A	0.80 ± 0.02 abc A	0.74 ± 0.04 a A
28	0.39 ± 0.05 b B	0.62 ± 0.02 c A	0.44 ± 0.01 b B
37	0.17 ± 0.02 c C	0.98 ± 0.03 a A	0.59 ± 0.06 ab B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

MDA content in rice flag leaf (μmol/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
		Guixiangzhan	
16	11.35 ± 0.38 b A[a]	7.59 ± 0.38 d B	10.97 ± 0.40 bc A
19	11.25 ± 0.83 b A	10.19 ± 0.75 c A	10.55 ± 0.12 c A
22	13.47 ± 0.58 a A	12.16 ± 0.65 ab A	11.33 ± 0.92 abc A
28	13.76 ± 0.13 a A	10.75 ± 0.19 bc B	12.72 ± 0.01 ab A
37	12.09 ± 0.72 ab A	12.85 ± 0.48 a A	13.18 ± 0.99 a A
		Peizaruanxiang	
16	11.91 ± 0.52 bc A	11.65 ± 0.31 b A	8.57 ± 0.51 cd B
19	11.14 ± 0.20 c A	13.16 ± 0.51 ab A	6.77 ± 0.87 d B
22	14.18 ± 0.87 a A	14.10 ± 0.47 a A	8.65 ± 0.44 c B
28	13.16 ± 0.76 ab A	14.04 ± 0.07 a A	14.37 ± 0.18 a A
37	13.48 ± 0.30 ab A	13.34 ± 0.82 a A	11.53 ± 0.63 b B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

Soluble protein content in brown rice grain (mg/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
		Guixiangzhan	
16	10.68 ± 0.35 a A[a]	10.62 ± 0.58 a A	10.66 ± 0.67 a A
19	11.23 ± 0.03 a A	10.31 ± 0.27 a A	10.60 ± 0.47 a A
22	10.78 ± 0.18 a A	11.16 ± 0.31 a A	10.45 ± 0.15 a A
28	10.60 ± 0.11 a A	10.30 ± 0.28 a A	10.11 ± 0.04 a A
37	10.55 ± 0.38 a A	10.19 ± 0.05 a A	10.56 ± 0.22 a A
		Peizaruanxiang	
16	12.05 ± 0.15 a A	11.77 ± 0.14 a A	8.31 ± 0.49 a B
19	11.26 ± 0.56 a A	11.72 ± 0.02 a A	8.15 ± 0.22 a B
22	11.42 ± 0.72 a A	11.40 ± 0.04 a A	8.23 ± 0.38 a B
28	11.92 ± 0.49 a A	11.19 ± 0.05 a A	7.81 ± 0.11 a B
37	11.67 ± 0.73 a A	11.43 ± 0.47 a A	8.10 ± 0.22 a B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

Soluble protein content in rice flag leaf (mg/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	25.08 ± 0.12 a A[a]	19.73 ± 0.62 a B	12.33 ± 0.64 a D
19	24.75 ± 0.53 a A	21.23 ± 0.87 a B	12.52 ± 0.16 a D
22	25.39 ± 0.35 a A	19.11 ± 0.06 a B	12.17 ± 0.42 a D
28	24.88 ± 0.26 a A	20.87 ± 0.76 a B	11.94 ± 0.45 a D
37	25.02 ± 0.08 a A	20.56 ± 0.76 a B	11.93 ± 0.27 a D
	Peizaruanxiang		
16	31.17 ± 0.97 a A	17.14 ± 0.29 a B	14.56 ± 0.56 a C
19	31.38 ± 0.42 a A	16.39 ± 0.69 a B	14.03 ± 0.11 a C
22	30.12 ± 0.91 a A	16.62 ± 0.45 a B	13.76 ± 0.72 a C
28	30.46 ± 0.22 a A	17.10 ± 0.77 a B	14.34 ± 0.15 a C
37	30.25 ± 0.80 a A	16.60 ± 0.52 a B	14.44 ± 0.21 a C

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

Proline content in brown rice grains (μg/g)			
Rice density (hills/m^2)	Number of days after heading (DAH)		
	7 DAH	14 DAH	21 DAH
	Guixiangzhan		
16	65.20 ± 0.72 a A[a]	22.14 ± 0.45 a B	18.51 ± 1.15 a B
19	53.66 ± 1.40 b A	21.49 ± 1.11 ab B	19.29 ± 0.72 a B
22	50.68 ± 0.91 bc A	19.80 ± 0.39 bc B	16.82 ± 1.69 a B
28	54.31 ± 0.91 b A	19.03 ± 0.78 c B	19.16 ± 1.01 a B
37	47.96 ± 2.18 c A	19.16 ± 0.47 c B	17.21 ± 0.94 a B
	Peizaruanxiang		
16	40.95 ± 1.82 a A	21.10 ± 0.34 a B	17.60 ± 0.26 a C
19	36.67 ± 0.69 ab A	23.05 ± 2.36 a A	13.97 ± 0.67 b B
22	36.15 ± 2.38 b A	25.51 ± 1.28 a B	13.71 ± 0.56 b C
28	27.46 ± 0.56 c A	23.96 ± 1.66 a B	12.80 ± 0.22 b C
37	29.79 ± 1.24 c A	21.23 ± 0.91 a B	13.58 ± 0.39 b C

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

3.2.3.4 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, in Guixiangzhan the concentration of 2-AP gradually decreased from 10 DAH (5.24 μg/g) and seemed to stabilize at 40 DAH (2.12 μg/g), a reduction rate of 60% **(Figure 3.2.3)**. Similarly, 2-AP content in Peizaruanxiang showed the same trend, but went through maxima at 20 DAH (0.83 μg/g) before significantly ($P<0.05$) dropping below the initial concentration and continuing to decrease before the end of the experiment with a content of 0.34 μg/g at 50 DAH.

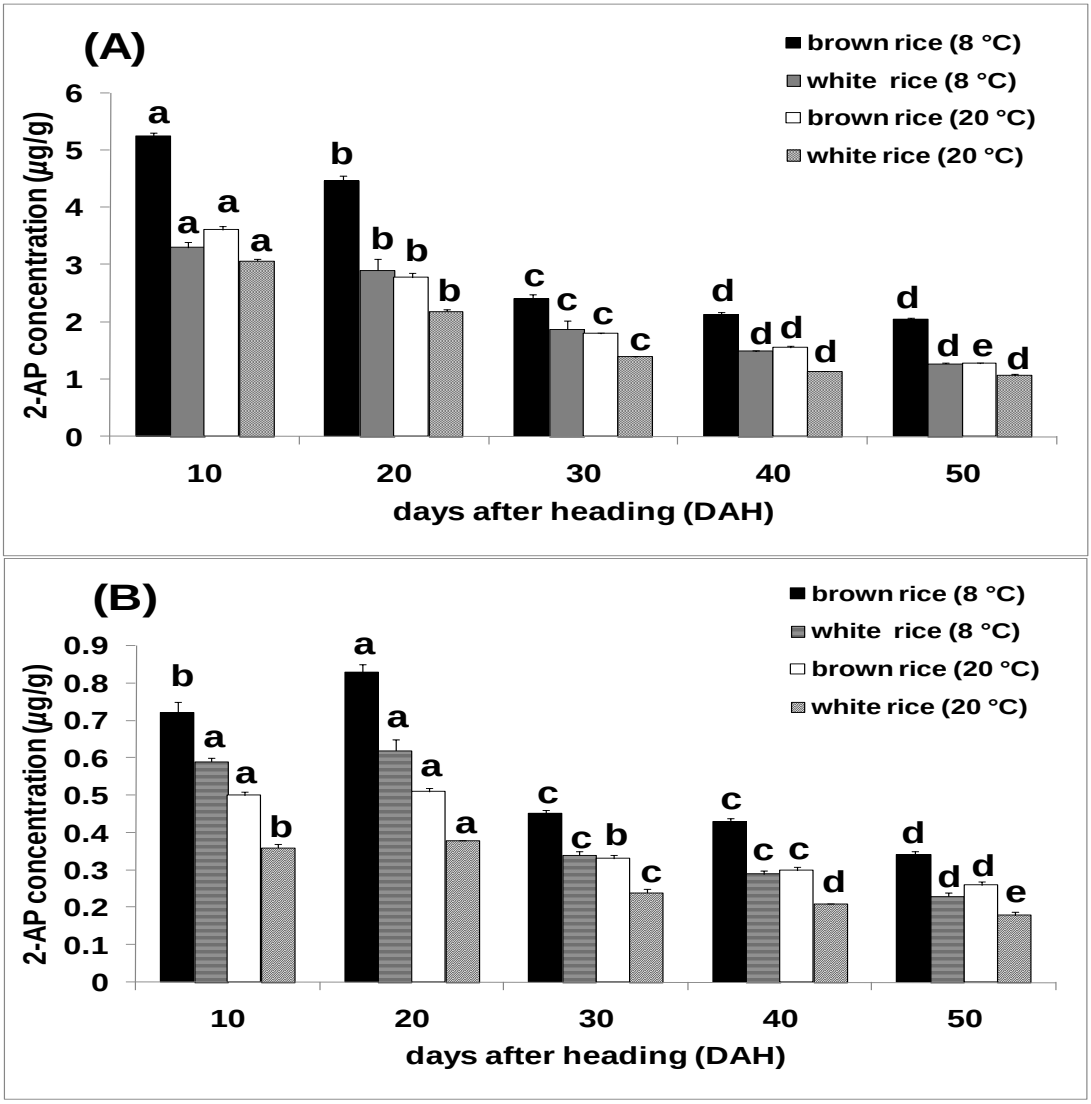


Figure 3.2.3 Change in 2-AP concentration in (A) Guixiangzhan and (B) Peizaruanxiang rice cultivars harvested at different dates after heading (DAH) and subjected for 3 months to different storage temperatures (8 and 20 ℃). Vertical bars (with standard deviations as error bars) with different letters are significantly different ($P<0.05$) according to Duncan's multiple range test.

Table 3.2.9 Means for grain yield and yield attributes of Guixiangzhan and Peizaruanxiang rice cultivars harvested at different dates after heading (DAH) during the late season

Harvest date (DAH)	Ripened spikelets/Panicle	Total spikelets/Panicles	Grain-filling percentage (%)	Grain weight (g/1,000)	Grain yield (t/ha)
			Guixiangzhan		
20	61.36 ± 3.17 a[a]	84.67 ± 3.48 a	72.45 ± 1.65 b	25.33 ± 0.03 b	4.97 ± 0.25 a
30	61.07 ± 0.48 a	82.00 ± 2.65 a	74.59 ± 1.83 ab	26.37 ± 0.13 a	5.14 ± 0.06 a
40	61.28 ± 0.67 a	79.00 ± 2.08 a	77.64 ± 1.50 a	26.30 ± 0.10 a	5.15 ± 0.04 a
50	61.45 ± 3.23 a	81.00 ± 4.73 a	75.92 ± 0.63 ab	26.30 ± 0.17 a	5.16 ± 0.25 a
			Peizaruanxiang		
20	88.74 ± 0.96 b	130.33 ± 4.10 a	68.20 ± 1.93 c	22.17 ± 0.15 a	6.01 ± 0.03 b
30	104.55 ± 2.27 a	129.00 ± 1.73 a	81.04 ± 0.92 a	21.83 ± 0.23 a	6.97 ± 0.13 a
40	97.54 ± 4.51 ab	127.33 ± 4.10 a	76.53 ± 1.06 b	21.80 ± 0.26 a	6.49 ± 0.27 ab
50	91.82 ± 2.21 b	124.00 ± 2.65 a	74.04 ± 0.43 b	21.73 ± 0.20 a	6.10 ± 0.20 b

[a] Results are mean ± standard deviation (n = 3). Different letters following data in a column for each parameter indicate significant differences ($P<0.05$ using Duncan's multiple range test).

3.2.3.5 Effect of harvesting time on rice yield and quality

Harvesting time in this study negatively influenced grain weight mainly for harvesting at 20 DAH (25.33 g) for Guixiangzhan **(Table 3.2.9)**. Weight patterns for harvesting at 20 to 50 DAH were similar to the more traditional 30 DAH practiced in South China. Grain yield in Peizaruanxiang increased rapidly from 10 DAH (4.29 t/ha) until it reached a maximum at 30 DAH (6.97 t/ha) and changed little thereafter. In Guixiangzhan, grain yields were not affected by delayed harvesting. For the two cultivars, harvest timing affected the percent of whole and total milled rice **(Table 3.2.10)**.

Table 3.2.10 Main effects of the harvesting time on the milling quality of Guixiangzhan and Peizaruanxiang rice cultivars (DAH)

Harvest date (DAH)	Brown rice yield (%)	Milled rice yield (%)	Head rice yield (%)
	Guixiangzhan		
20	81.82 ± 0.19 a[a]	70.47 ± 0.20 bc	61.77 ± 0.45 a
30	82.58 ± 0.21 a	70.85 ± 0.47 b	61.26 ± 0.12 ab
40	82.57 ± 0.21 a	72.72 ± 0.30 a	60.12 ± 0.29 b
50	82.40 ± 0.30 a	72.51 ± 0.10 a	60.31 ± 0.17 b
	Peizaruanxiang		
20	83.94 ± 0.29 a	73.18 ± 0.20 b	51.60 ± 0.19 c
30	84.29 ± 0.18 a	73.45 ± 0.13 b	66.64 ± 0.31 a
40	83.42 ± 0.37 a	75.14 ± 0.50 a	60.08 ± 0.33 b
50	83.95 ± 0.36 a	75.62 ± 0.14 a	60.48 ± 0.64 b

[a] Results are expressed as mean ± standard deviation (n = 3). 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.

The lowest head rice rate was observed harvesting at 10 DAH for the two fragrant rice cultivars. On the other hand, the percentage of head rice reached a maximum at an intermediate harvest date, and then declined rapidly with delays in harvesting. Maximum head rice rate was attained for Guixiangzhan at 20 DAH (61.77%). After that there was slightly decrease gradually from 61.26% at 30 DAH to 60.31% at 50 DAH. Similarly, harvesting at 30 DAH gave maximum head rice recovery for Peizaruanxiang (66.64%), while harvesting after that period resulted in increase of broken grains. **Table 3.2.11** shows the ANOVA results for the grain vitreosity of the two rice cultivars with the harvesting time. Grain vitreosities were highest for early and harvest dates for Guixiangzhan, and normal harvest dates for Peizaruanxiang.

Table 3.2.11 Variation in grain vitreosity, amylose content and protein content of Guixiangzhan and Peizaruanxiang rices harvested at different dates after heading (DAH)

Harvest date (DAH)	Grain vitreosity (%)	% area with chalkiness	Amylose content (%)	Protein content (%)
		Guixiangzhan		
20	91.00 ± 1.00 a[a]	11.67 ± 1.20 b	20.60 ± 0.03 a	9.17 ± 0.24 a
30	89.67 ± 0.67 b	21.00 ± 1.00 a	20.57 ± 0.16 a	9.27 ± 0.17 a
40	90.00 ± 0.58 ab	22.67 ± 0.88 a	20.20 ± 0.09 b	9.25 ± 0.22 a
50	83.33 ± 0.33 b	21.67 ± 0.67 a	20.22 ± 0.04 b	9.03 ± 0.22 a
		Peizaruanxiang		
20	84.67 ± 0.67 b	25.67 ± 0.33 b	29.02 ± 0.27 a	9.69 ± 0.28 a
30	81.67 ± 0.88 bc	23.00 ± 0.58 c	28.95 ± 0.27 a	9.90 ± 0.07 a
40	80.00 ± 1.53 c	27.67 ± 0.88 ab	28.84 ± 0.19 a	9.50 ± 0.24 a
50	78.67 ± 1.45 c	28.00 ± 1.00 ab	28.88 ± 0.18 a	9.57 ± 0.29 a

[a] Means (n = 3) ± standard deviation in the same column (for each rice cultivar) with different letters are significantly different (P<0.05) according to Duncan's multiple range test.

It is observed from the same table that harvest date had a very small significant effect on protein contents in the two cultivars. However, they did not respond in the same way to harvest date in relation to amylose content. Amylose content did not show much variation for Peizaruanxiang, but decrease with harvest date for Guixiangzhan, from 20.60% at 20 DAH to 20.22 at 50 DAH. Guixiangzhan samples were classified as having high amylose contents (28.37 to 28.88%) while Peizaruanxiang samples have intermediate contents (20.20 to 20.60%).

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

In our study, free proline was present at similar levels in rice leaves harvested from 10 to 50 DAH (0.40; 0.37 μmol/g), with a background similar with the two cultivars. In rice grains, however, proline had the highest concentration at the flowering stage. From **Figure 3.2.4**, it can be seen that generally the level of free proline is high at 10 DAH (0.23; 0.22 μmol/g) and then decreased gradually to maturity (0.14; 0.12 μmol/g). It is important to note the low amount of free proline present in the rice grain compared to that present in leaves for the two cultivars, approximately 41.74% decrease for Guixiangzhan and 39.44% for Peizaruanxiang at 10 DAH.

Figure 3.2.5 presents the evolution of the activity of one enzyme, namely proline oxidase (POX) involved in the proline biosynthesis pathway. Knowledge of the activity of POX can give us a general view on the total proline level in the leaves and grains. POX activity was present in the highest proportions in rice leaves at all harvesting periods with no consistent reduction in activity observed due to harvest date (Data not shown). In rice grains, however, POX activity

was present in high proportions at 10 DAH (10.94; 11.01 μmol/min/mg), but decreases gradually to 6.28 and 6.42 μmol/min/mg at maturity for Guixiangzhan and Peizaruanxiang, respectively.

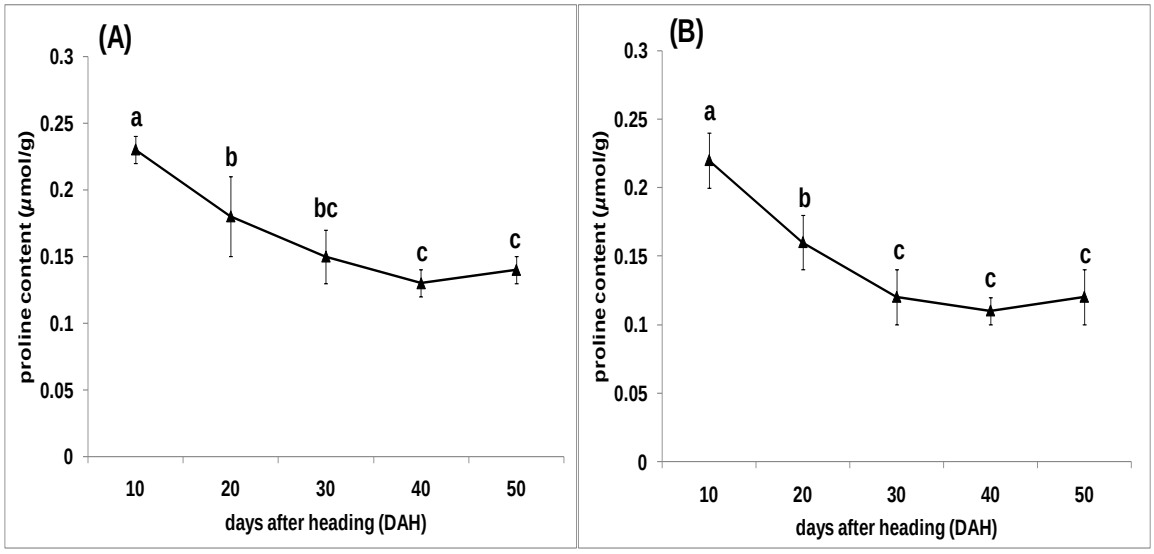


Figure 3.2.4 Time-content relationship for proline in brown grains of (A) Guixiangzhan and (B) Peizaruanxiang. Plotted points with different letters above are significantly different (P<0.05, Duncan's multiple range test). Vertical bars represent the standard deviations.

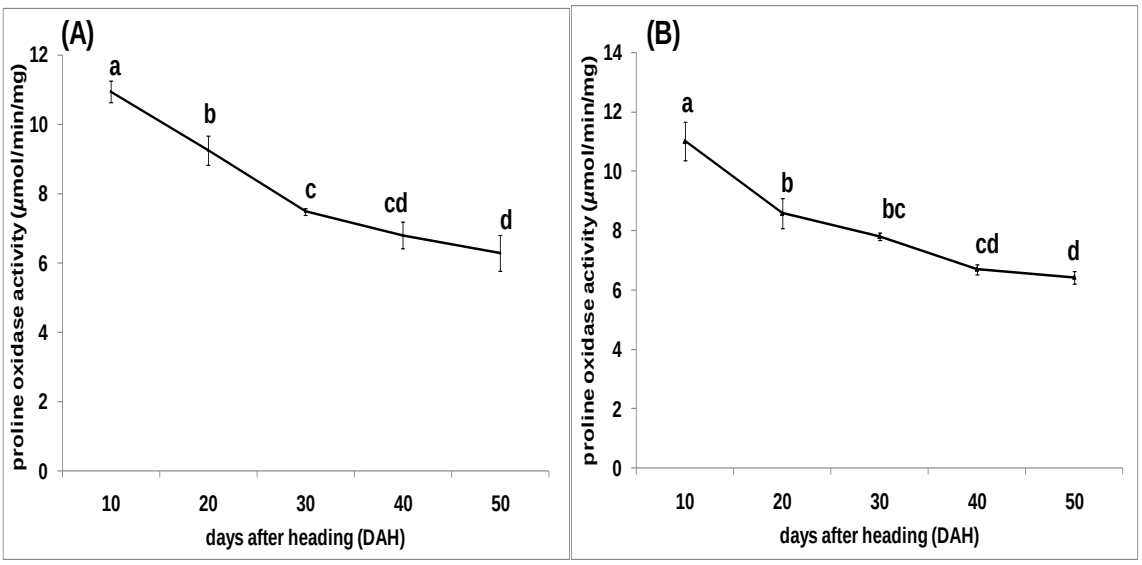


Figure 3.2.5 Time-activity relationship for proline oxidase in brown grains of (A) Guixiangzhan and (B) Peizaruanxiang. Plotted points with different letters above are significantly different (P<0.05, Duncan's multiple range test). Vertical bars represent the standard deviations.

As the content of free proline decreases, the content of 2-AP too decreases from 10 DAH to 50 DAH **(Figure 3.2.6)**.

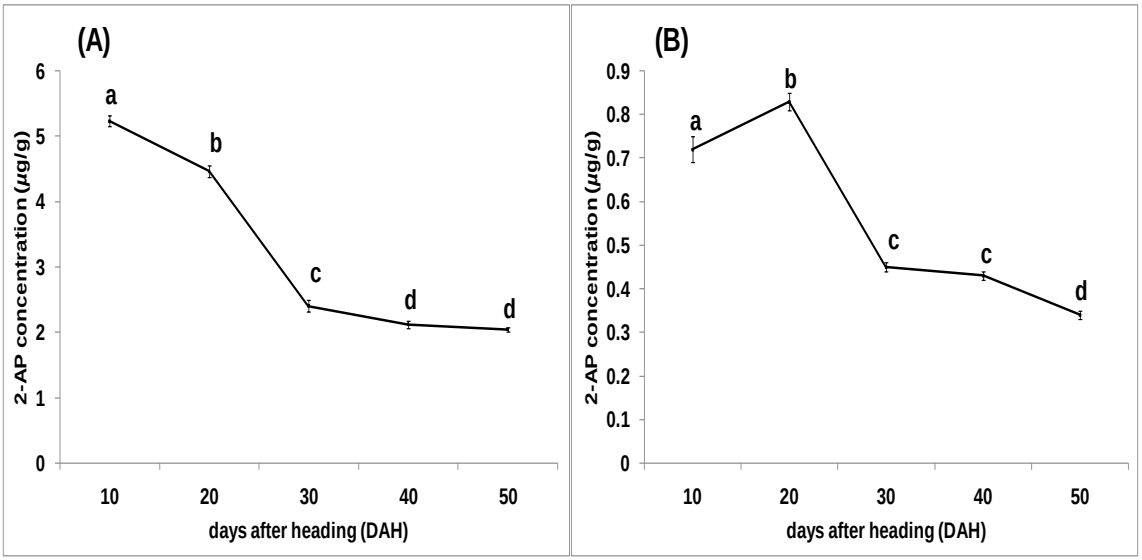


Figure 3.2.6 Time-content relationship for 2-AP in brown grains of (A) Guixiangzhan and (B) Peizaruanxiang. Plotted points with different letters above are significantly different (P<0.05, Duncan's multiple range test). Vertical bars represent the standard deviations.

Table 3.2.12 reports the correlation between proline content, proline oxidase activity and 2-AP in the two rices harcested at different dates after heading.

Table 3.2.12 Correlation between proline content (PRO), proline oxidase activity (POX) and 2-acetyl-1-pyrroline content (2-AP) in Guixiangzhan and Peizaruanxiang rice grains harvested at 10, 20, 30, 40 et 50 days after heading

Pairwise comparison	Number of values	Test of significance (2-tailed)	Pearson correlation coefficient (r)
	Guixiangzhan		
PRO vs POX	5	0.005	0.973**
PRO vs 2-AP	5	0.011	0.955*
POX vs 2-AP	5	0.003	0.981**
	Peizaruanxiang		
PRO vs POX	5	0.080	0.964**
PRO vs 2-AP	5	0.140	0.755
POX vs 2-AP	5	0.133	0.764

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

3.2.3.7 Effect of planting season on 2-acetyl-1-pyrroline content

Plants harvested during the late season (early November) resulted in significantly higher levels of 2-AP compared to the early season (mid-June), with 4.57 and 6.42 ng/g 2-AP for Guixiangzhan, and, 2.07 and 2.90 ng/g 2-AP for Peizaruanxiang. Brown rice samples were also milled to yield white rice. After this transformation however, no season effect on 2-AP content was evidenced using the KOH method, with concentrations ranging from 3.04 to 3.15 ng/g for Guixiangzhan and 1.82 to 2.09 ng/g for Peizaruanxiang **(Figure 3.2.7)**.

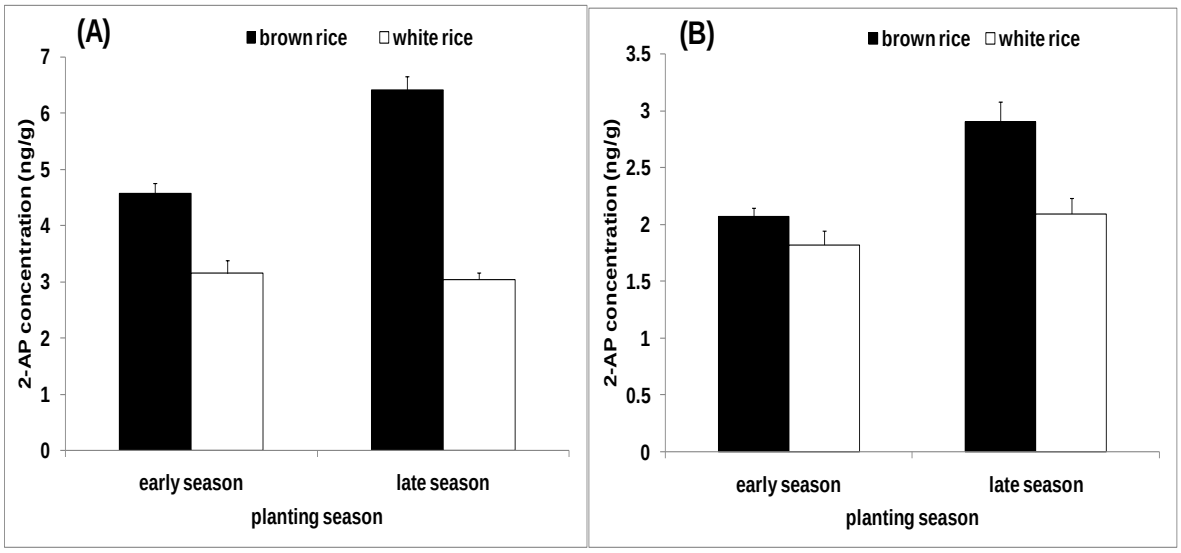


Figure 3.2.7 Overall effect of planting season on the concentration of 2-AP in (A) Guixiangzhan and (B) Peizaruanxiang rice cultivars. Data points charted have standard deviations as error bars.

3.2.3.8 Effect of planting season on rice yield and quality

The mean total head rice yields of the two rice cultivars were also significantly different across planting seasons, low for the early season (48.38%; 55.54%) and high for the late season (65.15%; 65.72%) **(Table 3.2.13)**. Marginal reduction of vitreosity was also observed with plant harvested during the late season. Rice yield and other quality traits, however, were less affected by planting season.

Table 3.2.12 Seasonal variation of yield and quality parameters in Guixiangzhan and Peizaruanxiang rice cultivars

Parameters	Early season	Late season
	Guixiangzhan	
Grain yield (t/ha)	5.18 ± 0.47	5.09 ± 0.28
Grain weight (g/1,000)	26.30 ± 0.13	25.92 ± 0.58
Head rice yield (%)	48.38 ± 1.78	65.15 ± 0.52
Grain vitreosity (%)	88.33 ± 0.33	92.33 ± 0.33
Amylose content (%)	14.56 ± 0.86	19.51 ± 0.51
Protein content (%)	7.79 ± 0.27	8.01 ± 0.21
	Peizaruanxiang	
Grain yield (t/ha)	4.77 ± 0.41	6.44 ± 0.14
Grain weight (g/1,000)	20.08 ± 0.48	21.70 ± 0.12
Head rice yield (%)	55.54 ± 1.39	65.72 ± 0.25
Grain vitreosity (%)	80.00 ± 0.58	85.33 ± 0.88
Amylose content (%)	27.29 ± 0.81	28.20 ± 0.57
Protein content (%)	9.28 ± 0.16	8.09 ± 0.29

[a] Results are mean ± standard deviation. Each determination is the mean of three replications.

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

Fragrant rices harvested in June and kept for 6 months at -4 ℃ contained up to 4 times 2-AP in all forms (brown and white), compared to those kept at 30 ℃. Generally, there was a significant decrease in Guixiangzhan and Peizaruanxiang overall volatile component concentrations after storage at 30 ℃ (**Figure 3.2.8**).

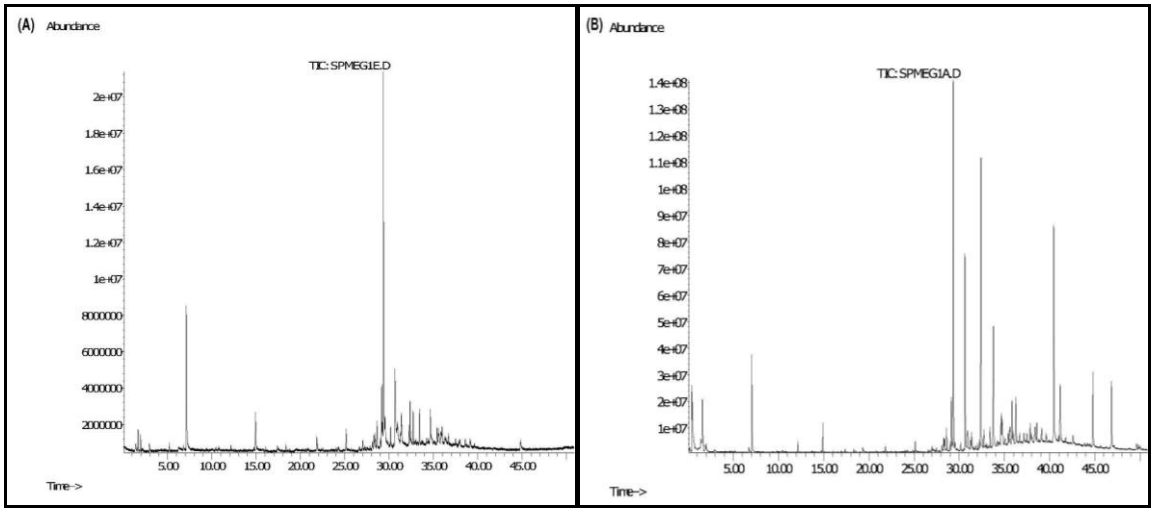
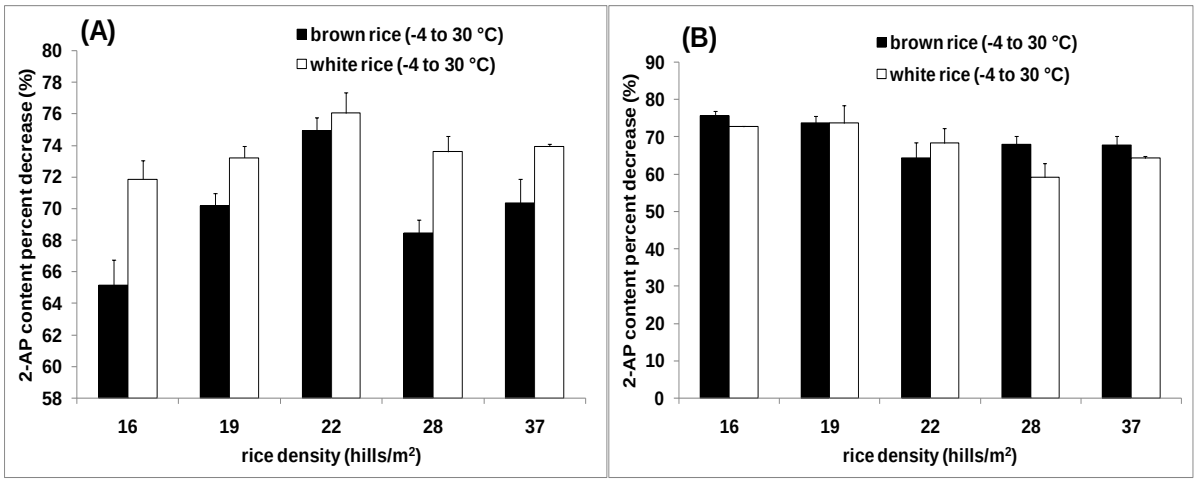


Figure 3.2.8 Chromatogram of total volatiles isolated using SPME from brown rice grains of Guixiangzhan stored for 6 months at (A) 30 ℃ and (B) -4 ℃.

High losses of 2-AP occurred under very warm conditions of 30 ℃, from 3.73 to 1.30 μg/g for Guixiangzhan and from 0.69 to 0.17 μg/g for Peizaruanxiang (**Figure 3.2.9A, Figure 3.2.9B**). 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 ℃ compared to 8 ℃ **(Figure 3.2.9C, Figure 3.2.9D)**.



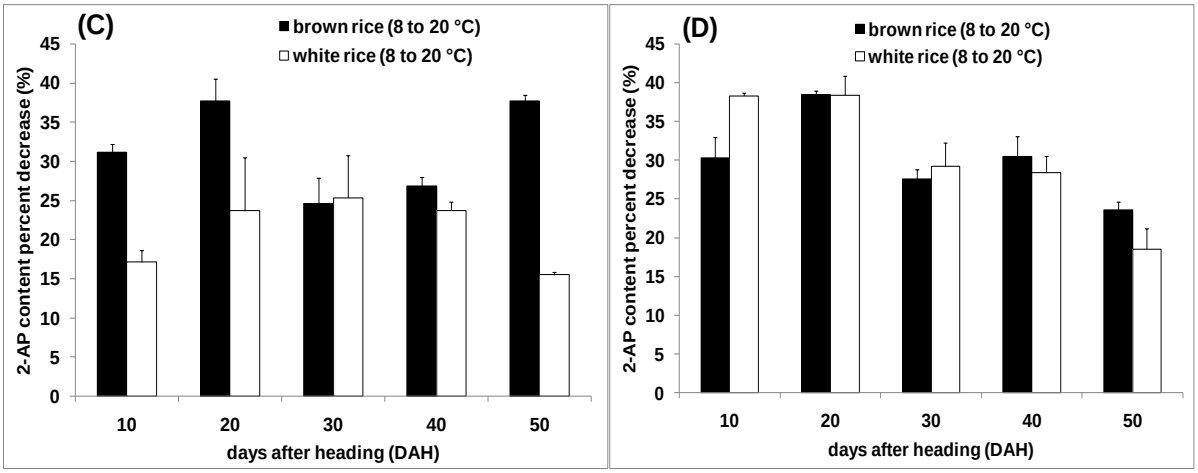


Figure 3.2.9 Percent decrease of 2-AP due to storage temperature in Guixiangzhang (A, C) and Peizaruanxiang (B, D) brown rice grains obtained from different treatments.

3.2.3.10. Effect of milling degree on 2-acetyl-1-pyrroline content

It can be seen 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 (**Figure 3.2.10**).

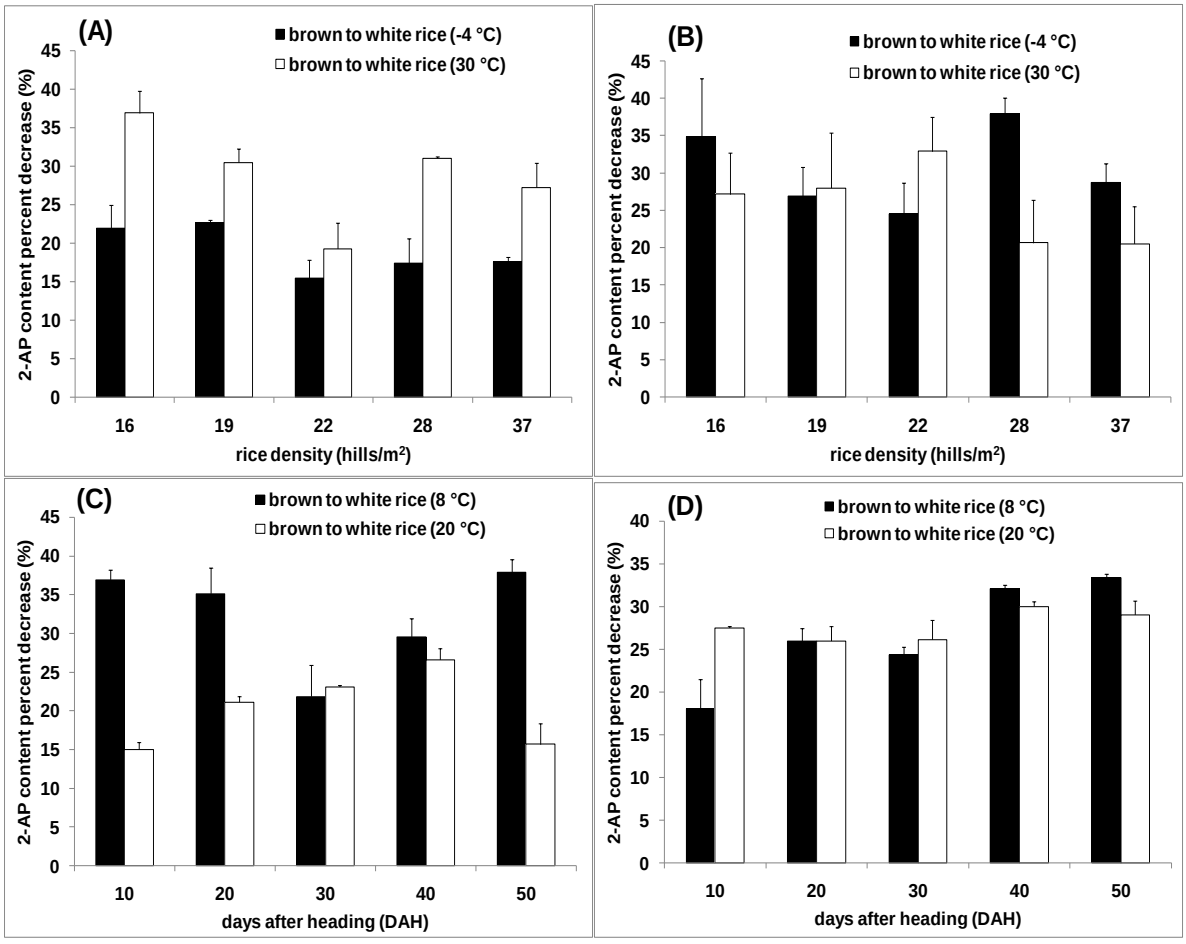


Figure 3.2.10 Percent decrease of 2-AP due to milling in Guixiangzhang (A, C) and Peizaruanxiang (B, D) brown rice grains obtained from different treatments.

3.2.4 Discussion

3.2.4.1 Planting density rates for optimum aroma and quality of transplanted rice

Rice flavor is a complex combination of taste and odor sensations. However its good smell stem primarily from its 2-AP content. 2-AP is related to a group of compounds having an acetyl group in close association with a nitrogen generally in a heterocyclic ring, and with an odor character frequently referred to as cracker or popcorn-like (Gu et al. 2002). 2-AP content has often been equated with the aroma quality of rice. Therefore, it was chosen to monitor whether or not pre- and post-harvest factors affect the aroma and flavor of Guixiangzhan and Peizaruanxiang.

During the early and late seasons of 2008, 2-AP content significantly ($P<0.05$) decreases 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. Particularly grain-filling rate and panicle density was stable as rice density increased. This provides evidence for the compensatory nature of rice to fill voids in the canopy by producing larger amounts of biomass (more reproductive tillers at low densities) as illustrated in the increase of the number of panicles/m^2. Other researchers have reported similar trends in panicle density and filled-grain panicles (Bond et al. 2008). Contrary to findings by some authors (Zeng and Shannon, 2000; Bond et al. 2008), planting density brought about a non-significant ($P<0.05$) difference in the protein content in Guixiangzhan and Peizaruanxiang.

Zeng and Shannon (2000) have reported that the maintenance of a critical level of rice plant population in the field was necessary to maximize stresses and diseases resistance. Data from this study and reporting POX, POD, SOD activities, MDA, proline, soluble protein content in rice brown grains and flag leaves suggest that defense systems are at the best at reduced seeding rates.

Taken together, our results indicate that planting density can be reduced from the currently recommended 28 hills/m^2 to 19-16 hills while maintaining optimum yield and quality, particularly in relation to aroma and flavor attributes. With a potential seed shortage for some cultivars and rising seed costs, this possibility to lower seeding rates and maintain high yield levels would be particularly important for farmers in respect to production costs since aromatic rice seeds are more expensive than those of the conventional cultivars.

3.2.4.2 Estimation of the optimum harvesting time for rice based on aroma content

As part of this study, we examined the effects of varying harvest dates on rice aroma and flavor, and particularly the content in 2-AP. In our study, 2-AP content in fragrant rice

decreased with harvesting time in both Guixiangzhan and Peizaruanxiang. The first growth stage of the rice plants were samples were taken was 10 days after heading (DAH). A strong characteristic flavor could be detected from the fragrant rice cultivars at this stage. At 50 DAH, however, plants were no longer exhibiting their fresh aroma. Our results corroborate with those obtained by Itani et al. (2004) in Japan using an early-heading and a late-heading rice cultivars. Flavor was considered to be rich in immature rice but poor in over-ripened rice. During grain development in the early-heading cultivar, the 2-AP concentration in the brown rice reached a peak at four or five weeks after heading and then decreased rapidly to 20% of the maximum at seven or eight weeks after heading.

Our results suggest that early harvesting, 10 DAH has the greatest chance to recover aroma; however, whiteness studies suggested that harvesting at 10 DAH may not be beneficial for appearance acceptability of the two cultivars. Samples harvested 20 to 40 DAH were similar in appearance. When choosing the optimum harvesting time for rice, aroma content and whiteness are not the only criteria involved; optimum paddy yield and grain qualities have to be taken into consideration (McCauley and Way, 2002).

Based on data presented in **Figure 3.2.3** and **Table 3.2.8** to **3.2.10**, 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

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

In our study, particular attention was given to the study of the effect of harvest time on the activity of proline oxidase and the concentration of free proline in rice grain because of its possible involvement in the biosynthesis of 2-AP and hence the flavor production pathway. Proline mediated stimulation of aroma has been observed in rice (Yoshihashi et al. 2002) and microorganisms (Schieberle, 1990; Muench et al. 1997; Costello et al. 2002; Thimmaraju et al. 2005; Adams and De Kimpe, 2007). Although a significant correlation was found between 2-AP content and proline content in Guixiangzhan rice grains, there was however only a weak correlation between 2-AP content and proline oxidase activity and between proline content and proline oxidase activity **(Table 3.2.11, Appendix 7).** In Peizaruanxiang, no significant correlation was found between the three parameters as shown by the calculated linear correlation coefficients and the plotted linear regressions **(Table 3.2.11, Appendix 8)**. This suggests that

free proline level cannot be considered as an indicator of fragrance is rice grains.

3.2.4.4 Seasonal variation of aroma content and rice quality parameters

Data including the concentration of 2-AP in Guixiangzhan and Peizaruanxiang rice plants grown over two seasons (early 2008 and late 2008) were compared to determine if there was an overall effect of the planting season on the concentration of 2-AP in rice grains. Samples were analyzed directly after harvest using the KOH method coupled to GC (Tang and Wu, 2006). Although the method have been reported as less sensitive compared to steam distillation, dichloromethane solvent extraction or headspace sampling (Wongpornchai et al. 2004), it could however clearly detect planting season difference in 2-AP content. Plants harvested during the late season (early November) resulted in significantly higher levels of 2-AP compared to the early season (mid-June). Possibly the differences in daily mean temperatures, accumulative rainfall and mean solar radiations between the two seasons were large enough to have any significant effect on 2-AP content. In general, the early season showed increasing daily mean temperature from 14.67-20.68 ℃ during germination to 28.49-27.90 ℃ during ripening and maturity phases, while the late season showed decreasing temperatures from 31.37-29.03 ℃ to 23.77-27.53 ℃ **(Table 3.2.1)**. It becomes evident that grains ripened under a low temperature regime (late season) had higher 2-AP. This result is reasonable and is supported by the general comments that high ripening temperatures lower the content of 2-AP in rice (Itani et al. 2004). This could also suggest that advancing the sowing date to allow seed ripening to coincide with the cool and dry segment of late November-early December could improve aroma content and other seed qualities.

Planting season affected only two quality traits in Guixiangzhang and Peizaruanxiang, head rice yield and grain vitreosity. The low head rice for the early season seems related to the hot, sunny, and dry days followed by humid or dewy nights of late June and early July, when the rice is approaching maturity. Meanwhile, the declining temperature and increased precipitation after late October might contribute to the improved head rice yield observed for Guixiangzhan and Peizaruanxiang during the late season. Marginal reduction of vitreosity was also observed with plant harvested during the late season (Lee et al. 1996; Sheehy et al. 2006). These results may be compared with those of Lam and Proctor (2003) who reported that fully milled rice has better aroma and flavor that broken rice. Indeed, grains harvested during the early season were chalkier and contained 2-AP in lower concentrations, compared to grains obtained from the late harvest. Rice yield and other quality traits, however, were less affected by planting season.

It was reported that protein content and amylose content correlate highly and negatively

with sweet taste in rice (Champagne et al. 2008). In particular, the linear amylose of starch is able to form inclusion complexes with a wide variety of volatile compounds that may affect the intensity of perceived aromas. The interactions of aroma compounds with lipids and proteins affecting their volatility have also been reported (Arvisenet et al. 2002; Jouquand et al. 2006). This study did not find any relationship between amylose and protein content, and aroma or flavor using planting season, harvest date and planting density.

3.2.4.5 Aroma stability during storage

During storage of the two cultivars, 2-AP content reduced over time and appeared to be significantly affected by the storage 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 ℃. It was also found that rough rice stored at frozen temperatures resulted in consistently whiter milled rice color over time (Meullenet et al. 2000; Lam and Proctor, 2003; Moonsor and Proctor, 2004). 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 displays the highest rate of decrease at the beginning of storage (Wongpornchai et al. 2004) and is inversely correlated with fat acidity at an early stage of storage (Yoshihashi et al. 2005). This demonstration highlights the necessity to focus on storage protocols for a successful development of Guixiangzhan and Peizaruanxiang cultivars.

3.2.4.6 Aroma preservation with milling

Results obtained previously provided an opportunity to compare the 2-AP content found in the headspace of unmilled brown rice and milled white rice. 2-AP content decreased with milling, which was in disagreement with two previous studies (Bergman et al. 2000; Grimm et al. 2001), which showed little difference in the 2-AP contents of unmilled and milled rice. Rice is normally consumed after it has been milled and cooked without undergoing any other processing that might improve aroma quality (Piggott et al. 1991). Therefore, the degree of milling is very important for aroma and flavor preservation.

3.2.5 Conclusion

In our study, two aromatic rice cultivars were subjected to three pre-harvest treatments (ripening temperature, planting density, and harvest date) and different storage conditions (3 to 6 months at -4, 8, 20, and 30 ℃) and their overall effects on aroma content evaluated. Highest

2-AP concentrations were obtained for Guixiangzhan and Peizaruanxiang with the lowest planting density of 16 hills/m^2 (3.73; 0.69 μg/g), the earliest harvesting time of 10 days after heading (5.24; 0.72 μg/g), a low ripening temperature of 25 ℃ (7.12; 2.42 ng/g), the shortest storage time of 3 months (2.40; 0.45 μg/g) and the coolest temperature of -4 ℃ (3.42; 0.49 μg/g). After milling, 2-AP content decreased up to 1.5-fold in both Guixiangzhan and Peizaruanxiang. These findings indicate that manipulating pre and post-harvest treatments can greatly improve the specific attributes of aromatic rices. It is assumed from the study that keeping aromatic rice under refrigeration, milling it at a low degree and consuming 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 show that altering sowing dates to allow the critical stages of seed maturation to coincide with favorable field environments (cool and dry segment of the year) as well as 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.

3.3 Change in the level of some odor-active volatile compounds of aromatic rice after application of growth regulators

3.3.1 Introduction

Each volatile component has a minimum detectable level called its odor threshold. Compounds present in concentrations above the threshold contribute to overall flavor. Related to the notion of odor thresholds is that of odor activity value (OAV) (Buttery et al. 1988), which was latter on extended to a screening method referred to as aroma extract dilution analysis (AEDA) (Jezussek et al. 2002). The two methods have been widely used to identify possible contributors to rice aroma and flavor.

Of the over 300 volatile compounds identified from various cultivars of aromatic and non-aromatic rice, a relatively small number has been recognized as odor-active compounds. Some of them are found as natural constituents of rice grains while others are formed during cooking (Grosch and Schieberle, 1997; Zhou et al. 1999). Broadly odor-active compounds in rice can be classified into 4 groups which are (1) nitrogen-containing compounds like 2-acetyl-1-pyrroline (2-AP, popcorn-like), benzothiazole (nutty), (2) maillard reaction products like 2-phenylethanol and phenylacetic acid (rose-like), 2-aminoacetophenone (naphthalene, floor polish), (3) lipid degradation products like hexanal (green), octanal (citrus-like), nonanal (floral, fruity), decanal (soapy), (*E*)-2-hexenal (apple, green), (*E*)-2-nonenal (fatty, tallowy), (*E*,*E*)-2,4-decadienal (fatty), 4,5-Epoxy-(*E*)-2-decenal (metallic), 2-pentylfuran (beany), vanillin (vanilla-like), 1-pentanol (plastic), 1-hexanol (green), (4) thermally induced products like 3-hydroxy-4,5-dimethyl-2(5H)-furanone (seasoning-like), bis-(2-methyl-3-furyl)-disulfide (meaty), 2-methoxy-4-vinyphenol (spicy, clove-like), 4-vinylguaiacol and 4-vinylphenol (phenolic, medicinal) (Buttery et al. 1988; Petrov et al. 1996; Widjaja et al. 1996b; Buttery et al. 1999; Jezussek et al. 2002; Yang et al. 2008a; Maraval et al. 2008). Quantitative and qualitative differences in these critical odor-active compounds are thought to collectively create the aroma perceived and account for differences among flavor types (Yang et al. 2008c; Champagne et al. 2008).

Rice aroma and flavor are determined by genetics factors, but are also very much dependent on environmental conditions during production and pre- and post-harvest practices. Drying method and conditions (Meullenet et al. 1999; Apintanapong and Noomhorm, 2003; Wongpornchai et al. 2004), rough rice storage conditions (Ishitani and Fushimi 1994; Tava and Bocchi, 1999; Meullenet et al. 2000; Yoshihashi et al. 2005; Sirisoontaralak and Noomhorm, 2006), milling degree (Piggott et al. 1991; Monsoor and Proctor, 2004), milled rice storage

temperature and time (Piggott et al. 1991; Widjaja et al. 1996a; Lam and Proctor 2003; Laohakunjit and Kerdchoechuen, 2007), preparation and cooking methods (Crowhurst and Creed, 2001; Monsoor and Proctor, 2002; Bett-Garber et al. 2007; Srisawas and Jindal, 2007; Tulyathan et al. 2008) all affect the content of 2-AP in rice.

Concerning pre-harvest factors, aromatic rice cultivated at a high altitude have a stronger aroma than that cultivated at a low altitude (Yoshihashi et al. 2004; Bradbury et al. 2008). The concentration of 2-AP, the compound imparting the popcorn character to aromatic rice flavor, was observed to be higher in brown rice ripened at a lower temperature than that which ripened at a high temperature (Lee et al. 1996; Dutta et al. 1999; Itani et al. 2004). In India, Pakistan, Thailand, China and Italy, areas with highest grain contents of volatile compounds are suspected of being dry and sandy (Hou et al. 1988; Huang, 1990; Lorieux et al. 1996; Bocchi et al. 1997; Itani et al. 2004). There is now considerable evidence that most pre-harvest treatments have an influence on the sensory and flavor quality of rice. Late harvest for example, lowers the concentration of 2-AP (Itani et al. 2004; Champagne et al. 2008). Draining paddy fields early can cause moisture stress in grains before they are physiologically mature, affecting metabolic processes and, in turn, volatile flavor compounds (Meullenet et al. 1999; Champagne et al. 2008). Aroma of cooked milled rice was also reported to be adversely influenced by nitrogen (Dutta et al. 1999; Suwanarit et al. 1996; Wilkie et al. 2004), zinc and lanthanum applications (Singh et al. 2000; Tang and Wu, 2006).

In rice, application of growth regulators has been used extensively for a variety of purposes which include improved growth and yield, and survival under different stress conditions (Sudria, 2001; Mander, 2003; Ghosh et al. 2003; Duan et al. 2009). Foliar application of growth regulators on rice plants is standard practice in most Asian countries. This is reflected by the high number of papers published on the subject in the recent past, especially from India, China, and Japan. Despite that extensive literature however, there is no report of an attempt made to evaluate the effects of spraying growth hormones on the aroma quality of rice grains. Hence, in the present study, the possible effect of growth regulators applied as foliar spray on the volatile components of rice was determined. Two aromatic rice cultivars (Guixiangzhan and Peizaruanxiang) grown in Guangzhou, P.R. China were selected for the study and received at 25% heading a foliar application of three growth regulators and a combination of growth regulators. The influence of the different treatments on odor-active compounds in brown and white rice grains was analyzed using a static headspace method coupled to GC. In addition, yield, milling quality, grain appearance, protein and amylose content of the two cultivars as influenced by treatments with growth regulators was investigated.

3.3.2 Experimental

3.3.2.1 Plot area, rice cultivars and growth conditions

An experiment to determine the aroma and quality response of two Indica rice cultivars (Guixiangzhan and Peizaruanxiang) to different plant growth regulators was conducted during the early and late seasons of 2008, at the South China Agricultural University (SCAU) Research Farm in Guangzhou, P. R. China. The two cultivars accounted for the major aromatic rice cultivars grown in the region. The plot areas were located on a sandy loan soil. The previous crop grown in the field was rice.

Pre-germinated seeds were sown on seedling trays and grown to raise uniform seedlings. Sixteen-day-old seedlings of the two rice genotypes were transplanted to the paddy field. Transplanting was done manually with hill spacing of 20 cm × 20 cm, and with three seedlings per hill. The row to row and plant to plant distances were 0.20 m and 0.18 m, respectively, which gave a total stand of 28 plants/m^2 **(Appendix 6)**.

The daily maximum and minimum air temperatures and precipitation data were recorded by the weather station located near the experimental site. The mean monthly temperatures were 25.58 ℃ for the early season, and 28.50 ℃ for the late season, which was slightly warmer than the long-term averages for 2008 (**Figure 1.3**). During the growth duration, rainfall did not differ widely from normal ranges in the city, with an average of 14.38 mm during the early season and 5.10 mm during the late season.

Standard crop production practices were adopted and routine plant protection measures were taken to control the incidence of pests and diseases. A shallow flood was established 4 days after transplanting when the rice reached the five leaf stage. 5- to 10-cm water depth was maintained until 7 days before physiological maturity for each genotype, at which time the field was drained. Paddy fields were treated like all conventional cultivars with a common pesticide program. Nitrogen (N) (20 kg/ha through urea), phosphorus (P) (30 kg/ha through phosphoric oxide), and potassium (12 kg/ha through potassium chloride) were applied based on soil test results in all plots one days before transplanting. Zinc in the form of zinc sulfate (3 kg/ha) was also applied at basal due to the high soil pH at the chosen location. One week after transplantation, each area also received additional doses of nitrogen (30 kg/ha), phosphorus (45 kg/ha), and potassium (18 kg/ha).

3.3.2.2 Foliar application of plant growth regulators

Three different plant growth regulators (gibberellic acid, paclobutrazol, and 3-indole acetic acid), proline and zinc chloride were used **(Figure 3.3.1)**. Foliar application of growth

regulators was done after the emergence of approximately 25% of panicles by spraying plants uniformly to run off (approximately 100 mL/m^2) using a Gloria type hand sprinkler (Guangzhou, P.R. China) with constant flow.

The experimental design for all experiments was a randomized complete block with three replications, each consisting of a 16.20-m^2 plot size.

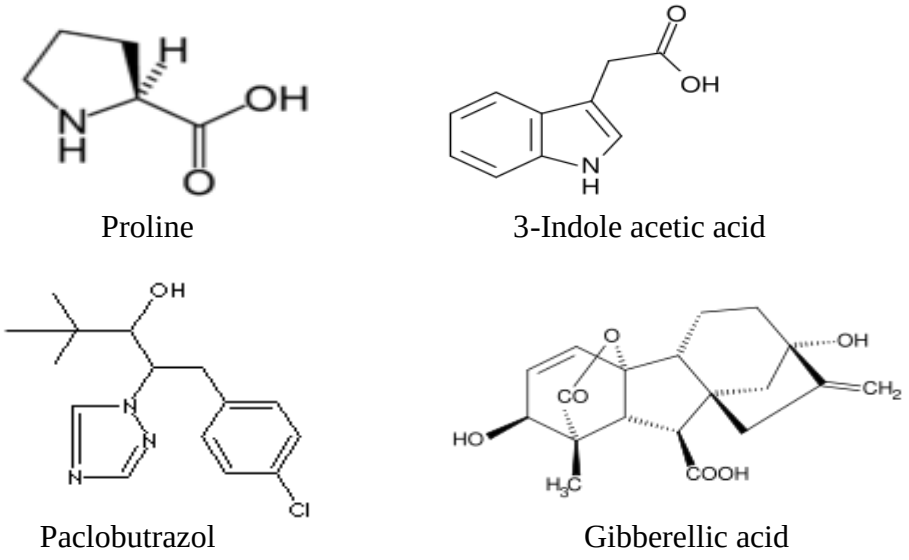


Figure 3.3.1 Chemical structure of proline and growth regulators sprayed on rice plants

Treatments included:

(1) a plot sprayed with distilled water and maintained as control (CTR),

(2) a plot sprayed with 40 mg/L gibberellic acid (GA3) prepared using 95% ethanol as surfactant,

(3) a plot sprayed with 25 mg/L paclobutrazol (PBZ),

(4) a plot sprayed with 25 mg/L 3-indole acetic acid (IAA), and

(5) a plot sprayed with a combination of paclobutrazol (15 mg/L), proline (120 mg/L) and zinc chloride (2000 mg/L), which we will refer to as PPZ.

The treatments were applied late in the afternoon. The concentration of each substance was selected on the basis of previous experiments conducted since 2003 by our laboratory to establish optimum dosages for various rice cultivars, including the two aromatic cultivars used in this study, and on actual practices employed by farmers in Guangzhou. GA3 was selected for improved paddy yield, IAA for improved grain quality, and PBZ for lodging prevention and increased efficiency of mechanical harvest. In one of those studies (Duan et al. 2009), combined application of PBZ, proline and zinc chloride could remarkably reverse the effects of various environmental stresses such as salinity on rice, therefore was included in the study.

Table 3.3.1 Optimum concentrations of plant growth regulators sprayed on rice plants

Regulator	Common name	IUPAC name	Weight volume % (mg/L)	Molecular weight (g/mol)	Molar concentration (mol/L)	Per area concentration (mg/m^2)
GA3	Gibberellic acid 90%	(3*S*,3a*S*,4*S*,4a*S*,7*S*,9a*R*,9b*R*,12*S*)-7,12-dihydroxy-3-methyl-6-methylene-2-oxoperhydro-4a,7-methano-9b,3-propeno[1,2-*b*]furan-4-carboxylic acid	40	C19H2206 (346.38)	1.1548×10^{-4}	4
PBZ	Paclobutrazol 95%	(2*RS*,3*RS*)-1-(4-chlorphenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol)	25	C15H20ClN30 (293.80)	0.8509×10^{-4}	2.5
IAA	3-Indole acetic acid 99%	2-(1H-indol-3-yl)acetic acid	25	C10H9NO2 (175.18)	1.4271×10^{-4}	2.5
PPZ	Proline	(*S*)-pyrrolidine-2-carboxylic acid	120	C5H9NO2 (115.13)	1.0423	12
	$ZnCl_2$	zinc (II) chloride	2000	ZnCl2 (136.32)	14.6714	200
	Paclobutrazol 95%	(2*RS*,3*RS*)-1-(4-chlorphenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol)	15	C15H20ClN30 (293.80)	0.5105×10^{-4}	1.5

3.3.2.3 Harvesting, storage and parameters measured

At maturity when 95% of the grains turned yellow, a 2.6-m^2 area from the center three rows of each plot was harvested using a sickle. Yield components measured included average paddy yield and 1000-grain rough rice weight. The samples were threshed and dried to 120 g/kg moisture content then separated into two sets. The first set used for volatile analysis was kept in a refrigerator at 8 ℃ while the second set was left at room temperature for 3 months and used to determine rice quality parameters. Volatile studies were carried out considering 2-AP as the key aroma compound and lipid oxidation products as important volatile products occurring in rice after storage. Samples were aged approximately 3 months for the late harvest and 6 months for the early harvest. Brown rice grains and flag leaves harvested at 7, 14, 21, and 28 days after heading (DAH) were used for malondialdehyde (MDA) content determination as an additional lipid peroxidation marker in growing rice plants. POX, SOD, POD activities as well as proline, soluble protein contents were also determined.

3.3.2.4 Sensory evaluation protocol

Two tests were perfomed as decribed in the "Materials and Methods" section: a sniffing test on brown rice powder cooked without water and a hedonic test on cooked milled rice. Panelists were required to smell and tell which of the samples has the most intense odor.

3.3.2.5 Statistical analysis

A statistical one-way analysis of variance was performed to assess differences in odor-active compound concentrations, odor intensity, and yield and quality parameters between control and treated samples. All analyses were carried out using SPSS 15.0 (Chicago, IL). The difference of means was resolved by means of confidence intervals using Duncan's multiple range test at $P<0.05$. A 3-way analysis of variance was also conducted to estimate the effects of treatment/cultivar, season and replication on aroma compounds detected and rice odor. Season and replication were considered as random effects; therefore a compound *F*-test was used for calculating *F* values. Data presented are an average of duplicate determinations for each sample with three samples per treatment.

3.3.3 Results

3.3.3.1 Separation and identification of odor-active compounds

Static headspace (SHS) coupled to a GC was used to extract and identify volatile compounds. A selection was made between two detectors, FID for the determination of lipid

oxidation volatiles and other major volatiles **(Figure 3.3.2)**, and NPD for 2-AP **(Figure 3.3.3).**

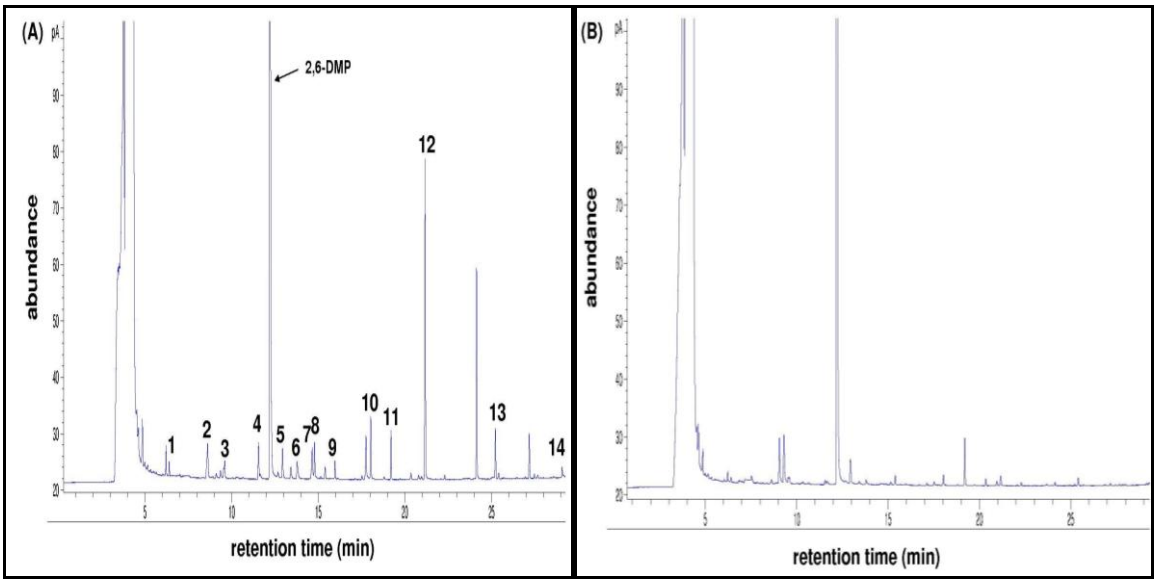


Figure 3.3.2 GC-FID profile of headspace volatiles in (A) Guixiangzhan and (B) Peizaruanxiang brown rice grains treated with distilled water. Numbers correspond to those in Table 3.3.2.

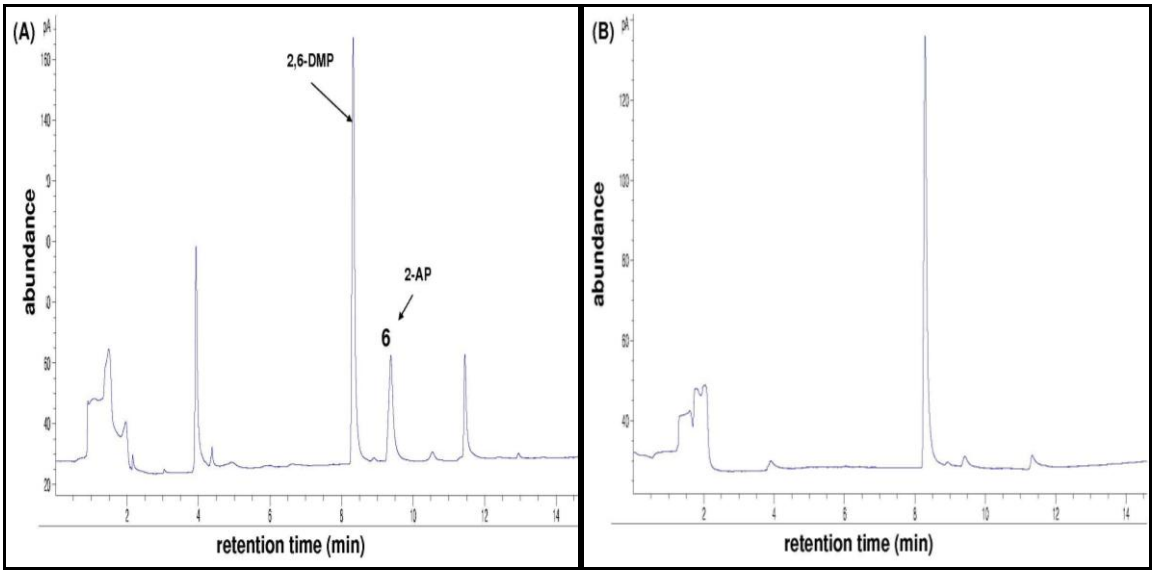
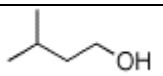
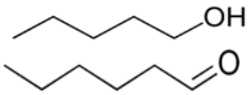
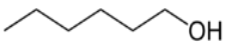
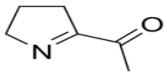
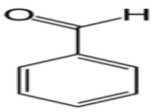
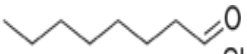
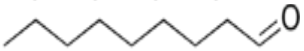
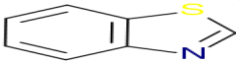


Figure 3.3.3 GC-NPD profile of headspace volatiles in (A) Guixiangzhan and (B) Peizaruanxiang brown rice grains treated with distilled water.

Overall, 14 compounds emanating from the headspace of the two rices were identified with some certainty and used in the study **(Table 3.3.2).** They belonged to the chemical classes of alcohols (6), aromatics (1), aldehydes (5), and nitrogen-containing compounds (2).

Table 3.3.2 Retention indexes and chemical structures of odor-active volatile compounds selected to monitor the effect of foliar application of plant growth regulators on rice aroma and flavor

Peak[a]	RI[b]	Volatile[c]	Retention time (min)	Molecular weight (g/mol)	Chemical structure
1	744	3-methyl-1-butanol	6.407	88.15	
2	787	1-pentanol	8.623	88.15	
3	803	Hexanal	9.601	100.16	
4	865	(*E*)-2-hexenal	11.558	98.14	
5	870	1-hexanol	12.937	102.17	
6	918	2-acetyl-1-pyrroline	13.776	111.14	
7	952	Benzaldehyde	14.650	106.12	
8	969	1-heptanol	14.788	116.20	
9	1005	Octanal	15.966	128.21	
10	1075	1-octanol	18.033	130.23	
11	1106	Nonanal	19.200	142.24	
12	1175	1-nonanol	21.163	144.26	
13	1206	Decanal	25.223	156.20	
14	1213	Benzothiazole	29.040	135.19	

[a] Compounds refers to those labeled in **Figure 3.3.1**. [b] Retention index given for HP-5MS column based on a series of *n*-hydrocarbons. [c] Retention times and GC Kovat's retention index found were consistent with those of authentic samples and enabled us to identify the odorants with some certainty.

The odor descriptions and thresholds of the different compounds identified in the headspace volatile of Guixiangzhan and Peizaruanxiang are given in **Table 3.3.3**.

The experiment was conducted over two seasons. At the exception of 1-octanol, 1-nonanol and benzaldehyde in Guixiangzhan, and, 1-nonanol, (*E*)-2-hexenal and decanal in Peizaruanxiang, all volatiles appeared to increase in concentration with storage duration in control samples, which was not the case in samples treated with growth regulators where most lipid-derived volatiles were substantially lower in abundance or remained at relatively unchanged levels. Despite this, however, there was no difference between the results of the two seasons relative to the effect of growth regulators. **Table 3.3.4, Table 3.3.5** (for the early season) **Table 3.3.6**, and **Table 3.3.7** (for the late season) show the concentrations of the 14 volatile components in Guixiangzhan and Peizaruanxiang after foliar treatment with growth regulators. Compositional differences in rice cultivars were found only for two compounds: benzaldehyde

and benzothiazole found in Guixiangzhan but not in Peizaruanxiang. There are also interesting differences between samples treated with growth regulators.

Table 3.3.3 Odor descriptions and odor thresholds of odor-active volatiles selected to monitor the effect of foliar application of plant growth regulators on rice aroma and flavor

Volatile	Odor description[a]	Odor threshold[b]	
		water (μg/L)	air (ng/L)
3-methyl-1-butanol	pungent, whiskey, malty	1800[c]	162[d]
1-pentanol	plastic, fusel, oil-like	4000[e]	153[f]
hexanal	green, grass-like, herbal	5[e]	1.1[f]
(*E*)-2-hexenal	apple, green	17[e]	3.1[f]
1-hexanol	vegetal, herbaceous, green	2500[e]	90[d]
2-acetyl-1-pyrroline	pleasant, popcorn-like, sweet	0.1[e]	0.02[g]
benzaldehyde	nutty, bitter, almond	350[e]	85[f]
1-heptanol	woody, sweet, green	3[e]	60[d]
octanal	slightly fruity, citrus-like	0.7[e]	0.4[f]
1-octanol	citrus, fruity, floral	110[e]	22[f]
nonanal	citrus, floral, fruity, fatty	1[e]	2.6[f]
1-nonanol	floral, citrus, fatty	50[e]	18[f]
decanal	sweet, waxy, floral, soapy	2[e]	2.6[f]
benzothiazole	nutty, rubber, solvent	80[e]	NA[h]

[a] Descriptors are compiled from the following references: Jezussek et al. (2002), Widjaja et al. (1996b), Chung et al. 2004; Maraval et al. (2008), Yang et al. (2008c). [b] Threshold values obtained from various sources: [c] Czerny et al. (2008), [d] Verschueren (2001), [e] Buttery et al. (1988), [f] Yang et al. (2008c), [g] Schieberle (1991), [h] NA = not available.

3.3.3.2 Influence of growth regulators on nitrogen-containing compounds

After application of growth regulators, 2-AP content appears to decrease in the two rices. In Guixiangzhan grown during the late season for instance, control samples had a 2-AP relative concentration of 6.25 ng/g. 2-AP in samples treated with plant growth regulators ranged in intensity from 1.90 ng/g (PPZ) to 4.95 ng/g (PBZ) **(Table 3.3.6)**. In Peizaruanxiang, GA3 treatment decreased the content of 2-AP by 31.58%, IAA by 20.47%, PBZ by 29.82%, and PPZ by 47.37% compared to the control (1.71 ng/g) **(Table 3.3.7)**. Similar trends were observed with samples grown during the early season with decreases ranging from 43 to 59% for Guixiangzhan **(Table 3.3.4)** and 35 to 56% for Peizaruanxiang **(Table 3.3.5)**. The 3-way ANOVA showed that there were significant ($P<0.05$) cultivar and treatment effects on 2-AP content. Significant ($P<0.05$) season-by-cultivar and season-by-treatment interactions were also observed **(Table 3.3.9).**

Quantification of 2-AP was performed using S-HS with a nitrogen-phosphorus detector

and the content in the early and late seasons samples calculated from a linear calibration curve plotted as a correlation of the peak area ratios between the compound (2-AP) and the internal standard (2,6-DMP), and concentrations of known amounts of synthetic 2-AP **(Figure 3.3.4)**. The findings were consistent with the analysis performed earlier using a flame ionization detector, were GA3, IAA, PBZ, and PPZ were shown to adversely influence 2-AP content in rice. When averaged over treatments, it seems GA3 and PPZ produced the lowest mean 2-AP, 1.95 and 1.89 μg/g in Guixiangzhan respectively, and 0.35 and 0.31 μg/g in Peizaruanxiang grown during the late season.

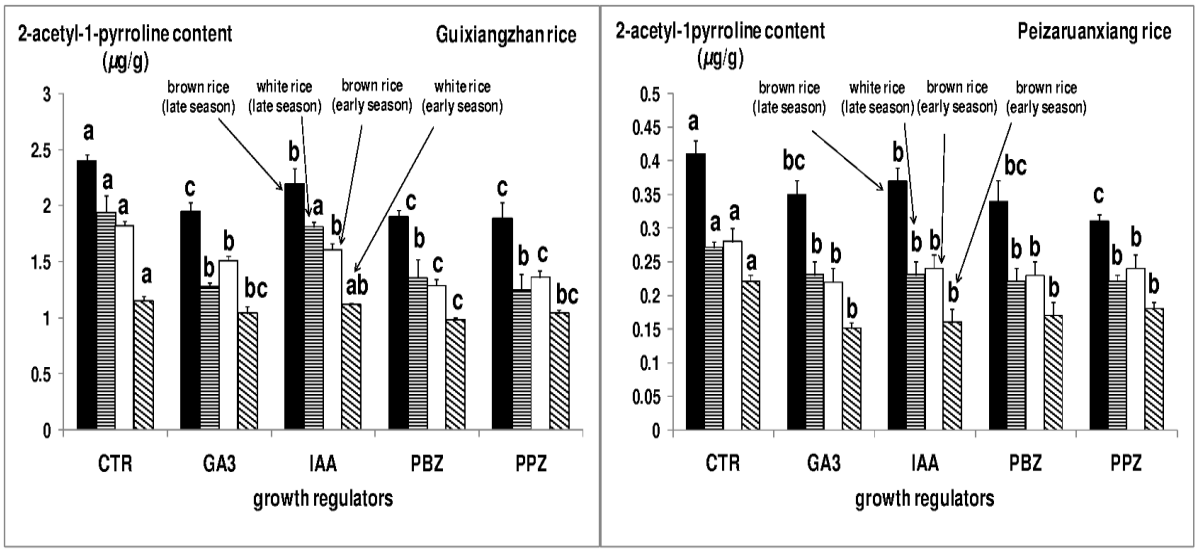


Figure 3.3.4 Comparison of concentrations of 2-AP in (A) Guixiangzhan and (B) Peizaruanxiang rice cultivars after foliar application of plant growth regulators. Vertical bars with different letters above are significantly different (P<0.05, Duncan's multiple range test). Capped bars represent the standard deviations.

The concentration of 2-AP in white rice can also be compared. For the brown rice the effect of the treatments was very noticeable, but this diminished gradually over milling. In the white rice, Duncan's multiple range test failed to detect a difference among the samples for the control and PBZ in Guixiangzhan, and IAA, PPZ in Peizaruanxiang. By comparing the mean intensities directly, however, it can be seen that samples treated with PBZ, IAA and PPZ were much lower in 2-AP compared to the control **(Figure 3.3.4)**.

The second nitrogen-containing compound, benzothiazole, was detected only in the control (3.08 ng/g) and samples treated with IAA (1.35 ng/g) and grown during the late season.

3.3.3.3 Influence of growth regulators on lipid oxidation aldehydes

Most aldehydes had either decreased or remained at relatively unchanged levels after application of growth regulators (**Table 3.3.4; Table 3.3.5; Table 3.3.6; Table 3.3.7**).

Table 3.3.4 Changes in relative concentrations of selected flavor molecular markers in the headspace vapor of Guixiangzhan brown rice treated with plant growth regulators during the early season

Compound	Relative concentration (ng/g)[a]				
	CTR	GA3	IAA	PBZ	PPZ
			Aliphatic Alcohols		
3-methyl-1-butanol	3.68 ± 0.33 a[b]	0.92 ± 0.05 b	0.90 ± 0.04 b	0.83 ± 0.01 b	1.07 ± 0.04 b
1-pentanol	12.28 ± 0.37 a	1.50 ± 0.03 c	2.13 ± 0.08 c	4.63 ± 0.22 b	2.10 ± 0.39 c
1-hexanol	9.37 ± 0.50 a	4.37 ± 0.34 b	4.78 ± 0.09 b	0.80 ± 0.13 c	1.62 ± 0.16 c
1-heptanol	10.57 ± 0.28 a	1.31 ± 0.25 b	0.85 ± 0.10 b	0.94 ± 0.20 b	1.39 ± 0.27 b
1-octanol	11.36 ± 0.35 a	4.86 ± 0.55 b	5.87 ± 0.36 b	2.94 ± 0.78 c	4.94 ± 0.53 b
1-nonanol	25.23 ± 1.34 a	8.19 ± 0.43 b	3.97 ± 0.07 c	4.19 ± 0.52 c	3.80 ± 0.11 c
			Aromatics		
benzaldehyde	ND[c]	ND	ND	ND	ND
			Aliphatic Aldehydes		
Hexanal	10.34 ± 0.29 a	2.22 ± 0.05 b	2.64 ± 0.53 b	1.87 ± 0.38 b	2.27 ± 0.08 b
(*E*)-2-hexenal	8.99 ± 0.55 a	5.24 ± 0.54 b	4.55 ± 0.66 b	4.74 ± 0.36 b	5.76 ± 0.46 b
Octanal	6.96 ± 0.35 a	1.10 ± 0.10 bc	0.93 ± 0.27 c	1.23 ± 0.21 bc	trace[d]
Nonanal	15.99 ± 0.36 a	8.14 ± 0.11 bc	6.04 ± 0.36 d	6.99 ± 0.79 cd	9.31 ± 0.02 b
Decanal	13.79 ± 0.30 a	1.08 ± 0.02 c	7.09 ± 0.02 b	1.13 ± 0.02 c	1.25 ± 0.16 c
			N-Containing Compounds		
2-acetyl-1-pyrroline	3.60 ± 0.11 a	1.46 ± 0.27 cd	2.05 ± 0.11 b	1.76 ± 0.04 bc	1.63 ± 0.03 bc
benzothiazole	3.62 ± 0.32	ND	ND	ND	ND

[a] Concentration of a compound in the headspace is expressed as internal standard equivalent (ng of 2,6-dimethylpyridine/g dry weight sample) ± standard deviation. [b] Values in the same row with different letters are significantly different ($P<0.05$) based on Duncan's multiple range test. [c] ND = not detected. [d] "trace" means the value obtained was below the detection limit of quantification (< 0.50 ng/g).

Table 3.3.5 Changes in relative concentrations of selected flavor molecular markers in the headspace vapor of Guixiangzhan brown rice treated with plant growth regulators during the late season

Compound	Relative concentration (ng/g)[a]				
	CTR	GA3	IAA	PBZ	PPZ
			Aliphatic Alcohols		
3-methyl-1-butanol	3.69 ± 0.59 a[b]	1.67 ± 0.29 b	0.91 ± 0.13 b	0.73 ± 0.07 b	0.60 ± 0.09 b
1-pentanol	12.65 ± 0.41 a	2.49 ± 0.22 b	2.39 ± 0.29 b	2.49 ± 0.09 b	2.87 ± 0.66 b
1-hexanol	10.74 ± 0.90 a	2.08 ± 0.09 b	1.69 ± 0.09 b	1.66 ± 0.03 b	1.99 ± 0.42 b
1-heptanol	9.69 ± 1.34 a	1.63 ± 0.03 b	0.95 ± 0.28 b	0.67 ± 0.07 b	0.79 ± 0.09 b
1-octanol	16.39 ± 1.81 a	3.48 ± 0.11 b	3.98 ± 0.17 b	3.42 ± 0.11 b	4.67 ± 0.95 b
1-nonanol	30.70 ± 5.22 a	6.60 ± 0.90 b	4.07 ± 0.53 b	3.43 ± 0.18 b	3.01 ± 0.50 b
			Aromatics		
benzaldehyde	4.22 ± 0.04	ND[c]	4.41 ± 0.20	ND	ND
			Aliphatic Aldehydes		
Hexanal	7.19 ± 1.15 a	1.49 ± 0.40 b	2.09 ± 0.53 b	2.00 ± 0.64 b	2.22 ± 0.05 b
(*E*)-2-hexenal	8.19 ± 0.40 a	2.91 ± 0.02 c	6.07 ± 0.01 b	6.15 ± 0.68 b	6.41 ± 0.29 b
Octanal	4.15 ± 0.48 a	0.77 ± 0.05 b	0.69 ± 0.03 b	0.65 ± 0.03 b	0.82 ± 0.07 b
Nonanal	13.59 ± 0.91 a	4.59 ± 0.48 c	14.19 ± 1.67 a	6.34 ± 0.18 bc	8.15 ± 0.88 b
Decanal	11.04 ± 1.08 a	3.16 ± 0.04 b	3.52 ± 0.04 b	2.68 ± 0.10 b	1.12 ± 0.08 b
			N-Containing Compounds		
2-acetyl-1-pyrroline	6.52 ± 0.41 a	2.37 ± 0.07 cd	3.46 ± 0.20 c	4.95 ± 0.31 b	1.90 ± 0.28 d
benzothiazole	3.08 ± 0.29	ND	1.35 ± 0.19	ND	ND

[a] Concentration of a compound in the headspace is expressed as internal standard equivalent (ng of 2,6-dimethylpyridine/g dry weight sample) ± standard deviation. [b] Values in the same row with different letters are significantly different ($P<0.05$) based on Duncan's multiple range test. [c] ND = not detected.

Table 3.3.6 Changes in relative concentrations of selected flavor molecular markers in the headspace vapor of Peizaruanxiang brown rice with plant growth regulators during the early season

Compound	Relative concentration (ng/g)[a]				
	CTR	GA3	IAA	PBZ	PPZ
			Aliphatic Alcohols		
3-methyl-1-butanol	1.22 ± 0.05 a[b]	0.42 ± 0.06 c	0.46 ± 0.02 c	0.60 ± 0.01 b	0.48 ± 0.03 c
1-pentanol	3.14 ± 0.21 a	1.51 ± 0.13 c	2.07 ± 0.02 b	1.75 ± 0.11 bc	1.55 ± 0.06 c
1-hexanol	3.37 ± 0.26 a	1.18 ± 0.09 b	1.21 ± 0.03 b	1.48 ± 0.17 b	1.30 ± 0.04 b
1-heptanol	1.35 ± 0.10 a	1.06 ± 0.03 b	1.09 ± 0.01 b	1.27 ± 0.15 ab	1.10 ± 0.01 ab
1-octanol	4.09 ± 0.12 a	3.02 ± 0.33 b	3.35 ± 0.20 b	3.07 ± 0.12 b	3.15 ± 0.12 b
1-nonanol	2.42 ± 0.36 a	trace	1.76 ± 0.15 b	1.69 ± 0.02 b	1.84 ± 0.06 ab
			Aromatics		
benzaldehyde	ND[d]	ND	ND	ND	ND
			Aliphatic Aldehydes		
Hexanal	6.61 ± 0.29 a	2.22 ± 0.08 c	2.34 ± 0.10 bc	2.17 ± 0.04 c	2.97 ± 0.44 b
(*E*)-2-hexenal	4.76 ± 0.13 a	1.74 ± 0.08 bc	2.12 ± 0.06 b	1.53 ± 0.24 c	2.14 ± 0.16 b
Octanal	2.20 ± 0.21 a	0.88 ± 0.10 b	0.89 ± 0.26 b	0.60 ± 0.32 b	1.08 ± 0.35 b
Nonanal	12.73 ± 0.70 a	5.78 ± 0.33 b	5.45 ± 0.51 b	5.30 ± 0.44 b	4.54 ± 0.17 b
Decanal	trace[c]	trace	trace	trace	trace
			N-Containing Compounds		
2-acetyl-1-pyrroline	1.15 ± 0.07 a	0.75 ± 0.11 b	0.74 ± 0.14 b	0.51 ± 0.03 b	0.59 ± 0.11 b
benzothiazole	ND	ND	ND	ND	ND

[a] Concentration of a compound in the headspace is expressed as internal standard equivalent (ng of 2,6-dimethylpyridine/g dry weight sample) ± standard deviation. [b] Values in the same row with different letters are significantly different ($P<0.05$) based on Duncan's multiple range test. [c] "trace" means the value obtained was below the detection limit of quantification (< 0.50 ng/g). [d] ND = not detected.

Table 3.3.7 Changes in relative concentrations of selected flavor molecular markers in the headspace vapor of Peizaruanxiang brown rice with plant growth regulators during the late season

Compound	Relative concentration (ng/g)[a]				
	CTR	GA3	IAA	PBZ	PPZ
			Aliphatic Alcohols		
3-methyl-1-butanol	0.99 ± 0.01 a[b]	0.66 ± 0.02 bc	0.70 ± 0.06 b	0.59 ± 0.03 c	0.57 ± 0.03 c
1-pentanol	2.83 ± 0.14 ab	1.43 ± 0.03 c	2.79 ± 0.16 ab	2.30 ± 0.32 b	2.90 ± 0.18 a
1-hexanol	1.84 ± 0.16 abc	1.56 ± 0.20 c	2.15 ± 0.12 a	1.58 ± 0.08 bc	1.82 ± 0.05 abc
1-heptanol	0.85 ± 0.01 a	0.95 ± 0.03 a	0.95 ± 0.06 a	trace[c]	Trace
1-octanol	3.47 ± 0.17 a	2.52 ± 0.29 b	3.97 ± 0.21 a	3.26 ± 0.20 ab	3.58 ± 0.36 a
1-nonanol	2.73 ± 0.03 a	1.70 ± 0.28 c	2.40 ± 0.26 ab	1.82 ± 0.09 bc	1.67 ± 0.22 c
			Aromatics		
benzaldehyde	ND[d]	ND	ND	ND	ND
			Aliphatic Aldehydes		
Hexanal	5.60 ± 0.78 a	1.57 ± 0.21 c	2.46 ± 0.38 bc	3.07 ± 0.51 b	2.65 ± 0.26 bc
(*E*)-2-hexenal	3.62 ± 0.38 b	1.83 ± 0.08 c	5.98 ± 0.42 a	5.21 ± 0.28 a	6.17 ± 0.34 a
Octanal	1.07 ± 0.05 a	0.68 ± 0.03 b	0.70 ± 0.06 b	0.64 ± 0.06 b	0.65 ± 0.06 b
Nonanal	11.14 ± 0.22 a	4.08 ± 0.56 c	9.80 ± 0.75 a	6.76 ± 0.34 b	4.43 ± 1.41 bc
Decanal	0.66 ± 0.02	Trace	Trace	Trace	ND
			N-Containing Compounds		
2-acetyl-1-pyrroline	1.71 ± 0.06 a	1.17 ± 0.07 bc	1.36 ± 0.06 b	1.20 ± 0.13 b	0.90 ± 0.09 c
benzothiazole	ND	ND	ND	ND	ND

[a] Concentration of a compound in the headspace is expressed as internal standard equivalent (ng of 2,6-dimethylpyridine/g dry weight sample) ± standard deviation. [b] Values in the same row with different letters are significantly different ($P<0.05$) based on Duncan's multiple range test. [c] "trace" means the value obtained was below the detection limit of quantification (< 0.50 ng/g). [d] ND = not detected.

Aldehydes, identified in decreasing order of their relative concentrations in Guixiangzhan were nonanal, decanal, hexanal, (*E*)-2-hexenal, octanal, and heptanal. Similar rankings from high to low concentrations among the components were observed in Peizaruanxiang. In concentration, nonanal described as citrus, floral, fruity (Buttery et al., 1988) was the most important aldehyde present in the two rices, 15.99 ng/g for Guixiangzhan and 12.73 ng/g for Peizaruanxiang samples maintained as control during the early season, 13.59 ng/g and 11.14 ng/g during the late season. The relative concentrations of hexanal and decanal changed by a larger factor relative to those of the other aldehydes with decreases of 5.0 to 12.8-fold in Guixiangzhan and 2.1 to 3.6-fold in Peizaruanxiang. The aldehydes underwent these changes after foliar application of all growth regulators, but they were greater for samples treated with gibberellic acid during the late season. This was generally the trend of change observed for the two types of rice. Exception to this trend is (*E*)-2-hexenal described as apple and green, which decreased in concentration in Guixiangzhan and Peizaruanxiang grown during the early season, but in Peizaruanxiang grown during the late season remained constant ($P<0.05$) after treatments with 3-indole acetic acid (5.98 ng/g), paclobutrazol (5.21 ng/g), and the regulator mixture (6.17 ng/g)

3.3.3.4 Influence of growth regulators on malondialdehyde content

Changes in the MDA content with time for both the control and treated samples in the two rices grown during the early season were plotted as shown in **Figure 3.3.5**.

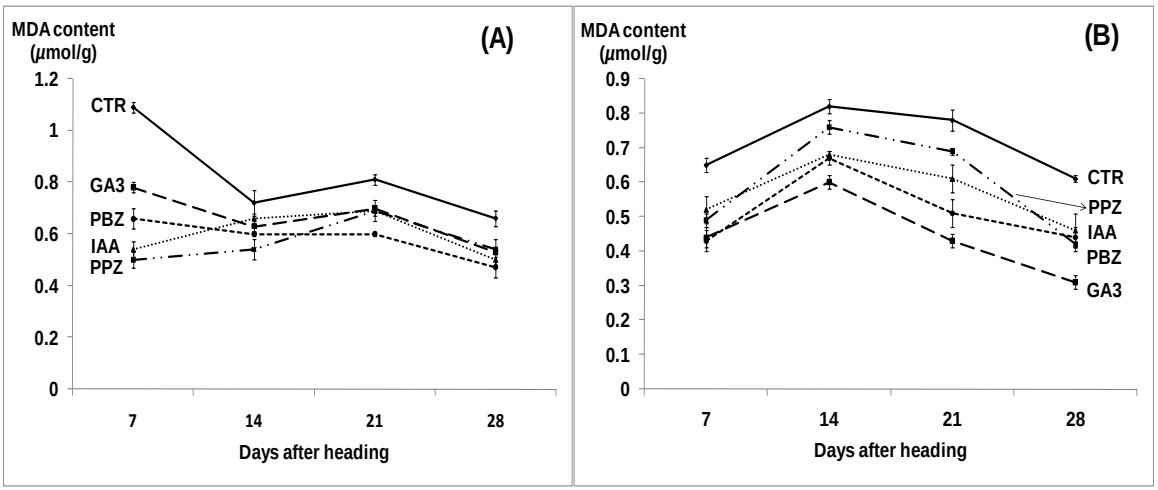


Figure 3.3.5 Evolution of malondialdehyde (MDA) level in (A) Guixiangzhan and (B) Peizaruanxiang brown grains after foliar application of plant growth regulators. All points for each day are significantly different (Duncan's multiple range test at $P<0.05$) from the control, except for those marked with an arrow. Capped vertical lines represent the standard deviations. In some cases the deviation bar is obscured by the symbol.

MDA was as a greater concentration in control samples treated with distilled water than samples treated with plant growth regulators for any harvesting time, with a background level similar for the two rices. In Peizaruanxiang, MDA concentrations reached a plateau 14 days after heading (DAH). There was initial increase in the concentrations of MDA at day 7, before they finally fell at concentrations slightly lower than the initial concentrations at day 28. In Guixiangzhan, MDA content decreased rapidly during the first 7 days, leveled up 21 DAH, and then decreased significantly ($P<0.05$) until day 28.

3.3.3.5 Influence of growth regulators on lipid oxidation alcohols

Generally most alcoholds decreased in content after foliar application of growth regulators. These decreases were generally in the order of GA3 > PPZ > PBZ > IAA in Peizaruanxiang **(Table 3.3.4; Table 3.3.5; Table 3.3.6; Table 3.3.7)**. In Guixiangzhan, the decrease in alcohols seems to be greater with plants treated with PBZ; the differences, however, are not significant ($P<0.05$). 1-Nonanol (30.70; 2.73 ng/g), 1-octanol (16.39; 3.47 ng/g), 1-pentanol (12.65; 2.83 ng/g) and 1-hexanol (10.74; 1.84 ng/g) were the major lipid-derived alcohols volatile in the two rices. 3-Methyl-1-butanol and 1-heptanol were produced in the least quantities throughout the study.

3.3.3.6 Influence of growth regulators on aromatic compounds

Benzaldehyde was chosen to monitor the influence of growth hormones on rice aromatic compounds. Benzaldehyde was detected only in Guixiangzhan samples grown during the late season (4.22 ng/g) **(Table 3.3.6).** Its absence from samples treated with GA3, PBZ and PPZ can be noted. Another aromatic compound (toluene) was also detected in the two cultivars (data not shown). Interestingly, toluene concentration increased in samples treated with plant growth regulators, and to a far greater extent in samples treated with PPZ, 9.69 and 11.10 ng/g in Guixiangzhan and Peizaruanxiang, respectively.

3.3.3.7 Influence of growth regulators on cooked rice odor

After cooking the samples for approximately 30 min, Guixiangzhan and Peizaruanxiang treated with distilled water possessed a strong characteristic aroma, which differed greatly from the odor of samples treated PBZ, PPZ, and GA3. That difference could be easily perceived in smelling evaluation, even from students not trained in sensory analysis. Only few sensory panelists, however, were able to detect a significant difference in rice odor due to treatment with IAA grown during the early season. Panelists gave scores from 3.11 to 3.87 for control samples and from 2.10 to 2.94 for samples treated with growth regulators **(Table 3.3.8).**

Table 3.3.8 Decrease in rice odor intensity after foliar application of growth regulators at 25% panicle emergence.

Growth regulator	Early season		Late season	
	Brown rice powder	Cooked milled rice	Brown rice powder	Cooked milled rice
		Guixiangzhan		
CTR	A [a]	3.20 ± 0.14 a [b]	A	3.87 ± 0.32 a
GA3	B	2.71 ± 0.26 b	B	2.31 ± 0.02 c
IAA	B	2.94 ± 0.03 ab	B	2.91 ± 0.28 b
PBZ	B	2.71 ± 0.03 b	B	2.72 ± 0.48 bc
PPZ	B	2.83 ± 0.29 b	B	3.04 ± 0.32 b
		Peizaruanxiang		
CTR	A	3.15 ± 0.09 a	A	3.11 ± 0.60 a
GA3	B	2.56 ± 0.09 b	B	2.20 ± 0.17 b
IAA	B	2.54 ± 0.43 b	B	2.10 ± 0.06 b
PBZ	B	2.40 ± 0.37 b	B	1.76 ± 0.65 b
PPZ	B	2.57 ± 0.09 b	B	2.23 ± 0.06 b

[a] A means "as strong as Guixiangzhan/Peizaruanxiang" B means "less strong than Guixingzhan/Peizaruanxiang" [b] Means (n = 3) ± standard deviation in the same column (for each rice cultivar) with different letters are significantly different (P<0.05) according to Duncan's multiple range test.

The same result was obtained after heating the samples at 80 ℃ for 20 min while performing an SPME extraction on brown rice powder **(Figure 3.3.6)**.

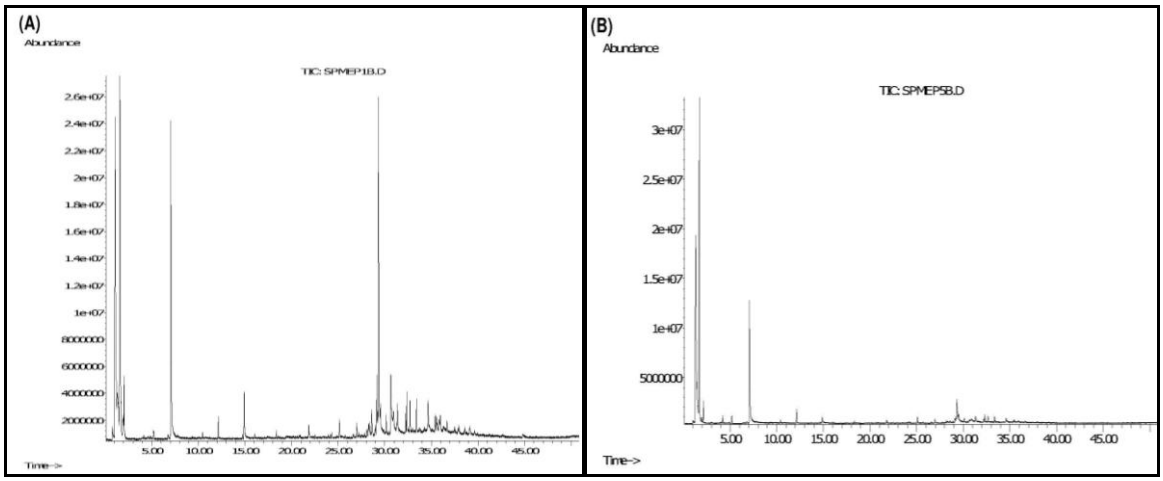


Figure 3.3.6 Chromatograms by GC/MS of Guixiangzhan brown rice comparing SPME headspace volatiles in (A) samples treated with distilled water and (B) samples treated with paclobutrazol.

As shown by the calculated F values **(Table 3.3.9)**, there were no significant (P<0.05) season effects on rice odor. Season-by-cultivar and season-by-treatment interactions were, however, significant.

Table 3.3.9 Calculated *F* values from ANOVA for treatment (CTR, GA3, IAA, PBZ, PPZ), cultivar (Guixiangzhan, Peizaruanxiang) and season (early season, late season) effects on flavor molecular markers and rice odor intensity

	Regulator	Cultivar	Season	Regulator × Cultivar	Regulator × Season	Cultivar × Season	Regulator × Cultivar × Season
3-methyl-1-butanol	122.74	194.45	0.48*	55.84	4.48	0.16*	2.28*
1-pentanol	367.85	424.87	3.21*	245.73	6.09	1.92*	8.09
1-hexanol	145.53	214.37	17.80	70.37	10.67	23.62	10.87
1-heptanol	162.76	185.02	8.97	138.53	1.68*	1.45*	0.68*
1-octanol	68.96	130.35	0.71*	54.01	4.86	0.57*	7.85
1-nonanol	430.38	1012.03	3.96*	376.09	7.01	0.01*	8.31
benzaldehyde	1927.34	5138.15	5138.15	1927.34	1927.34	5138.15	1927.34
Hexanal	133.41	1.08*	12.88	9.40	8.77	6.02	2.74
(*E*)-2-hexenal	59.64	176.13	62.07	4.41	22.05	53.26	0.70*
Octanal	147.08	83.49	36.11	70.77	14.96	3.47*	4.68
Nonanal	83.16	57.15	0.82*	4.02	26.31	0.43*	3.06
Decanal	98.67	340.79	1.73*	87.01	3.02	0.50	5.25
2-AP brown rice FID	115.45	922.27	304.67	59.21	23.45	86.67	15.28
2-AP brown rice NPD	47.45	8792.31	436.10	27.81	1.08*	189.39	1.07*
2-AP white rice NPD	29.60	4020.17	227.18	20.47	12.27	137.16	11.96
benzothiazole	189.18	290.72	2.96*	189.18	11.13	2.96*	11.13
Odor intensity	20.36	37.27	2.18*	1.36*	3.29	9.80	0.99*

* Correlation is non significant at the 0.05 level

3.3.3.8 Influence of growth regulators on proline content, lipid peroxidation and antioxidative systems of rice

It can be seen from **Table 3.3.10** the activities of SOD, POD, and POX were enhanced by the four treatments.

Table 3.3.10 Effect of growth regulators on SOD, POD, POX activities in brown rice grains and flag leaves harvested at different days after heading (DAH)

SOD activity in brown rice grains (Unit/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
		Guixiangzhan		
CTR	169.37 ± 7.73 d B[a]	173.24 ± 9.50 d B	213.22 ± 4.77 c A	181.30 ± 5.15 c B
GA3	218.60 ± 6.43 bc B	284.14 ± 8.06 c A	283.42 ± 6.68 b A	176.21 ± 6.28 c C
IAA	251.06 ± 2.57 a C	307.30 ± 9.71 bc A	280.63 ± 4.08 b B	225.61 ± 2.29 ab D
PBZ	229.79 ± 8.31 b C	329.42 ± 8.24 ab A	291.36 ± 7.07 b B	224.67 ± 2.29 b C
PPZ	206.63 ± 3.20 c D	339.37 ± 6.63 a A	318.18 ± 6.72 a B	241.37 ± 7.82 a C
		Peizaruanxiang		
CTR	180.80 ± 5.50 d B	269.51 ± 2.22 c A	179.94 ± 7.91 d B	160.40 ± 4.48 c C
GA3	316.18 ± 6.63 b A	308.29 ± 9.11 a A	255.25 ± 7.42 c B	225.61 ± 6.74 b C
IAA	261.21 ± 6.06 c B	289.21 ± 5.54 b A	294.28 ± 2.33 b A	211.80 ± 5.02 b C
PBZ	254.53 ± 6.19 c C	287.63 ± 5.12 b AB	302.85 ± 6.18 b A	268.42 ± 6.72 a BC
PPZ	342.16 ± 4.91 a A	310.43 ± 2.78 a B	336.98 ± 6.05 a A	257.00 ± 8.08 a C

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

SOD activity in rice flag leaf (Unit/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
		Guixiangzhan		
CTR	191.74 ± 6.00 d B[a]	327.08 ± 15.38 c A	310.16 ± 7.52 c A	303.58 ± 2.62 c A
GA3	242.10 ± 7.20 c B	408.93 ± 18.18 ab A	383.58 ± 5.48 b A	394.19 ± 6.74 b A
IAA	259.94 ± 2.46 bc C	369.54 ± 06.90 b B	413.80 ± 8.81 a A	410.76 ± 3.48 a A
PBZ	264.75 ± 6.19 b B	409.79 ± 06.70 a A	409.64 ± 6.00 a A	400.19 ± 5.76 ab A
PPZ	311.01 ± 5.94 a B	408.85 ± 11.32 ab A	411.56 ± 8.90 a A	405.36 ± 3.91 ab A
		Peizaruanxiang		
CTR	257.21 ± 15.12 c B	324.90 ± 09.45 c A	330.88 ± 15.24 d A	316.43 ± 5.76 c A
GA3	295.27 ± 09.33 b B	414.74 ± 07.88 a A	398.95 ± 07.39 bc A	410.63 ± 3.39 b A
IAA	332.46 ± 12.60 a C	381.04 ± 03.02 b AB	362.35 ± 15.10 cd BC	402.09 ± 4.40 b A
PBZ	295.96 ± 10.08 b C	378.80 ± 06.95 b B	409.78 ± 11.29 ab A	414.58 ± 5.28 b A
PPZ	298.16 ± 07.83 ab B	431.02 ± 11.41 a A	445.83 ± 07.65 a A	430.91 ± 6.19 a A

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

POD activity in brown rice grain (Unit/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
	Guixiangzhan			
CTR	12.01 ± 0.45 c A[a]	9.06 ± 0.88 c B	8.45 ± 0.41 cd BC	6.62 ± 0.37 d C
GA3	20.82 ± 1.02 a A	13.69 ± 0.34 ab B	13.92 ± 0.55 a B	15.41 ± 0.66 a B
IAA	23.81 ± 1.07 a A	15.50 ± 0.74 a B	9.03 ± 0.23 c C	11.25 ± 0.50 b C
PBZ	16.82 ± 1.51 b A	14.26 ± 0.69 ab AB	11.86 ± 0.50 b B	12.22 ± 0.69 b B
PPZ	16.20 ± 1.13 b A	12.41 ± 0.44 b B	7.72 ± 0.11 d C	9.25 ± 0.53 c C
	Peizaruanxiang			
CTR	2.61 ± 0.28 c B	6.49 ± 0.38 b A	1.70 ± 0.12 c C	1.78 ± 0.11 d C
GA3	8.27 ± 0.86 a A	7.66 ± 0.23 ab A	3.68 ± 0.23 b B	3.93 ± 0.09 b B
IAA	6.26 ± 0.33 b B	7.52 ± 0.14 ab A	4.18 ± 0.47 b C	2.74 ± 0.13 c D
PBZ	6.56 ± 0.29 b B	8.38 ± 0.79 a A	3.74 ± 0.39 b C	2.41 ± 0.19 c C
PPZ	5.28 ± 0.38 b B	7.16 ± 0.89 ab A	5.49 ± 0.39 a AB	4.42 ± 0.18 a B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

POD activity in rice flag leaf (Unit/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
	Guixiangzhan			
CTR	168.80 ± 2.79 c A[a]	160.41 ± 0.74 d A	110.24 ± 4.25 c B	160.75 ± 2.62 b A
GA3	255.32 ± 3.51 a A	240.98 ± 4.50 a A	183.78 ± 6.88 a B	250.09 ± 2.84 a A
IAA	194.16 ± 5.21 b A	204.56 ± 3.38 b A	167.08 ± 7.76 a B	114.15 ± 3.49 c C
PBZ	251.60 ± 4.46 a A	209.32 ± 1.75 b B	145.02 ± 1.11 b D	171.19 ± 5.74 b C
PPZ	202.51 ± 6.43 b A	187.55 ± 2.24 c AB	146.81 ± 8.07 b C	170.86 ± 6.12 b B
	Peizaruanxiang			
CTR	103.81 ± 1.94 d D	157.17 ± 1.78 a A	138.68 ± 3.28 c B	117.68 ± 4.07 d C
GA3	115.35 ± 2.57 c D	154.00 ± 6.09 a C	190.23 ± 4.71 a A	175.02 ± 3.47 a B
IAA	124.46 ± 0.67 b B	157.14 ± 3.46 a A	158.39 ± 1.58 b A	149.86 ± 4.60 b A
PBZ	166.39 ± 3.46 a A	122.31 ± 3.88 b B	124.97 ± 5.30 cd B	173.41 ± 4.75 a A
PPZ	168.29 ± 1.24 a A	153.81 ± 5.85 a A	123.82 ± 6.81 d B	136.44 ± 3.40 c B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

POX activity in brown rice grains (μmol/min/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
			Guixiangzhan	
CTR	115.84 ± 2.74 d A[a]	75.58 ± 5.62 d B	39.05 ± 4.00 d C	29.46 ± 3.52 a C
GA3	135.95 ± 1.76 c B	252.86 ± 6.47 b A	61.19 ± 2.98 c C	32.83 ± 2.89 a D
IAA	214.88 ± 4.60 b B	275.71 ± 3.66 a A	114.88 ± 4.05 b C	28.90 ± 4.54 a D
PBZ	135.49 ± 6.26 c B	230.57 ± 8.84 c A	115.62 ± 2.74 b B	37.27 ± 4.86 a C
PPZ	233.58 ± 3.74 a B	286.93 ± 6.61 a A	141.14 ± 2.24 a C	35.33 ± 2.93 a D
			Peizaruanxiang	
CTR	112.98 ± 4.18 d A	105.66 ± 2.19 d A	47.82 ± 7.94 a B	26.78 ± 3.57 a C
GA3	152.61 ± 5.96 bc A	149.91 ± 6.94 c A	46.09 ± 4.65 a B	26.18 ± 2.95 a C
IAA	171.28 ± 3.88 a A	45.97 ± 4.33 e B	45.91 ± 4.22 a B	22.45 ± 2.38 a C
PBZ	138.65 ± 7.77 c B	192.77 ± 3.71 b A	49.48 ± 7.18 a C	23.95 ± 6.48 a D
PPZ	161.25 ± 6.44 ab B	234.96 ± 4.32 a A	45.11 ± 4.72 a C	21.56 ± 6.81 a D

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

POX activity in rice flag leaf (μmol/min/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
			Guixiangzhan	
CTR	63.81 ± 4.60 d A[a]	44.65 ± 3.79 b B	30.17 ± 1.82 b C	43.60 ± 0.24 b B
GA3	126.57 ± 5.87 c A	43.76 ± 4.84 b C	69.96 ± 4.40 a B	64.91 ± 7.34 a B
IAA	150.65 ± 5.33 b A	44.68 ± 6.18 b B	57.35 ± 5.92 a B	52.92 ± 3.60 ab B
PBZ	145.33 ± 7.44 b A	60.61 ± 3.44 a B	57.84 ± 3.05 a B	60.58 ± 0.99 a B
PPZ	170.57 ± 4.99 a A	43.42 ± 1.10 b C	60.30 ± 7.86 a BC	63.56 ± 7.53 a B
			Peizaruanxiang	
CTR	41.76 ± 4.27 cd A	36.93 ± 5.97 c A	34.84 ± 2.73 c A	32.87 ± 2.30 d A
GA3	120.66 ± 1.96 a A	102.00 ± 7.82 a B	52.06 ± 2.42 b D	69.90 ± 5.07 c C
IAA	74.85 ± 7.22 b A	68.94 ± 3.39 b A	69.34 ± 5.67 a A	65.93 ± 3.98 c A
PBZ	36.75 ± 3.21 d D	55.47 ± 4.51 b C	73.62 ± 5.06 a B	121.71 ± 7.29 b A
PPZ	47.97 ± 2.83 c C	59.90 ± 2.13 b BC	64.73 ± 4.31 ab B	166.91 ± 5.89 a A

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

On the other hand, proline content increased, MDA content decreased, while total soluble proteins content remained stable after treatment with the four growth regulators **(Table 3.3.11).**

Table 3.3.11 Effect of growth regulators on MDA, soluble proteins, proline contents in brown rice grains and flag leaves harvested at different days after heading (DAH)

MDA content in brown rice grains (μmol/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
		Guixiangzhan		
CTR	1.09 ± 0.01 a A[a]	0.72 ± 0.03 a C	0.81 ± 0.01 a B	0.66 ± 0.02 a C
GA3	0.78 ± 0.01 b A	0.63 ± 0.01 b C	0.70 ± 0.01 b B	0.53 ± 0.03 b D
IAA	0.54 ± 0.04 d B	0.66 ± 0.01 ab A	0.69 ± 0.01 b A	0.50 ± 0.04 b B
PBZ	0.66 ± 0.03 c A	0.60 ± 0.02 bc A	0.60 ± 0.04 c A	0.47 ± 0.03 b B
PPZ	0.50 ± 0.03 d B	0.54 ± 0.04 c B	0.69 ± 0.02 b A	0.54 ± 0.01 b B
		Peizaruanxiang		
CTR	0.65 ± 0.02 a B	0.82 ± 0.02 a A	0.78 ± 0.03 a A	0.61 ± 0.01 a B
GA3	0.44 ± 0.03 b B	0.60 ± 0.02 d A	0.43 ± 0.02 c B	0.31 ± 0.02 b C
IAA	0.52 ± 0.04 b BC	0.68 ± 0.01 c A	0.61 ± 0.04 b AB	0.46 ± 0.05 b C
PBZ	0.43 ± 0.02 b C	0.67 ± 0.01 c A	0.51 ± 0.01 c B	0.44 ± 0.03 b C
PPZ	0.49 ± 0.02 b C	0.76 ± 0.02 b A	0.69 ± 0.01 b B	0.42 ± 0.02 c C

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

MDA content in rice flag leaf (μmol/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
		Guixiangzhan		
CTR	15.25 ± 0.10 a A[a]	10.34 ± 0.22 a C	11.38 ± 0.29 a B	9.21 ± 0.30 a D
GA3	10.89 ± 0.15 b A	8.86 ± 0.18 b B	10.25 ± 0.28 b A	7.37 ± 0.27 bc C
IAA	7.37 ± 0.77 d B	9.23 ± 0.17 b A	9.76 ± 0.03 b A	5.92 ± 0.43 d B
PBZ	9.12 ± 0.43 c A	8.44 ± 0.27 bc A	8.47 ± 0.58 c A	6.52 ± 0.40 cd B
PPZ	7.08 ± 0.40 d B	7.57 ± 0.53 c B	9.6 ± 0.28 b A	7.55 ± 0.12 b A
		Peizaruanxiang		
CTR	8.96 ± 0.22 a B	11.58 ± 0.12 a A	10.87 ± 0.39 a A	8.03 ± 0.19 a C
GA3	6.27 ± 0.43 bc B	8.47 ± 0.24 d A	6.15 ± 0.34 c B	5.22 ± 0.75 b B
IAA	7.21 ± 0.55 b BC	9.54 ± 0.10 c A	8.43 ± 0.63 b AB	6.49 ± 0.62 b C
PBZ	5.83 ± 0.24 c C	9.48 ± 0.21 c A	7.13 ± 0.19 c B	6.13 ± 0.41 b C
PPZ	6.05 ± 0.32 bc C	10.70 ± 0.28 b A	9.56 ± 0.17 b B	5.69 ± 0.20 b C

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

Soluble protein content in brown rice grains (mg/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
		Guixiangzhan		
CTR	10.68 ± 0.35 a A[a]	10.62 ± 0.58 a A	10.66 ± 0.67 a A	10.45 ± 0.17 a A
GA3	11.23 ± 0.03 a A	10.31 ± 0.27 a A	10.60 ± 0.47 a A	11.12 ± 0.26 a A
IAA	10.78 ± 0.18 a A	11.16 ± 0.31 a A	10.45 ± 0.15 a A	10.95 ± 0.42 a A
PBZ	10.60 ± 0.11 a A	10.30 ± 0.28 a A	10.11 ± 0.04 a A	10.22 ± 0.11 a A
PPZ	10.55 ± 0.38 a A	10.19 ± 0.05 a A	10.56 ± 0.22 a A	10.97 ± 0.50 a A
		Peizaruanxiang		
CTR	12.05 ± 0.15 a A	11.77 ± 0.14 a A	8.31 ± 0.49 a B	8.71 ± 0.49 a B
GA3	11.26 ± 0.56 a A	11.72 ± 0.02 a A	8.15 ± 0.22 a B	7.86 ± 0.38 a B
IAA	11.42 ± 0.72 a A	11.40 ± 0.04 a A	8.23 ± 0.38 a B	8.31 ± 0.47 a B
PBZ	11.92 ± 0.49 a A	11.19 ± 0.05 a A	7.81 ± 0.11 a B	7.78 ± 0.28 a B
PPZ	11.67 ± 0.73 a A	11.43 ± 0.47 a A	8.10 ± 0.22 a B	8.72 ± 0.20 a B

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

Soluble protein content in rice flag leaf (mg/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
		Guixiangzhan		
CTR	25.08 ± 0.12 a A[a]	19.73 ± 0.62 a B	12.33 ± 0.64 a D	17.69 ± 0.40 a C
GA3	24.75 ± 0.53 a A	21.23 ± 0.87 a B	12.52 ± 0.16 a D	18.30 ± 0.29 a C
IAA	25.39 ± 0.35 a A	19.11 ± 0.06 a B	12.17 ± 0.42 a D	17.14 ± 0.89 a C
PBZ	24.88 ± 0.26 a A	20.87 ± 0.76 a B	11.94 ± 0.45 a D	18.06 ± 0.82 a C
PPZ	25.02 ± 0.08 a A	20.56 ± 0.76 a B	11.93 ± 0.27 a D	17.84 ± 0.45 a C
		Peizaruanxiang		
CTR	31.17 ± 0.97 a A	17.14 ± 0.29 a B	14.56 ± 0.56 a C	14.61 ± 0.41 a C
GA3	31.38 ± 0.42 a A	16.39 ± 0.69 a B	14.03 ± 0.11 a C	14.73 ± 0.44 a C
IAA	30.12 ± 0.91 a A	16.62 ± 0.45 a B	13.76 ± 0.72 a C	14.47 ± 0.49 a BC
PBZ	30.46 ± 0.22 a A	17.10 ± 0.77 a B	14.34 ± 0.15 a C	14.17 ± 0.47 a C
PPZ	30.25 ± 0.80 a A	16.60 ± 0.52 a B	14.44 ± 0.21 a C	14.48 ± 0.37 a C

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

Proline content in brown rice grains (μg/g)				
Growth regulator	Number of days after heading (DAH)			
	7 DAH	14 DAH	21 DAH	28 DAH
		Guixiangzhan		
CTR	22.16 ± 0.43 e A[a]	19.08 ± 0.81 c B	17.14 ± 0.58 b B	13.41 ± 0.90 ab C
GA3	35.30 ± 0.43 b A	22.00 ± 0.43 b B	19.41 ± 0.49 a C	14.22 ± 0.32 ab D
IAA	26.70 ± 1.29 d A	22.49 ± 0.43 b B	18.76 ± 0.71 ab C	12.76 ± 0.71 b D
PBZ	32.05 ± 1.01 c A	20.70 ± 0.43 bc B	18.76 ± 0.43 ab B	14.87 ± 0.43 a C
PPZ	41.13 ± 0.65 a A	26.70 ± 0.97 a B	19.24 ± 0.32 a C	14.54 ± 0.28 ab D
		Peizaruanxiang		
CTR	25.08 ± 1.41 cd A	18.43 ± 0.28 b B	16.81 ± 0.58 a B	12.92 ± 0.32 a C
GA3	23.62 ± 1.06 d A	19.57 ± 0.71 b B	16.48 ± 0.84 a C	11.46 ± 0.71 a D
IAA	31.24 ± 0.43 b A	23.43 ± 0.58 a B	16.97 ± 0.84 a C	11.46 ± 0.58 a D
PBZ	27.03 ± 0.71 c A	17.95 ± 0.49 b B	15.19 ± 0.58 a C	11.95 ± 0.86 a D
PPZ	35.30 ± 1.27 a A	22.16 ± 0.43 a B	16.97 ± 0.28 a C	12.44 ± 0.32 a D

[a] Means followed by the same lower-case letter in the same column and by the same upper-case letter in the same row do not differ significantly at the 0.05 level (Duncan's multiple range test).

3.3.3.9 Influence of growth regulators on rice yield and quality

Highest grain yield was obtained with gibberellic acid during the two growing seasons, 6.41 and 5.71, 7.60 and 6.44 t/ha in Guixiangzhan and Peizaruanxiang, respectively **(Table 3.3.12; Table 3.3.13)**. However, yield correlated negatively with 1000 grains weight after treatment with GA3. Of all the three plant growth regulators, IAA could highly influence the yield components for better yield and quality. Treatment with IAA increased 1000-grain weight (27.23; 22.12 g) **(Table 3.3.12; Table 3.3.13)**, head rice yield (65.48; 66.34 %) **(Table 3.3.14; Table 3.3.15)**, and grain vitreosity (72.73; 84.36%) **(Table 3.3.16; Table 3.3.17)**. It also appeared that amylose content (20.38; 25.76%), and protein content (8.68; 9.06%) improved following treatment with IAA **(Table 3.3.16; Table 3.3.17)**. PBZ seemed to produce the highest head rice rate (65.96%) and grain vitreosity (84.45%) in Peizaruanxiang and may indicate a cultivar effect. However, the same positive effects were not noted for the other parameters measured.

The results showed that cultivar affected all the yield and quality parameters measured **(Table 3.3.18)**. There were significant ($P<0.05$) treatment with regulator effects on 9 attibutes including the total number of ripened spikelets, the grain-filling percentage, 1000-grain weight, the average paddy yield, the head rice rate, the grain vitreosity, the % area with chalkiness, the protein and MDA contents. Significant season ($P<0.05$) effects were also observed on not less that 9 yield and quality attributes.

Table 3.3.12 Means for grain yield and five yield attributes of Guixiangzhan and Peizaruanxiang rice cultivars after foliar application of plant growth regulators during the early season

Growth regulator	Panicles/Hill	Ripened spikelets/Panicle	Total spikelets/Panicles	Grain-filling percentage (%)	Grain weight (g/1,000)	Grain yield (t/ha)
			Guixiangzhan			
CTR	10.27 ± 0.41 a[a]	61.02 ± 1.15 b	109.65 ± 2.67 a	55.66 ± 0.30 b	25.11 ± 0.82 a	4.72 ± 0.28 b[a]
GA3	10.40 ± 0.10 a	73.43 ± 2.68 a	113.88 ± 0.22 a	64.47 ± 2.24 a	26.42 ± 0.57 a	5.71 ± 0.15 a
IAA	10.17 ± 0.27 a	59.46 ± 2.40 b	110.45 ± 1.35 a	53.79 ± 1.66 b	27.80 ± 0.42 a	4.94 ± 0.06 b
PBZ	10.02 ± 0.29 a	62.08 ± 2.33 b	109.06 ± 0.88 a	56.96 ± 2.58 b	26.25 ± 1.26 a	4.94 ± 0.17 b
PPZ	10.18 ± 0.11 a	61.45 ± 1.28 b	108.70 ± 2.92 a	56.68 ± 2.63 b	26.10 ± 1.02 a	4.97 ± 0.34 b
			Peizaruanxiang			
CTR	11.70 ± 0.36 a	107.24 ± 2.41 b	183.25 ± 1.78 a	58.51 ± 0.75 b	21.14 ± 0.35 bc	5.18 ± 0.14 c
GA3	11.36 ± 0.07 a	127.97 ± 3.71 a	174.01 ± 6.51 a	73.84 ± 4.35 a	20.22 ± 0.19 c	6.44 ± 0.15 a
IAA	11.19 ± 0.46 a	106.40 ± 2.89 b	175.91 ± 8.41 a	60.60 ± 1.20 b	22.67 ± 0.10 a	5.68 ± 0.22 b
PBZ	11.56 ± 0.10 a	104.67 ± 2.75 b	181.70 ± 5.68 a	57.82 ± 3.43 b	21.52 ± 0.64 ab	5.52 ± 0.09 bc
PPZ	11.62 ± 0.13 a	106.52 ± 2.16 b	170.80 ± 8.46 a	59.72 ± 2.68 b	21.78 ± 0.46 ab	5.37 ± 0.07 bc

[a] Results are mean ± standard deviation (n = 3). Different letters following data in a column for each parameter indicate significant differences ($P<0.05$ using Duncan's multiple range test).

Table 3.3.13 Means for grain yield and five yield attributes of Guixiangzhan and Peizaruanxiang rice cultivars after foliar application of plant growth regulators during the late season

Growth regulator	Panicles/Hill	Ripened spikelets/Panicle	Total spikelets/Panicles	Grain-filling percentage (%)	Grain weight (g/1,000)	Grain yield (t/ha)
			Guixiangzhan			
CTR	10.30 ± 0.72 a[a]	57.75 ± 1.90 b	82.00 ± 4.93 a	70.87 ± 4.15 b	25.92 ± 0.58 ab	5.09 ± 0.28 b[a]
GA3	11.20 ± 0.62 a	72.38 ± 1.07 a	84.67 ± 3.48 a	85.67 ± 2.26 a	26.21 ± 0.27 ab	6.41 ± 0.43 a
IAA	10.30 ± 0.21 a	60.77 ± 1.30 b	82.00 ± 2.65 a	74.16 ± 0.91 ab	27.23 ± 0.16 a	5.20 ± 0.20 b
PBZ	11.63 ± 0.64 a	60.38 ± 2.89 b	79.00 ± 2.08 a	76.58 ± 4.60 ab	25.61 ± 0.74 b	5.55 ± 0.12 ab
PPZ	10.67 ± 0.84 a	60.03 ± 2.36 b	81.00 ± 4.73 a	74.58 ± 5.12 ab	26.05 ± 0.54 ab	5.47 ± 0.34 b
			Peizaruanxiang			
CTR	11.40 ± 0.21 a	96.85 ± 3.90 b	124.67 ± 2.90 a	77.72 ± 2.13 c	21.70 ± 0.12 b	6.44 ± 0.14 b
GA3	11.17 ± 0.55 a	126.02 ± 5.96 a	130.33 ± 4.10 a	96.63 ± 2.18 a	21.09 ± 0.18 c	7.60 ± 0.17 a
IAA	10.33 ± 0.72 a	110.01 ± 5.03 b	129.00 ± 1.73 a	85.24 ± 3.30 b	22.12 ± 0.06 a	6.75 ± 0.49 ab
PBZ	11.10 ± 0.44 a	105.63 ± 5.07 b	127.33 ± 4.10 a	82.87 ± 1.30 bc	21.96 ± 0.09 ab	6.44 ± 0.28 b
PPZ	10.30 ± 0.40 a	99.98 ± 3.43 b	124.00 ± 2.65 a	80.58 ± 1.09 bc	21.98 ± 0.17 ab	6.57 ± 0.25 b

[a] Results are mean ± standard deviation (n = 3). Different letters following data in a column for each parameter indicate significant differences ($P<0.05$ using Duncan's multiple range test).

Table 3.3.14 Main effects of foliar application of plant growth regulators on the milling quality of Guixiangzhan and Peizaruanxiang rice cultivars during the early season

Growth regulator	Brown rice yield (%)	Milled rice yield (%)	Head rice yield (%)
	Guixiangzhan		
CTR	79.41 ± 0.33 a[a]	65.55 ± 1.73 a	48.35 ± 0.13 c
GA3	78.49 ± 1.47 a	69.75 ± 2.42 a	48.46 ± 0.25 bc
IAA	80.26 ± 0.96 a	63.81 ± 1.78 a	48.99 ± 0.02 a
PBZ	78.86 ± 0.73 a	68.76 ± 2.13 a	48.84 ± 0.06 ab
PPZ	79.23 ± 0.22 a	67.88 ± 2.12 a	48.78 ± 0.10 ab
	Peizaruanxiang		
CTR	81.70 ± 2.28 a	72.60 ± 1.00 a	56.17 ± 0.37 b
GA3	82.20 ± 0.98 a	69.97 ± 2.42 a	55.74 ± 0.45 b
IAA	79.26 ± 2.14 a	70.77 ± 1.57 a	62.02 ± 0.41 a
PBZ	80.04 ± 0.50 a	72.43 ± 2.68 a	60.96 ± 0.18 a
PPZ	79.55 ± 1.87 a	70.70 ± 1.80 a	61.11 ± 0.26 a

[a] Results are expressed as mean ± standard deviation (n = 3). 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.

Table 3.3.15 Main effects of foliar application of plant growth regulators on the milling quality of Guixiangzhan and Peizaruanxiang rice cultivars during the late season

Growth regulator	Brown rice yield (%)	Milled rice yield (%)	Head rice yield (%)
	Guixiangzhan		
CTR	80.82 ± 0.35 a[a]	69.19 ± 0.22 a	65.15 ± 0.52 a
GA3	80.87 ± 0.28 a	69.13 ± 0.47 a	63.28 ± 0.56 b
IAA	81.13 ± 0.11 a	69.60 ± 0.29 a	65.48 ± 0.13 a
PBZ	80.76 ± 0.47 a	69.12 ± 0.78 a	64.44 ± 0.57 ab
PPZ	81.05 ± 0.47 a	69.61 ± 0.60 a	64.72 ± 0.90 ab
	Peizaruanxiang		
CTR	82.24 ± 0.32 a	71.70 ± 0.33 ab	65.72 ± 0.25 ab
GA3	81.93 ± 0.23 a	70.43 ± 0.78 b	63.19 ± 0.40 c
IAA	82.67 ± 0.28 a	72.11 ± 0.39 a	66.34 ± 0.46 a
PBZ	82.45 ± 0.29 a	71.92 ± 0.33 a	65.96 ± 0.50 a
PPZ	82.53 ± 0.22 a	71.98 ± 0.18 a	64.49 ± 0.44 bc

[a] Results are expressed as mean ± standard deviation (n = 3). 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.

Table 3.3.16 Variation in grain vitreosity, amylose content and protein content of Guixiangzhan and Peizaruanxiang rice cultivars after foliar application of plant growth regulators during the early season

Growth regulator	Grain vitreosity (%)	% area with chalkiness	Amylose content (%)	Protein content (%)
		Guixiangzhan		
CTR	88.33 ± 0.33 b[a]	26.60 ± 0.33 a	17.60 ± 0.35 ab	7.90 ± 0.19 b
GA3	86.33 ± 0.33 c	25.73 ± 0.62 a	16.13 ± 0.23 b	7.71 ± 0.37 b
IAA	90.67 ± 0.88 a	25.87 ± 0.53 a	17.78 ± 0.43 a	8.70 ± 0.04 a
PBZ	88.00 ± 0.58 bc	23.72 ± 0.16 b	16.60 ± 0.75 ab	8.04 ± 0.02 ab
PPZ	88.33 ± 0.33 b	23.85 ± 0.25 b	16.17 ± 0.43 b	7.78 ± 0.30 b
		Peizaruanxiang		
CTR	78.33 ± 0.33 b	29.63 ± 0.27 a	28.10 ± 0.06 a	8.11 ± 0.05 b
GA3	80.00 ± 0.58 b	29.54 ± 0.03 a	26.13 ± 0.93 b	7.39 ± 0.28 c
IAA	83.67 ± 0.33 a	28.93 ± 0.08 b	28.27 ± 0.27 a	8.83 ± 0.04 a
PBZ	82.33 ± 0.88 a	28.53 ± 0.10 b	26.87 ± 0.57 ab	7.97 ± 0.40 bc
PPZ	84.00 ± 0.58 a	27.99 ± 0.09 c	27.47 ± 0.48 ab	8.24 ± 0.12 ab

[a] Means (n = 3) ± standard deviation in the same column (for each rice cultivar) with different letters are significantly different (P<0.05) according to Duncan's multiple range test.

Table 3.3.17 Variation in grain vitreosity, amylose content and protein content of Guixiangzhan and Peizaruanxiang rice cultivars after foliar application of plant growth regulators during the late season

Growth regulator	Grain vitreosity (%)	% area with chalkiness	Amylose content (%)	Protein content (%)
		Guixiangzhan		
CTR	89.33 ± 0.33 ab[a]	30.33 ± 1.67 a	19.51 ± 0.51 ab	8.01 ± 0.21 b
GA3	86.33 ± 0.88 b	27.00 ± 1.53 ab	18.89 ± 0.49 b	7.02 ± 0.23 c
IAA	90.67 ± 0.88 a	26.33 ± 1.45 ab	20.38 ± 0.24 a	8.63 ± 0.08 a
PBZ	87.67 ± 1.20 ab	24.67 ± 1.20 b	20.62 ± 0.23 a	8.07 ± 0.20 b
PPZ	87.33 ± 1.33 b	25.67 ± 1.45 b	19.64 ± 0.44 ab	8.00 ± 0.10 b
		Peizaruanxiang		
CTR	80.33 ± 0.88 b	31.00 ± 0.58 a	28.20 ± 0.57 a	8.09 ± 0.29 bc
GA3	83.00 ± 1.15 ab	32.00 ± 0.58 a	28.27 ± 0.94 a	7.77 ± 0.24 c
IAA	81.33 ± 0.88 b	28.00 ± 1.53 bc	25.76 ± 0.97 b	9.06 ± 0.34 a
PBZ	85.67 ± 0.67 a	26.00 ± 1.00 c	28.24 ± 0.79 a	8.63 ± 0.33 abc
PPZ	80.00 ± 1.53 b	30.67 ± 0.67 ab	28.04 ± 0.46 ab	8.78 ± 0.21 ab

[a] Means (n = 3) ± standard deviation in the same column (for each rice cultivar) with different letters are significantly different (P<0.05) according to Duncan's multiple range test.

Table 3.3.18 Calculated *F* values from ANOVA for treatment (CTR, GA3, IAA, PBZ, PPZ), cultivar (Guixiangzhan, Peizaruanxiang) and season (early season, late season) effects on rice yield and quality parameters

	Regulator	Cultivar	Season	Regulator × Cultivar	Regulator × Season	Cultivar × Season	Regulator × Cultivar × Season
Panicles/Hill	1.26*	11.36	0.00*	0.56*	0.85*	10.06	0.68*
Ripened spikelets/Panicle	22.23	973.35	1.80*	2.48*	1.15*	0.05*	0.76*
Total spikelets/Panicles	0.20*	896.88	446.98	0.43*	0.47*	36.86	0.61*
Grain-filling percentage (%)	17.38	27.36	284.97	1.15*	0.72*	2.22*	0.07*
Grain weight (g/1,000)	4.96	363.23	0.13*	1.66*	0.73*	0.79*	0.32*
Grain yield (t/ha)	13.76	67.13	53.51	0.46*	0.16*	8.39	0.25*
Brown rice yield (%)	0.19*	9.75	15.82	0.70*	0.48*	0.02*	1.02*
Milled rice yield (%)	0.58*	25.93	3.95	1.40*	1.12*	2.13*	0.80*
Head rice yield (%)	33.81	920.32	3611.17	14.27	11.15	753.93	9.82
Grain vitreosity (%)	11.57	302.84	0.20*	6.56	4.09	0.40*	2.25*
% area with chalkiness	9.96	65.27	7.89	1.81*	2.89	1.65*	1.27*
Amylose content (%)	1.73*	1324.17	42.33	1.93*	3.79	26.83	2.15*
Protein content (%)	16.61	8.29	1.80*	0.63*	0.91*	4.36	0.88*
MDA content 7 DAH	114.09	246.08	310.74	17.34	15.54	47.60	20.66
MDA content 14 DAH	53.08	9.76	1686.89	18.05	2.54	48.89	17.44
MDA content 21 DAH	74.15	21.95	1057.39	7.62	15.41	43.19	9.88
MDA content 28 DAH	47.36	37.02	4.52	11.23	8.29	12.50	2.38*

* Correlation is non significant at the 0.05 level

3.3.4 Discussion

3.3.4.1 Separation and identification of odor-active compounds

Studies involving the isolation and identification of odor-active compounds in rice grains have been performed for decades. However, other than lipid oxidation products and 2-acetyl-1-pyrroline (2-AP), researchers have not been successful in conclusively identifying specific volatile compounds or classes of compounds that contribute to other desirable or undesirable aroma or flavor attributes in rice (Buttery et al. 1988; Zhou et al. 1999; Jezussek et al. 2002; Champagne et al. 2008; Maraval et al. 2008; Yang et al. 2008a, 2008c). Due to their relative importance, 2-AP and lipid-derived volatiles were selected to make decisions on the overall effect of foliar application of plant growth regulators at approximately 25% panicle emergence on rice aroma and flavor. It should be kept in mind that our intent was not to assess the relative importance of each compound to the overall aroma of the two rice cultivars, but just to compare major compounds previously identified in rice.

A selection of methods is available for the collection and concentration of volatiles components of rice, each with advantages and limitations. In our study, static headspace (SHS) coupled to a GC appeared a suitable choice for the isolation of compounds of interest. A selection was made between two detectors, FID for the determination of lipid oxidation volatiles and other major volatiles, and NPD for 2-AP. Overall, 14 compounds emanating from the headspace of the two rices were identified with some certainty and used in the study. They belonged to the chemical classes of alcohols (6), aromatics (1), aldehydes (5), and nitrogen-containing compounds (2).

3.3.4.2 Influence of growth regulators on nitrogen-containing compounds

Considering nitrogen-containing compounds first, particular attention may be focused on 2-AP (pleasant, popcorn-like, and sweet) found in many rice cultivars, but with higher contents in fragrant rices. A good correlation between the concentration of 2-AP in various rice samples and sensory intensity has been established (Paule and Powers, 1989; Hori et al. 1994). Therefore, the compound has been widely used as a good indicator of aroma and flavor quality. The human olfactory thresholds for 2-AP are extremely low, in the range of 0.01 ng/L in air, and 0.1 μg/L in water.

Interestingly all treatments with plant growth regulators decreased the level of 2-AP compared to controls treated with distilled water (CTR), with a behavior similar to both cultivars. Studies into the biological formation of 2-AP have shown that it can be derived from either proline (Schieberle, 1990; Muench et al. 1997; Costello et al. 2002; Yoshihashi et al. 2002; Thimmaraju et al. 2005; Adams and De Kimpe, 2007), or glutamic acid (Huang et al.

2008). In the first case, the nitrogen in the pyrroline ring of proline becomes the nitrogen in the pyrroline ring of 2-AP while the carboxyl group is removed and replaced with an acetyl group from another source (Yoshihashi et al. 2002). Although the smell of aromatic rice have been reported to stem primarily from its 2-AP content, it is important to note that this compound only confers the popcorn-like odor. Therefore, a decrease of the magnitude determined would not necessarily contribute to diminished fragrance, as the unique aromas found across diverse rice cultivars cannot be accounted for simply by the variation in 2-AP concentration. Recent studies in odor-active compounds have shown that aldehydes for example, made up over 90% of all the odor-active compounds in rice even though the relative proportion varied among samples (Suzuki et al. 1999; Yang et al. 2008c). Similarly data showed 2-AP as a key odorant in brown rices, but only in those available in some Asian countries (Jezussek et al. 2002; Maraval et al. 2008). So it will be premature to conclude that foliar application of plant growth regulators has an adverse effect on aroma/flavor.

Of the identified components in Guixiangzhan, benzothiazole is a nitrogen-containing component with a nutty and rubber scent at low concentration. It was selected as one of the flavor indicators of Basmati rice (Yang et al. 2008c). In our study, its content also decreased after application of growth regulators. Its average odor intensity, however, is reported to be very low (Yang et al. 2008c).

3.3.4.3 Influence of growth regulators on lipid oxidation aldehydes

The aldehydes have low odor thresholds and are considered to be important contributors to the overall aroma in many rice cultivars. This is supported by the fact that most aldehydes contribute to off-flavor and increase in concentration during storage (Grosch and Schieberle, 1997; Zhou et al. 1999). When changes to lipid-derived rice volatiles after foliar application of plant growth regulators are considered, it can be seen that most aldehydes had either decreased or remained at relatively unchanged levels. In concentration, nonanal (citrus, floral, fruity) was the most important aldehydes in the two rices. Its concentration was highest throughout the study relative to hexanal; it may thus have a great impact on the global aroma of the two Chinese's cultivars. Hexanal, which has been reported to be produced non-enzymatically or by an unknown pathway from linoleic acids via 9-D-hydroperoxy-10,12-(*E,Z*)-octadecadienoic acid (Suzuki et al. 1999) is considered one of the most potent odorants in rice after 2-AP. Hexanal contributes rancid, unpleasant notes on high dilution, but exhibits green, grass-like odors at low concentrations (Widjaja et al. 1996b; Zhou et al. 1999). Hexanal was found to have the highest contribution to milled rice odor during early storage contrary to octanal (slightly fruity, citrus-like), which contributes more during early storage (Lam and Proctor, 2003). Heptanal commonly contribute

floral, fruity and fatty aromas and its increase may hence have an indirect influence on the global aroma of rice. Another compound, (*E*)-2-hexenal (apple, green) followed a reduction rate in Guixiangzhan but increased in Peizaruanxiang and may indicate a cultivar effect. Results obtained over two growing seasons also indicate that some growth regulators could have a selective effect on the metabolism of volatile compounds like gibberellic acid on 1-hexanol, 1-octanol, 1-nonanol, (*E*)-2-hexenal, and nonanal, which increased in concentration over time.

3.3.4.4 Influence of growth regulators on malondialdehyde content

The aldehydes are thought to mainly be produced via lipid oxidation and decomposition. Lipid oxidation in rice occurs by oxidative reaction of unsaturated fatty acids mediated via enzymatic, thermal, or light reactions and by thermal mediated oxidative reactions of saturated fatty acids (Grosch and Schieberle, 1997; Suzuki et al. 1999; Zhou et al. 1999; Yang et al. 2008a). Of all the unsaturated fatty acids, oleic and linoleic acids are the most effective substrates for rice lipoxygenase-3 and therefore, acts as the precursors for most of the lipid-derived odor-active compounds in rice. For example, heptanal, octanal, nonanal, and decanal, are produced by oleate hydroperoxide decomposition; linoleate decomposition produce hexanal (Lam and Proctor, 2003). Another evidence of inhibition of lipid oxidation in our study is the decrease in malondialdehyde (MDA) level, a non-volatile product of lipid peroxidation. Clearly, the effect presented here, i.e., retardation of lipid peroxidation due to plant growth regulators adds weight to results found above, supporting the inhibition of flavor formation by plant growth regulators. It may be postulated that the other lipid-derided odor-active products in rice like (*E*,*E*)-2,4-decadienal and 2-pentylfuran also decreased in concentration.

3.3.4.5 Influence of growth regulators on lipid oxidation alcohols

Considering the changes that occurred in volatile alcohols after foliar application of plant growth regulators, it can be seen that there was generally a decrease in levels of most of the alcohols as was the case with the aldehydes. All these alcohols are produced mainly by the reduction of their corresponding aldehydes and methylketones (Suzuki et al. 1999; Zhou et al. 1999). The most critical odorant among all the alcohols was 1-hexanol which has more pleasant aroma descriptors (vegetal, herbaceous, and green) (Widjaja et al. 1996b). A clear discrimination between aromatic and non-aromatic cultivars was shown for this component by three papers (Petrov et al. 1996; Yang et al. 2008c; Maraval et al. 2008) with 1-hexanol concentration significantly higher for scented rice samples. In these three studies, 1-pentanol (plastic, fusel, oil-like) was also identified as discriminants. 1-Pentanol produced from linoleate decomposition (Lam and Proctor, 2003) was reported to be higher in non-aromatic rices (Petrov et al. 1996; Widjaja et al.

1996b; Yang et al. 2008c; Maraval et al. 2008), to be a major volatile in rice cakes (Buttery et al. 1999), to be one of the three major volatile components during normal LOX-3 rice storage (Suzuki et al. 1999), and has regularly been cited as occurring in significantly high concentration in rice. Consequently, the relative ratio of these compounds may hence have an indirect influence on the global aroma of rice.

Most of the oxidation products discussed so far have been tagged as likely causing rancid and stale flavor, and are reported to increase in concentration over time (Lam and Proctor, 2003; Wongpornchai et al., 2004). The results presented in **Table 3.3.4** and **Table 3.3.6**, then **Table 3.3.5** and **Table 3.3.7** show that the potential for rancidity development during rice storage was higher in control samples than in samples treated with growth regulators This constitutes an interesting result and provides evidence for the ability of growth regulators to reduce off-flavor development in rice grain during storage, probably by inducing various enzymatic actions and preventing lipid hydrolysis, leading to decreases in volatile compounds, or their conversion into non-volatiles compounds.

3.3.4.6 Influence of growth regulators on aromatic compounds

Among the nine compounds found by Petrov et al. (1996) to discriminate fragrant and non-fragrant rices, benzaldehyde was detected in Guixiangzhan (4.22 ng/g). It is the simplest representative of the aromatic aldehydes with a characteristic and pleasant almond-like odor (Widjaja et al. 1996b). It was absent from samples treated with GA3, PPZ and PBZ. Benzaldehyde would most likely be formed from oxidation reactions of cinnamic acid or phenylacetaldehyde (Grosch and Schieberle, 1997). Industrially benzaldehyde is primarily made from toluene by a number of different processes. Toluene has a paint and ethereal-like aroma (Verschueren, 2001). Its contribution to the flavor of rice has been established only by one paper (Yang et al. 2008a).

3.3.4.7 Influence of growth regulators on cooked rice odor

Growing Guixiangzhan and Peizaruanxiang rice cultivars with growth regulators decreased the aroma content and changed the sensory properties to a level that could be easily detected by taste panel evaluation of brown rice powder cooked without addition of water. Key thermally induced odorants in rice have also been assigned and include 2-methoxy-4-vinyphenol (spicy), 4-vinylguaiacol, and 4-vinylphenol (phenolic, medicinal) (Maraval et al., 2008; Yang et al., 2008c). These results indicate a decrease in their concentrations. Therefore, rice grown with growth regulators is expected to have significant decrease in flavor that may negatively affect consumer acceptance.

3.3.4.8 Influence of growth regulators on proline content, lipid peroxidation and antioxidative systems of rice

In a general sense, plant growth regulators are transported from one part of the rice plant to another, where they elicit a specific response. One such response is the role that GA3, PBZ, and IAA play in the alleviation of adverse effects of various stresses (Ghosh et al. 2003; Mander, 2003). Based on the results presented above**,** it is evident that coincidently with high salinity for example, all the four treatments will be effective to enhance the capacity of Peizaruanxiang and Guixiangzhan to scavenge and control the production of damaging species of active oxygen**.**

3.3.4.9 Influence of growth regulators on rice yield and quality

More detailed insights into the beneficial effect of plant growth regulators can be obtained when stress tolerance are considered together with yield and quality parameters. It can be seem from **Table 3.3.7** to **Table 3.3.9** that for most of the yield and quality parameters measured, there were one or more treatments with growth regulators under which an improvement was found. For example, highest grain yield was obtained with GA3, 6.41 and 7.60 t/ha in Guixiangzhan and Peizaruanxiang, respectively **(Table 3.3.7)**. The aromatic cultivars sprayed with GA3 had greener foliage and were generally higher plants, compared with PBZ-treated plants which had a slightly yellowing of the foliage with narrow, short and fewer leaves. In rice, the panicle bears a large number of spikelets during ontogeny, but all of them do not reach maturity to produce good quality grains (Yang et al. 2007). The spikelets located on the apical primary branches reach anthesis first and fill properly to produce larger and heavier grains, compared to the proximal spikelets. The proximal spikelets are either sterile or fill poorly to produce grains unsuitable for human consumption (McCauley and Way, 2002; Sheehy et al. 2006; Bond et al. 2008). The action of exogenous GA3 in this study may be responsible for the positional variation in development of spikelets on the panicle.

3.3.4.10 Modes of action of plant growth regulators

Leaf-applied growth regulators can enter the leaf either by penetration of the cuticle or via the stomatal pathway and affect rice growth by way of several mechanisms. Paclobutrazol for example, has been widely used with marked success to reduce plant height and increase chlorophyll content in aromatic rice plants. Paclobutrazol is translocated primarily apo-plastically through the xylem to its site of action where it decreases the rate of cell division and elongation by the inhibition of *ent*-kaurene oxidase, which catalyzes the sequential oxidations from *ent*-kaurene to *ent*-kaurenoic acid in the early sequence of gibberellic acid biosynthesis (Yim et al. 1997). Gibberellic acid is associated with the stimulation of the

formation of hydrolytic enzymes in germinating cereal grain and the induction of carbohydrate translocation (Mander, 2003). The most significant physiological effect of exogenous gibberellic acid on rice plants is to break dwarfism and stimulate the elongation of leaves and stems. The pathways of formation of volatile aroma compounds in the rice plant are, therefore, very important to understand the mechanisms of action of growth regulators.

Although L-proline was identified as the principal precursor of 2-acetyl-1-pyrroline in rice (Yoshihashi et al. 2002), the biochemical pathways of 2-acetyl-1-pyrroline synthesis is presently not well elucidated (Bourgis et al. 2008). Fragrance in rice is reported to be due to an eight-base pair deletion in exon 7 of a gene on chromosome 8 encoding a putative betaine aldehyde dehydrogenase 2. Chen et al. (2008) suggested that the functional betaine aldehyde dehydrogenase 2 inhibits 2-acetyl-1-pyrroline biosynthesis in non-fragrant rice by converting 4-aminobutyraldehyde to 4-aminobutyric acid while the non-functional enzyme result in 4-aminobutyraldehyde accumulation leading to the formation of 2-acetyl-1-pyrroline in fragrant rice. Bradbury et al. (2008a) instead suggested γ-aminobutyraldehyde as the effective substrate for betaine aldehyde dehydrogenase 2. On the contrary, Huang et al. (2008) did not propose any direct role for betaine aldehyde dehydrogenase 2. Their work led to the conclusion that Δ^1-pyrroline-5-carboxylate, usually the immediate precursor of proline synthesized from glutamate, reacts directly with methylglyoxal to form 2-acetyl-1-pyrroline. No matter the pathway proposed, however, decreases in the levels of volatile compounds in rice found after treatment with growth regulators can mostly be explained by side activities on enzymes involved in the biosynthesis of 2-acetyl-1-pyrroline and other volatile compounds and the alteration of the mechanisms involved in their transport. In the case of betaine aldehyde dehydrogenase 2-dependant 2-acetyl-1-pyrroline synthesis, decreases in 2-acetyl-1-pyrroline accumulation in response to growth regulators could be attributed to an activation of betaine aldehyde dehydrogenase 2. In the second case, foliar application of growth regulators would have reduced the activity of enzymes involved in proline biosynthesis from glutamine and which include glutamine synthetase, glutamate synthase, and Δ^1-pyrroline-5-carboxylate synthetase. Other enzymatic activities cannot, however, be excluded. This hypothesis should be tested in the light of recent findings which showed that at least one other mutation drives the accumulation of 2-acetyl-1-pyrroline (Bourgis et al. 2008; Fitzgerald et al. 2008).

The major deteriorating mechanism occurring in rice being lipid oxidation and decomposition, the low level observed for lipid-derived volatiles can be explained by the inhibition of lipooxygenase and lipase activities and hence a reduction of the rate of oxidation of unsaturated fatty acids like oleic and linoleic acids (Suzuki et al. 1999). The same comment might hold true for benzaldehyde which is reported to originate from oxidation reactions of

cinnamic acid or phenylacetaldehyde.

In any case, the possibility of other mechanisms operating in parallel should also be considered. Interaction of aroma compounds with other compounds such as starch matrices, lipids and proteins, may increase or decrease aroma retention even during cooking (Champagne, 2008). Storage tests indicated that 2-acetyl-1-pyrroline can exist as a complex within the hydrophobic region of crystalline starch (Yoshihashi et al. 2002). Growth regulators can well affect the matrix composition and the migration process of aroma molecules which, in turn, will affect the flavor perceived. It has also been demonstrated that the application of exogenous auxins like 3-indole acetic acid alters the gland formation process, the number of glands produced and their integrity in many plants by inhibiting the formation of trichomes, which would affect the biosynthesis of volatile compounds and their secretion from glands and mesophyll cells to intercellular spaces (Sudria et al. 2001).

We would have expected a positive effect of proline application on the aroma quality of the two fragrant rice cultivars because of its possible involvement in the biosynthesis 2-acetyl-1-pyrroline (Yoshihashi et al. 2002). This was, however, not the case in our work. This was probably because proline was applied along with zinc chloride and paclobutrazol, the latter being predominant in its activity. Another explanation is that it is a biosynthetic pathway in aromatic rice grains which leads to the formation of 2-acetyl-1-pyrroline from proline and not the level of free proline.

3.3.5 Conclusion

Changes in rice aroma after treatment with gibberellic acid (GA3), paclobutrazol (PBZ), 3-indole acetic acid (IAA) and a mixture of paclobutrazol, proline, and zinc chloride (PPZ) were examined using two aromatic rice cultivars grown in South China, namely Guixiangzhan and Peizaruanxiang. Applications were done when the crop had 25% of panicles emerged. The analysis focused on 14 odor-active compounds extracted and identified using static headspace coupled to GC. All treatments with plant growth regulators resulted in reduced aroma content that negatively affected overall flavor. In a confirmatory sensory evaluation, rice flavor was most intense in cooked rice from the control plots than samples treated with gibberellic acid, paclobutrazol and indole acetic acid. The difference between the aroma of control and treated samples was largely related to 2-acetyl-1-pyrroline (2-AP), the major rice aroma compound, and lipid oxidation volatiles. In Guixiangzhan, GA3 treatment decreased the content of 2-AP by 18.75%, IAA by 20.83%, PBZ by 8.75%, and PPZ by 21.25% compared to the control (2.40 μg/g.). In Peizaruanxiang, control samples had a 2-AP concentration of 0.41 μg/g. 2-AP in

treated samples ranged in intensity from 0.31 μg/g (PPZ) to 0.37 μg/g. (IAA). The decreases were generally in the order of GA3 > PPZ > PBZ > IAA. Our findings indicate that treatments with plant growth regulators inhibited the metabolic processes associated with volatile formation. Although the type of action of plant growth regulators can be highly variable depending on cultivar and time and mode of application, we recommended that normal agronomic practices are adhered to. If plant growth regulators are to be used for purposes such as improved yield and survival under stress conditions, then the aroma quality needs to be carefully monitored.

4 Conclusions and recommendations

Increased consumer demand for imported aromatic rice has spurred interest in the development of specific domestic cultivars with similar and/or unique flavor and scent. In this study, two of such cultivars grown in Guangzhou (South China) were evaluated for their fragrance potential. Furthermore, we were leaned to identify the specific combination of pre- and post-harvest practices that will allow growers and processors to foster conditions for high aroma yield in cooked rice.

The following may be concluded from the results of our investigation:

1. Genotype had the greatest effect on the 2-AP content of the two cultivars grown over two seasons in Guangzhou. The new line Peizaruanxiang is not as good as the old one, Guixiangzhang. Considering 2-AP as the molecular marker for flavor and aroma in rice, Guixiangzhan was found to have fragrance potential comparable to that of the Thailand cultivar KDML 105, similarity which is promising for the future development of South China scented cultivars.

2. High ripening temperatures during the early season lowered the content of 2-AP in the two rice cultivars. Advancing the sowing date to allow seed ripening to coincide with the cool and dry segment of November could improve aroma content and other seed qualities.

3. From the results of the study involving different planting densities, we recommend not to adhere to the current 20 cm × 20 cm. The use of lower planting densities will not only improve the flavor and aroma attribute, but will also maintain optimum yield and quality.

4. Irrespective of cultivar, 2-AP concentration reached a plateau at 0 to 10 days after heading (DAH), and then started to decrease logarithmically. However, taking into consideration yield and quality attributes, the best time we propose for harvesting transplanted rice is between 28 and 34 DAH for the early season and 32 and 38 DAH for the late season for Guixiangzhan and Peizaruanxiang.

5. Storage at colder temperatures of -4 ℃ and 8 ℃ was found to be an effective way to preserve the desirable character of fragrant rice, as monitored by the changes in the levels of 2-AP. The main question, however, is whether farmers and processors possess adapted storage facilities.

6. As a consequence of the changes in aroma character due to the storage time, it is advisable to consume fragrant rice within six months after harvest. This recommendation however, will depend on market economics.

7. There appears to be a great difference between the levels of 2-AP recovered from

milled and brown rice samples with 2-AP content significantly lower in white rice. Thus, it is quite important to preserve the content of 2-AP during the milling process of aromatic rice by establishing an optimum milling degree.

8. Spraying rice plants with growth regulators at panicle emergence may be a practical means of reducing off-flavor development in milled rice. However, 2-AP content is also reduced as a result of application of regulators which is an undesirable quality feature.

Acknowledgement

My sincere gratitude to:

1. God, the Omnipotent,
2. My family, for all the sacrifices made for me,
3. My principal supervisor, Prof. Xiangru Tang for its guidance, the Department of Crop Science and Technology of South China Agricultural University in China, and the Chinese Scholarship Council (CSC) for the stipend and operating funds,
4. Dr. Clautilde Teugwa Mofor, the Department of Biochemistry of the University of Yaoundé I in Cameroon, the Cameroonian Ministry of Higher Education, and the Cameroon Embassy in China, for allowing me to pursue my research in China through their mobility programme,
5. Prof. Sugunya Wongpornchai, the Department of Chemistry of Chiang Mai University in Thailand, and the Center of Excellence for Innovation in Chemistry (PERCHCIC), Commission on Higher Education, Ministry of Education, Thailand, for technical assistance,
6. Cheng Shifu and his wife, for the design, set up and maintenance of my field trials,
7. Meiyang Duan, Tinakorn Sriseadka, Jujing Xu, and Mutita Yangtaworn for helping me with sampling, harvesting and laboratory work,
8. James Aipa, Christ Paulus, Joseph Manda, Juan de Dios Leidonde, and Manuel Abeso; together in the laboratory we were, together in friendship we are, and together in success we will be,
9. David Deng Wei, Mina Chen Nana and Joe Lin Hao, I will never forget your daily advice and interest in all my academic and private life issues in China,
10. Martial Ndeffo, Edouard Akono, Ella Bella, Alain Ngoumkwa, Bernadin Dongsom, Rostand Nguo, and Lucien Tamno, for their constant friendship,
11. The International Students Office of South China Agricultural University,
12. All foreign students in South China Agricultural University, South China University of Technology, Sun Yat-Sen University, and Southern Medical University.
13. The National Natural Science Foundation of China (30671221), the Specialized Research Fund for the Doctoral Program of Higher Education of China (4100-C08023), and the Guangdong Provincial Nature Science Foundation (8151064201000017).

References

Adams P, De Kimpe N G. Formation of pyrazines and 2-acetyl-1-pyrroline by *Bacillus cereus*. Food Chemistry, **2007**, 101: 1230-1238.

Ahn S N, Bollich C N, Tanksley S D. RFLP tagging of a gene for aroma in rice. Theoretical and Applied Genetic, **1992**, 84: 825-828.

Apintanapong M, Noomhorm A. The use of spray drying to microencapsulate 2-acetyl-1-pyrroline, a major flavor component of aromatic rice. International Journal of Food Science and Technology, **2003**, 38: 95-102.

Arvisenet G, Le Bail P, Voilley A et al. Influence of physicochemical interactions between amylose and aroma compounds on the retention of aroma in food-like matrices. Journal of Agricultural and Food Chemistry, **2002**, 50: 7088-7093.

Awasthi, L. P.; Sharma, A. K.; Maurya, D. M. Histological variation among aromatic rice. International Rice Research Institute Notes, **1997**, 22: 33-34.

Bates L S, Waldren R P, Teare I D. Rapid determination of free proline for water-stress studies. Plant and soil, **1973**, 39: 205-207.

Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and applicable to acrylamide gels. Annals of Biochemistry, **1971**, 44: 276-287.

Bergman C J, Delgado J T, Bryant R et al. Rapid gas chromatographic technique for quantifying 2-acetyl-1-pyrroline and hexanal in rice (*Oryza sativa* L.). Cereal Chemistry, **2000**, 77: 454-458.

Berner D K, Hoff B J. Inheritance of scent in American long grain rice. Crop Science, **1986**, 26: 876-878.

Bett-Garber K L, Champagne E T, Ingram D A et al. Influence of water to rice ratio on cooked rice flavor and texture. Cereal Chemistry, **2007**, 84: 614-619.

Bhattacharjee P, Ranganathan T V, Singhal R S et al. Comparative aroma profiles using supercritical carbon dioxide and Likens-Nickerson extraction from a commercial brand of Basmati rice. Journal of the Science of Food and Agriculture, **2003**, 83: 880-883.

Bhattacharjee, P.; Singhal, R. S.; Kulkarni, P. R. Basmati rice: a review. Journal of the Science of Food and Agriculture, **2002**, 37: 1-12.

Bocchi S, Sparaciono A C, Sciorati F et al. Effect of soil characteristics on aromatic quality of rice. Informazioni Agrario, **1997**, 53: 60-62.

Bond J A, Walker T W, Ottis B V et al. Rice seeding and nitrogen rate effects on yield and yield components of two rice cultivars. Agronomy Journal, **2008**, 100: 393-397.

Bourgis F, Guyot R, Gherbi H et al. Characterization of the major fragrance gene from an aromatic japonica rice and analysis of its diversity in Asian cultivated rice. Theoretical and Applied Genetic, **2008**, 117: 353-368.

Bradbury L M T, Gillies S A, Brushett D J et al. Inactivation of an aminoaldehyde dehydrogenase is responsible for fragrance in rice. Plant Molecular Biology, **2008a**, 68: 439-449.

Bradbury L M T, Henry R J, Qingsheng J et al. A perfect marker for fragrance genotyping rice. Molecular Breeding, **2005**, 16: 279-283.

Bradbury M L T, Henry R J, Waters D L E. Flavor development in rice. *In* Biotechnology in Flavor Production. Havkin-Frenkel D, Belanger F C Eds, Oxon, UK: Blackwell Publishing Ltd, **2008b**. pp 130-146.

Bradford M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annals of Biochemistry, **1976**, 72: 248-254.

Buege J A, Aust S D. Microsomal lipid peroxidation. Methods in Enzymology, **1978**, 52: 302-310.

Buttery R G, Juliano B O, Ling L C. Identification of rice aroma compound 2-acetyl-1-pyrroline in Pandan leaves. Chem. Industries, **1983**, 478.

Buttery R G, Ling L C, Juliano B O. 2-Acetyl-1-pyrroline and its use for flavoring foods. U.S. Patent, **1985**, 4: 522,838.

Buttery R G, Orts W J, Takeoka G R et al. Volatile flavor components of rice cakes. Journal of Agricultural and Food Chemistry, **1999**, 47: 4353-4356.

Buttery R G, Turnbaugh J G, Ling L C. Contribution of volatiles to rice aroma. Journal of Agricultural and Food Chemistry, **1988**, 36: 1006-1009.

Champagne E T. Rice aroma and flavor: a literature review. Cereal Chemistry, **2008**, 85: 445-454.

Chen S, Wu J, Yang Y et al. The fgr gene responsible for rice fragrance was restricted within 69 kb. Plant Science, **2006**, 171: 505-514.

Chen S, Yang Y, Shi W et al. Badh2, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. Plant Cell, **2008**, 20: 1850-1861.

Chung H Y, Ma W C J, Kim J S et al. Odor-active headspace components in fermented red rice in the presence of a *Monascus* species Journal of Agricultural and Food Chemistry, **2004**, 52: 6557-6563.

Costello J P Henschke P A. Mousy off-flavor of wine: precursors and biosynthesis of the causative N-heterocycles 2-ethyltetrahydropyridine, 2-acetyltetrahydropyridie, and 2-acetyl-1-pyrroline by *Lactobacillus hilgardii* DSM20176. Journal of Agricultural and Food Chemistry, **2002**, 50: 7079-7087.

Crowhurst D G, Creed P G. Effect of cooking method and variety on the sensory quality of rice. Food Service Technology, **2001**, 1: 133-140.

Czerny M, Christlbauer M, Christlbauer M et al. Re-investigation on odor thresholds of key food aroma compounds and development of an aroma language based on odor qualities of defined aqueous odorant solutions. European Food Research and Technology, **2008**, 28: 265-273.

De Kimpe N G, Stevens C V, Keppens M A. Synthesis of 2-acetyl-1-pyrroline, the principal rice flavor component. Journal of Agricultural and Food Chemistry, **1993**, 41: 1458-1461.

Duan M Y, Li G X, Tian H et al. Effects of regulators on aroma content and physiological characteristics of aromatic rice. Journal of South China Agricultural University (Chinese) **2009**, 30: 1-3.

Dutta R K, Lahiri B P, Khanam S et al. Aroma synthesis in Basmati rice in relation to temperature and nitrogen. Indian Journal of Plant Physiology, **1999**, 4: 215-218.

Fitzgerald M A, Hamilton N R S, Calingacion M N et al. Is there a second fragrance gene in rice? Plant Biotechnology Journal, **2008**, 6: 416-423.

George S P, Bastian D, Radhakrishan N V et al. Evaluation of aromatic rice varieties in Wayanad, Kerala. Journal of Tropical Agriculture, **2005**, 43: 67-69.

Ghiasvand A R, Setkova L, Pawliszyn J. Determination of flavor profile in Iranian fragrant rice samples using cold-fiber rice SPME–GC–TOF–MS. Fragrance Journal, **2007**, 22: 377-391.

Ghosh S, Penterman J N, Madey E et al. Paclobutrazol: its physiological effects and biochemical functions during plant stress. Physiology and Molecular Biology of Plants, **2003**, 9: 163-173.

Glaszmann J C. Isozymes and classification of Asian rice varieties. Theoretical and Applied Genetic, **1987**, 74: 21-30.

Grimm C C, Bergman C, Delgado J T et al. Screening for 2-acetyl-1-pyrroline in the headspace of rice using SPME/ GC-MS. Journal of Agricultural and Food Chemistry, **2001**, 49: 245-249.

Grosch W, Schieberle P. Flavor of cereal products: a review. Cereal Chemistry, **1997**, 74: 91-97.

Gu J. Identification of 2-acetylpyridine in Xiangjing-8618 rice and in Yahonkaoluo leaves. Food Chemistry, **2002**, 78: 163-166.

Hien N L, Yoshihashi T, Sarhadi W A et al. Sensory test for aroma and quantitative analysis of 2-acetyl-1-pyrroline in Asian aromatic rice varieties. Plant Production Science, **2006**, 9: 294-297.

Hofmann T, Schieberle P. New and convenient syntheses of the important roasty, popcorn-like smelling food aroma compounds 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine from their corresponding cyclic α-amino acids. Journal of Agricultural and Food Chemistry, 1**998**, 46: 616-619.

Hori K, Purboyo R B R A, Jo M et al. Comparison of sensory evaluation of aromatic rice by consumers in East and South-east Asia. Journal of Consumer Studies and Home Economics, **1994**, 18: 135-139.

Hou F F, Hong M C, Song S. Effect of soil texture on rice quality. Taichung District Agricultural Improvement Station, **1988**, 13: 242-248.

Huang S C. Soil characteristics influence on aromatic rice in Hunan. Hunan Agricultural Science (Chinese), **1990**, 4: 37-40.

Huang T C, Teng C S, Chang J L et al. Biosynthetic mechanism of 2-acetyl-1-pyrroline and its relationship with pyrroline-5-carboxylic acid and methylglyoxal in aromatic rice callus. Journal of Agricultural and Food Chemistry, **2008**, 56: 7399-7404.

Ishitani K, Fushimi C. Influence of pre- and post-harvest conditions on 2-acetyl-1-pyrroline concentration in aromatic rice. The Koryo, **1994**, 183: 73-80.

Itani T. Agronomic characteristics of aromatic rice cultivars collected from Japan and other countries. Japanese Journal of Crop Science, **2002**, 71: 67-75.

Itani T, Tamaki M, Hayata Y et al. Variation of 2-acetyl-1-pyrroline concentration in aromatic rice grains collected in the same region in Japan and factors affecting its concentration. Plant Production Science, **2004**, 7: 178-183.

Jezussek M, Juliano B O, Schieberle P. Comparison of key aroma compounds in cooked brown rice varieties based on aroma extract dilution analysis. Journal of Agricultural and Food Chemistry, **2002**, 50: 1101-1105.

Jin Q S, Waters D, Cordeiro G M et al. A single nucleotide polymorphism (SNP) marker linked to the fragrance gene in rice (*Oryza sativa* L.). Plant Science, **2003**, 165: 359-364.

Jouquand C, Ducruet V, Le Bail P. Formation of amylose complexes with C6-aroma compounds in starch dispersions and its impact on retention. Food Chemistry, **2006**, 96: 461-470.

Juliano B O, Villareal C P. Grain Quality Evaluation of World Rices. Manila, Philippines: International Rice Research Institute, **1993**. pp 205.

Kandpal R P, Vaidyanathan C S, Udaya K M et al. Alteration in the activities of the enzymes of proline metabolism in ragi (*Eleusine coracana*) leaves during water stress. Journal of Biosciences, **1981**, 3: 361-370.

Laguerre M, Mestres C, Davrieux F et al. Rapid discrimination of scented rice by solid phase micro-extraction, mass spectrometry, and multivariate analysis used as a mass sensor. Journal of Agricultural and Food Chemistry, **2007**, 55: 1077-1083.

Laksanalamai V, Ilangantileke S. Comparison of aroma compound (2-acetyl-l-pyrroline) on leaves from pandan (*Pandanum amaryllifolius*) and Thai fragrant rice (Khao Dawk Mali-105). Cereal Chemistry, **1993**, 70: 381-384.

Lam H S, Proctor A. Milled rice oxidation volatiles and odor development. Journal of Food Sciience, **2003**, 68: 2676-2681.

Laohakunjit N, Kerdchoechuen O. Aroma enrichment and the change during storage of non-aromatic milled rice coated with extracted natural flavor. Food Chemistry, **2007**, 101: 339-344.

Lee J I, Kim Y D, Shin J C et al. Effect of ripening temperature on quality, appearance and chemical quality characteristics of rice grain. RDA Journal of Agricultural Sciences, **1996**, 38: 1-9.

Lin C, Hsieh T, Hoff B. Identification and quantification of the "popcorn"-like aroma in Louisiana aromatic Delia rice (*Oryza sativa* L.). Journal of Food Science, **1990**, 55: 1466-1467.

Lorieux M, Petrov M, Huang N et al. Aroma in rice: genetic analysis of a quantitative trait. Theoretical and Applied Genetic, **1996**, 93: 1145-1151.

MacAdam J W, Nelson C J, Sharp R E. Peroxidase activity in the leaf elongation zone of tall fescue. Plant Physiology, **1992**, 99: 872-879.

Mahatheeranont S, Keawsaard S, Dumri K. Quantification of the rice aroma compound, 2-acetyl-l-pyrroline, in uncooked Khao Dawk Mali 105 brown rice. Journal of Agricultural and Food Chemistry, **2001**, 49: 773-779.

Mander L N. Twenty years of gibberellins research. Natural Product Reports, **2003**, 20: 49-69.

Maraval I, Mestres C, Pernin K et al. Odor-active compounds in cooked rice cultivars from Camargue (France) analyzed by GC-O and GC-MS. Journal of Agricultural and Food Chemistry, **2008**, 56: 5291-5298.

McCauley G N, Way M O. Drain and harvest timing effects on rice grain drying and whole-milled grain. Field Crops Research, **2002**, 74: 163-172.

Meullenet J F, Marks B P, Griffin K et al. Effects of rough rice drying and storage conditions on sensory profiles of cooked rice. Cereal Chemistry, **1999**, 76: 438-486.

Meullenet J F, Marks B P, Hankins J A et al. Sensory quality of cooked long-grain rice as affected by rough rice moisture content, storage temperature, and storage duration. Cereal Chemistry, **2000**, 77: 259-263.

Monsoor M A, Proctor A. Effect of water washing on the reduction of surface total lipids and FFA on milled rice. Journal of the American Oil Chemists' Society, **2002**, 79: 867-870.

Monsoor M A, Proctor A. Volatile component analysis of commercially milled head and broken rice. Journal of Food Science, **2004**, 69: 632-636.

Muench P, Hofmann T, Schieberle P. Comparison of key odorants generated by thermal treatment of commercial and self prepared yeast extracts: influence of the amino acid composition on odorant formation. Journal of Agricultural and Food Chemistry, **1997**, 45: 1338-1344.

Nadaf A B, Krishnan S, Wakte K V. Histochemical and biochemical analysis of major aroma compound (2-acetyl-1-pyrroline) in basmati and other scented rice (*Oryza sativa* L.). Current Science, **2006**, 92: 1533-1536.

Paule C M, Powers J J. Sensory and chemical examination of aromatic and non-aromatic rices. Journal of Food Science, **1989**, 54: 343-346.

Pawliszyn J. Solid Phase Micro-extraction: Theory and Practice. New York, USA: Wiley-VCH, **1997**. pp 12-264.

Petrov M, Danzart M, Giampaoli P et al. Rice aroma analysis: discrimination between a scented and non-scented rice. Science des Aliments, **1996**, 16: 347-360.

Piggott J R, Morrison W R, Clyne J. Changes in lipids and in sensory attributes on storage of rice milled to different degrees. International Journal of Food Science and Technology, **1991**, 26: 615-628.

Pinson S R M. Inheritance of aroma in six rice cultivars. Crop Science, **1994**, 34: 1151-1157.

Schieberle P. Primary odorants in popcorn. Journal of Agricultural and Food Chemistry, **1991**, 39: 1141-1144.

Schieberle P. The role of free amino acids present in yeast as precursors of the odorants 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine in wheat bread crust. Zeitschrift für Lebensmittel-Untersuchung und –Forschung, **1990**, 191: 206-209.

Sekhar B P S. Amino acid profiles in some scented rice varieties. Theoretical and Applied Genetic, **1982**, 62: 35-37.

Sheehy J E, Ferrer A B, Mitchell P L. Decline in rice grain yields with temperature: models and correlations can give different estimates. Field Crops Research, **2006**, 98: 151-156.

Singh R K, Singh U S, Khush G S. Aromatic rices. New Hampshire, USA: Science Publishers, Inc, **2000**. pp 292.

Sirisoontaralak P, Noomhorm A. Changes to physicochemical properties and aroma of irradiated rice. Journal of Stored Products Research, **2006**, 42: 264-276.

Smith C W, Dilday R H. Rice: origin, history, technology and production. Hoboken, New Jersey: John Wiley and Sons, Inc, **2003**. pp 642.

Srisawas W, Jindal V K. Sensory evaluation of cooked rice in relation to water-to-rice ratio and physicochemical properties. Journal of Texture Studies, **2007**, 38: 21-41.

Sriseadka T, Wongpornchai S, Kitsawatpaiboon P. Rapid method for quantitative analysis of the aroma impact compound, 2-acetyl-1-pyrroline, in fragrant rice using automated headspace gas chromatography. Journal of Agricultural and Food Chemistry, **2006**, 54: 8183-8189.

Sudria C, Palazon J, Cusido R et al. Effect of benzyladenine and indolebutyric acid on ultrastructure, glands formation, and essential oil accumulation in *Lavandula dentata* plantlets. Biologia Plantarum, **2001**, 44: 1-6.

Suwanarit A, Kreetapirom S, Buranakarn S et al. Effect of nitrogen fertilizer on grain qualities of Khao Dawk Mali 105. Kasetsart Journal of Natural Sciences, **1996**, 30: 458-474.

Suzuki Y, Ise K, Li C et al. Volatile components in stored rice (*Oryza sativa* L.) varieties with and without lipoxygenase-3 in seeds. Journal of Agricultural and Food Chemistry, **1999**, 47: 1119-1124.

Sweeney M, McCouch S. The complex history of the domestication of rice. Annals of Botany (Lond), **2007**, 100: 951-957.

Tanchotikul U, Hsieh T C Y. An improved method for quantification of 2-acetyl-l-pyrroline, a popcorn-like aroma, in aromatic rice by high-resolution gas chromatography/mass spectrometry/selected ion monitoring. Journal of Agricultural and Food Chemistry, **1991**, 39: 944-947.

Tang X R, Wu M. Effects of application of zinc, iron and lanthanum on contents of aroma in brown rice and proline in flag leaf of aromatic rice. Hybrid Rice (Chinese), **2006**, 21: 69-72.

Tava A, Bocchi S. Aroma of cooked rice (*Oryza sativa* L.): Comparison between commercial Basmati and Italian line B5-3. Cereal Chemistry, **1999**, 76: 526-529.

Thimmaraju R, Bhagyalakshmi N, Narayan M S et al. *In vitro* culture of *Pandanus amaryllifolius* and enhancement of 2-acetyl-l-pyrroline, the major flavoring compound of aromatic rice, by precursor feeding of L-proline. Journal of the Science of Food Agriculture, **2005**, 85: 2525-2534.

Tulyathan V, Srisupattarawanich N, Suwanagul A. Effect of rice flour coating on 2-acetyl-1-pyrroline and *n*-hexanal in brown rice cv. Jao Hom Supanburi during storage. Postharvest Biology and Technology, **2008**, 47: 367-372.

Verschueren K. *Handbook of environmental data on organic chemicals*. 4th ed. Vols. 1 and 2. New York: John Wiley and Sons, **2001**. pp. 28, 1221-1222.

Widjaja R, Craske J D. Wootton, M. Changes in the volatile components of paddy, brown and white fragrant rice during storage. Journal of the Science of Food Agriculture, **1996a**, 71: 218-224.

Widjaja R, Craske J D, Wootton M. Comparative studies on volatile components of non-fragrant and fragrant rices. Journal of the Science of Food Agriculture, **1996b**, 70: 151-161.

Wilkie K, Wootton M, Paton J E. Sensory testing of Australian fragrant, imported fragrant and non-fragrant rice aroma. International Journal of Food Properties, **2004**, 7: 27-36.

Wongpornchai S, Dumri K, Jongkaewwattana S et al. Effects of drying methods and storage time on the aroma and milling quality of rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105. Food Chemistry, **2004**, 87: 407-414.

Yang, J. C. Some critical considerations on rice high-yielding breeding in China. Frontiers of Agriculture in China, **2007**, 1: 389-396.

Yang D S, Lee K S, Jeong O Y et al. Characterization of volatile aroma compounds in cooked black rice. Journal of Agricultural and Food Chemistry, **2008a**, 56: 235-240.

Yang D S, Lee K S, Kim K J et al. Site of origin of volatile compounds in cooked rice. Cereal Chemistry, **2008b**, 85: 591-598.

Yang D S, Shewfelt R L, Lee K S et al. Comparison of odor-active compounds from six distinctly different rice flavor types. Journal of Agricultural and Food Chemistry, **2008c**, 56: 2780-2787.

Yang W, Peng S, Laza R C et al. Grain yield and yield attributes of new plant type and hybrid rice. Crop Science, **2007**, 47: 1393-1400.

Yau N J N, Liu T T. Instrumental and sensory analysis of volatile aroma of cooked rice. Journal of Sensory Studies, **1999**, 14: 209-233.

Yoshihashi T N, Nguyen T T H, Surojanametakul V et al. Effect of storage conditions on 2-acetyl-1-pyrroline content in aromatic rice variety, Khao Dawk Mali 105. Journal of Food Science, **2005**, 70: 34-37.

Yoshihashi T N, Nguyen T T H, Inatomi H. Precursors of 2-acetyl-l-pyrroline, a potent flavor compound of an aromatic rice variety. Journal of Agricultural and Food Chemistry, **2002,** 50: 2001-2004.

Yoshihashi T. Quantitative analysis on 2-acetyl-1-pyrroline of an aromatic rice variety by stable isotope dilution method and model studies on its formation during cooking. Journal of Food Science, **2002**, 67: 619-622.

Yoshihashi T, Nguyen T T H, Kabaki N. Area dependency of 2-acetyl-1-pyrroline content in an aromatic rice variety, KDML105. Japan Agricultural Research Quaterly, **2004**, 38: 105-109.

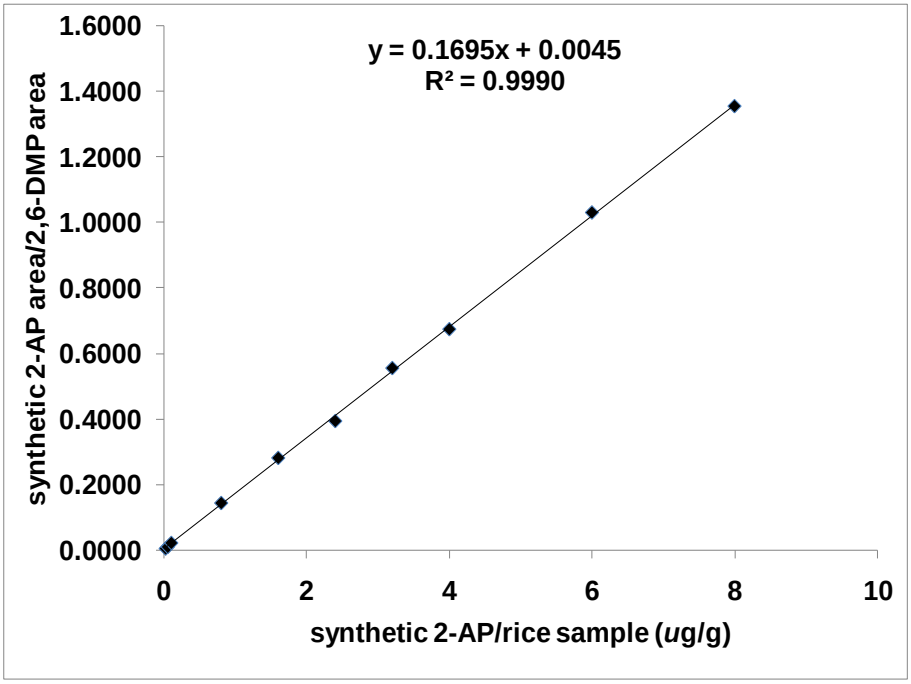
Zeng L H, Shannon M C. Effects of salinity on grain yield and yield components of rice at different seeding densities. Agronomy Journal, **2000**, 92: 418-423.

Zeng Z, Zhang H, Chen J Y et al. Flavor volatiles of rice during cooking analyzed by modified headspace SPME/GC-MS. Cereal Chemistry, **2008**, 85: 140-145.

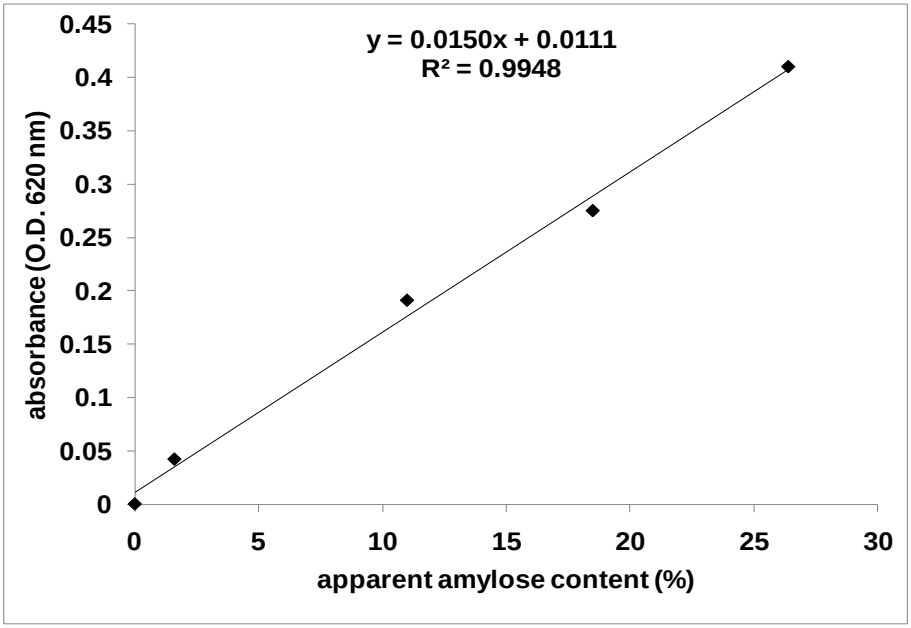
Zhou M, Robards K, Glennie-Holmes M et al. Analysis of volatile compounds and their contribution to flavor in cereals. Journal of Agricultural and Food Chemistry, **1999**, 47: 3941-3953.

Appendix

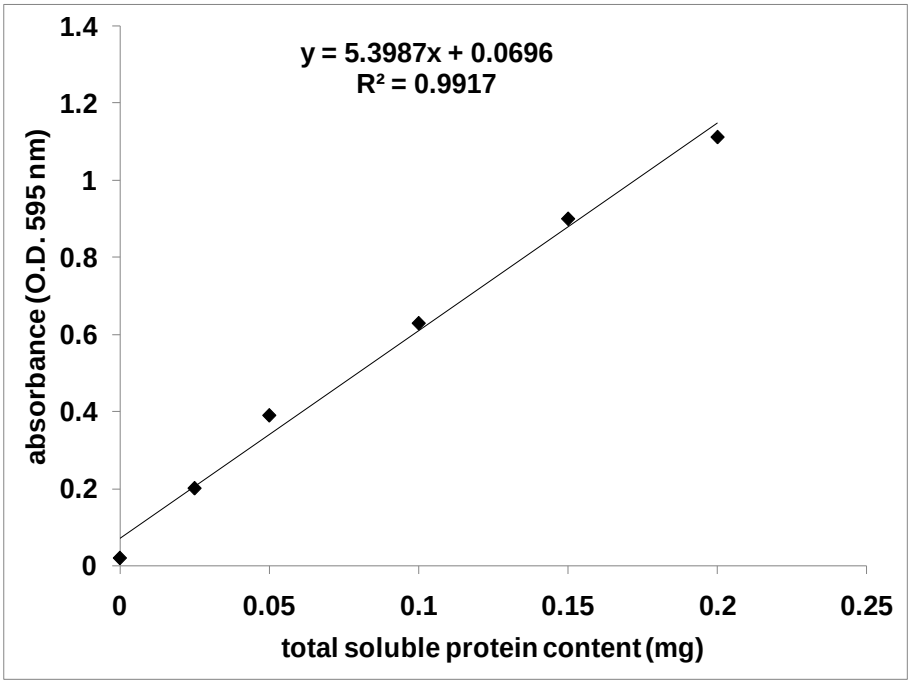
Appendix 1. Standard curve for 2-acetyl-1-pyrroline content determination



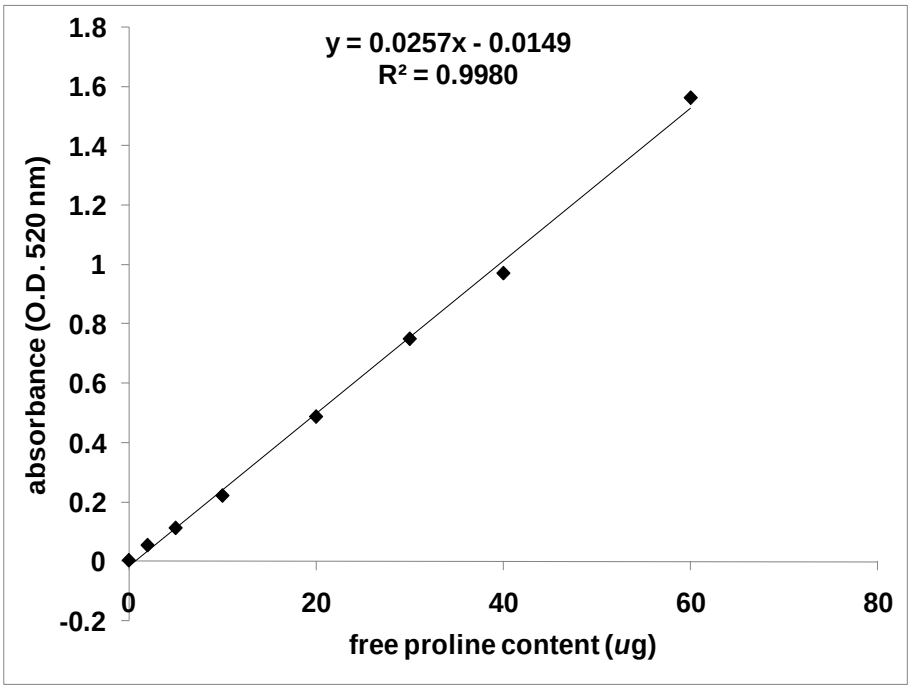
Appendix 2. Standard curve for amylose content determination



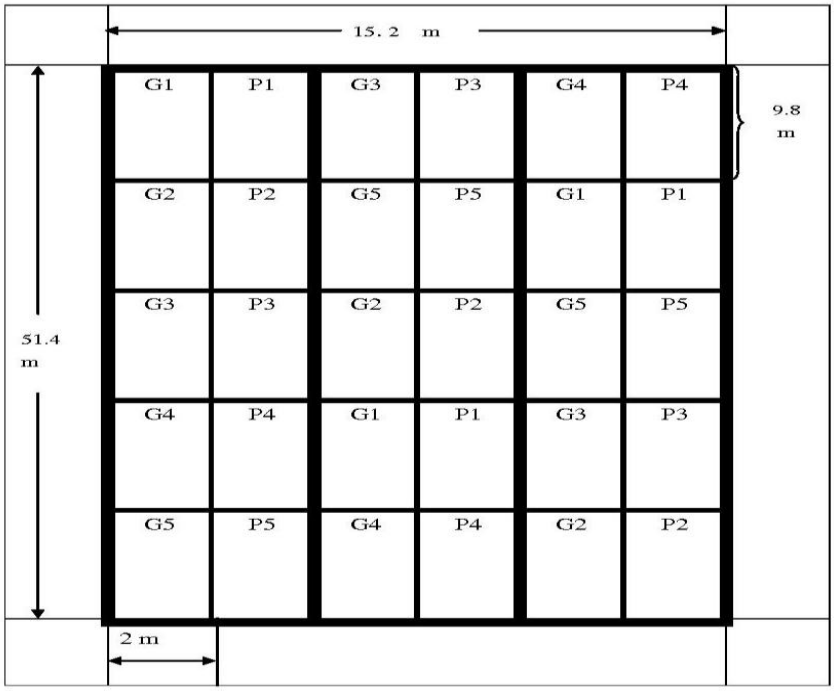
Appendix 3. Standard curve for total soluble protein content determination



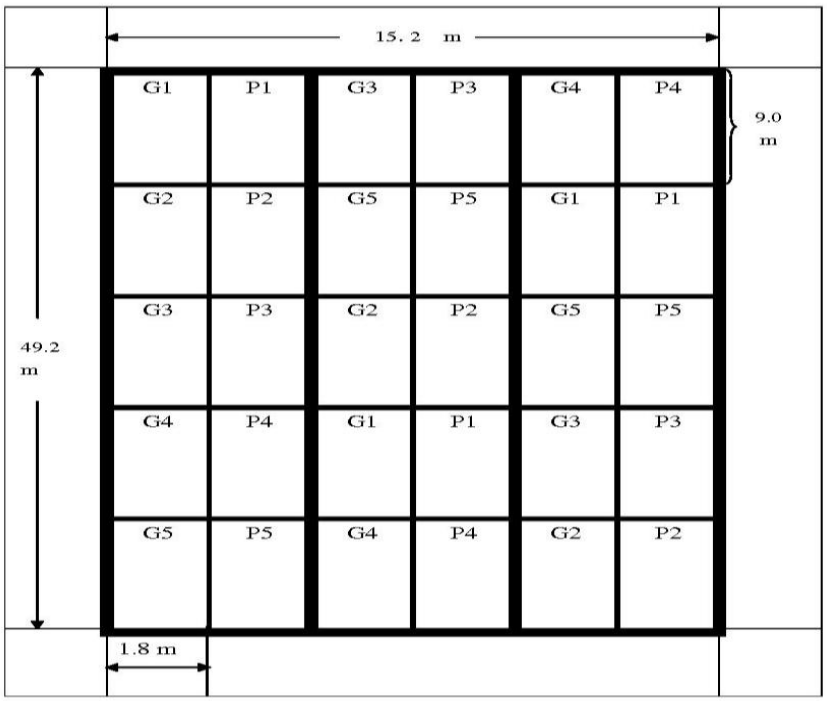
Appendix 4. Standard curve for free proline content determination



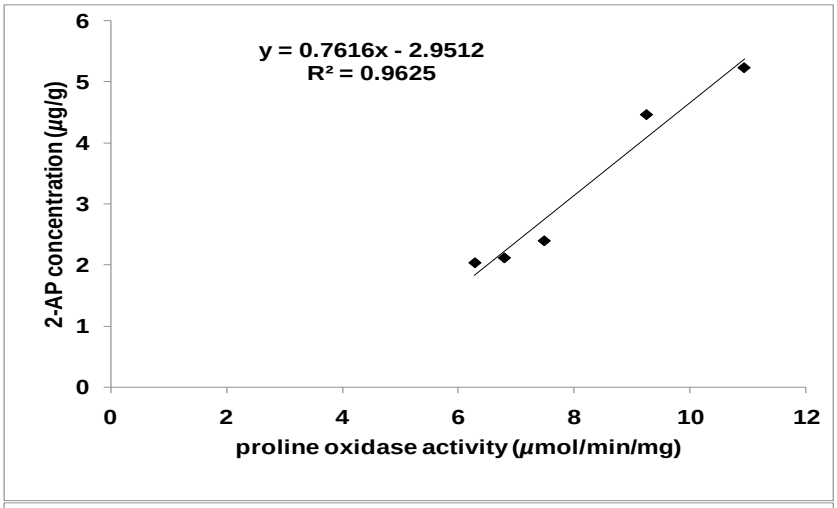
Appendix 5. Paddy field design for planting density study: G = Guixiangzhan, P = Peizaruanxiang, 1 = 20 cm × 15 cm, 2 = 20 cm × 20 cm, 3 = 20 cm × 25 cm, 4 = 20 cm × 30 cm, 5 = 20 cm × 35 cm

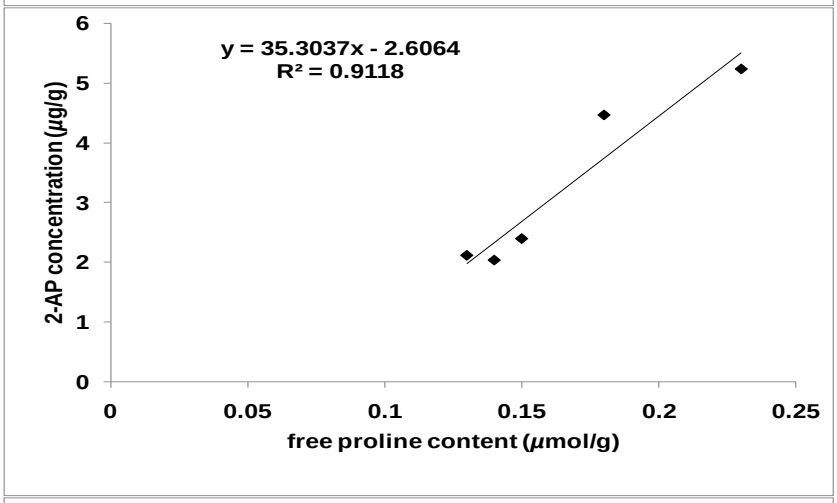


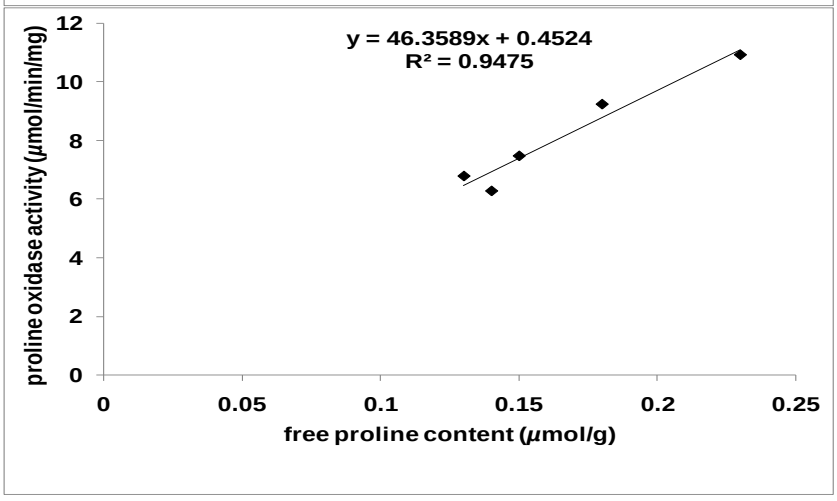
Appendix 6. Paddy field design for plant growth regulators study: G = Guixiangzhan, P = Peizaruanxiang, 1 = Control treated with distilled water (CTR), 2 = Gibberellic acid (GA3), 3= 3-Indole acetic acid (IAA), 4 = Paclobutrazol (PBZ), 5 = Combined paclobutrazol, proline and zinc chloride (PPZ)



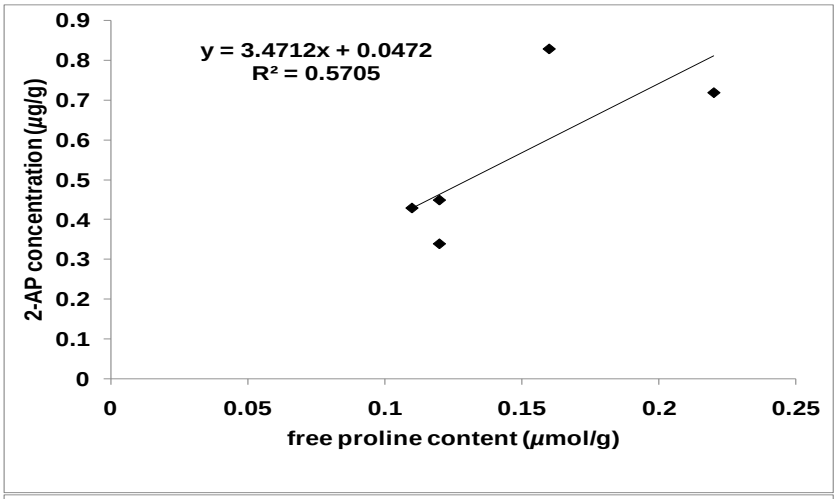
Appendix 7. Plotted linear regression between proline content, proline oxidase activity and 2-acetyl-1-pyrroline content in Guixiangzhan

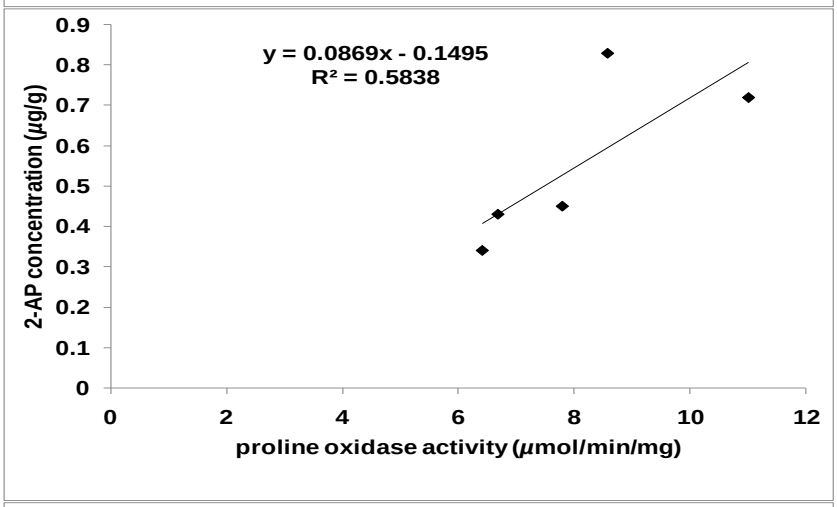


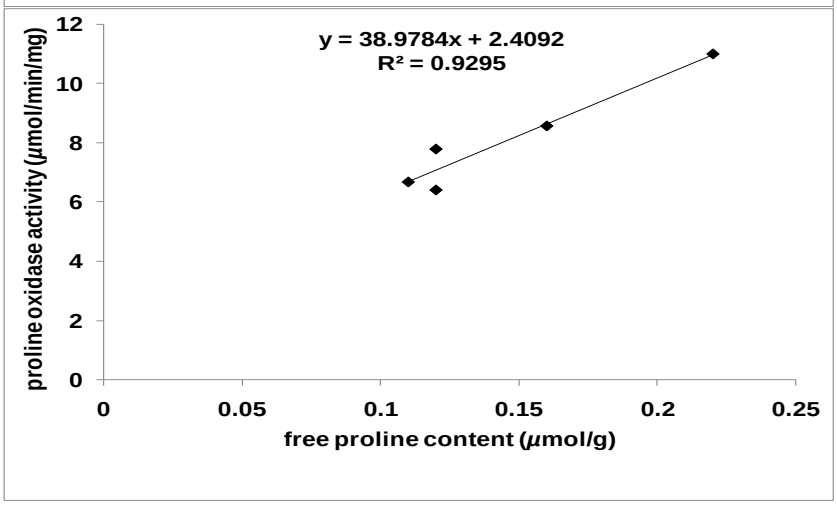




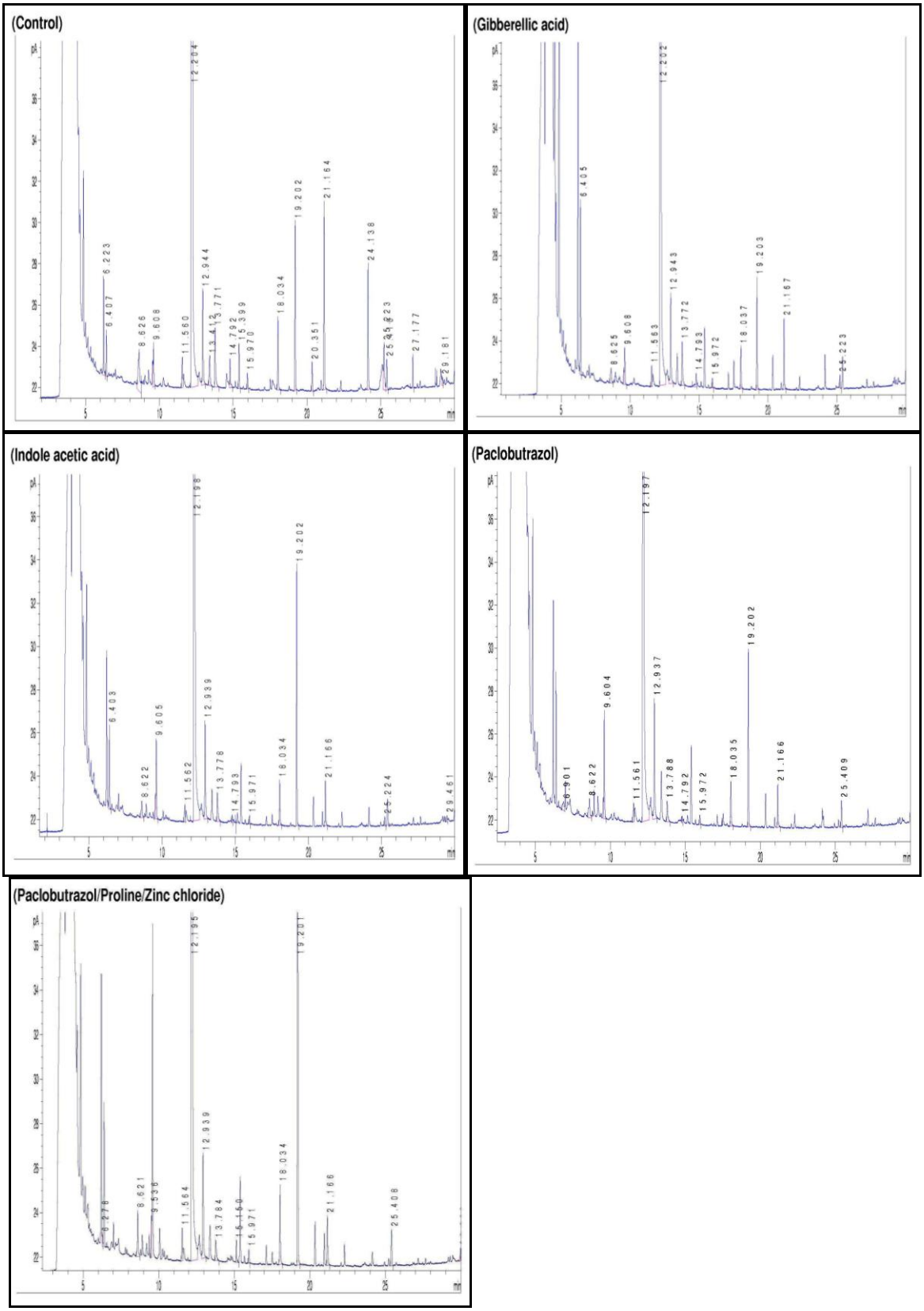
Appendix 8. Plotted linear regression between proline content, proline oxidase activity and 2-acetyl-1-pyrroline content in Peizaruanxiang



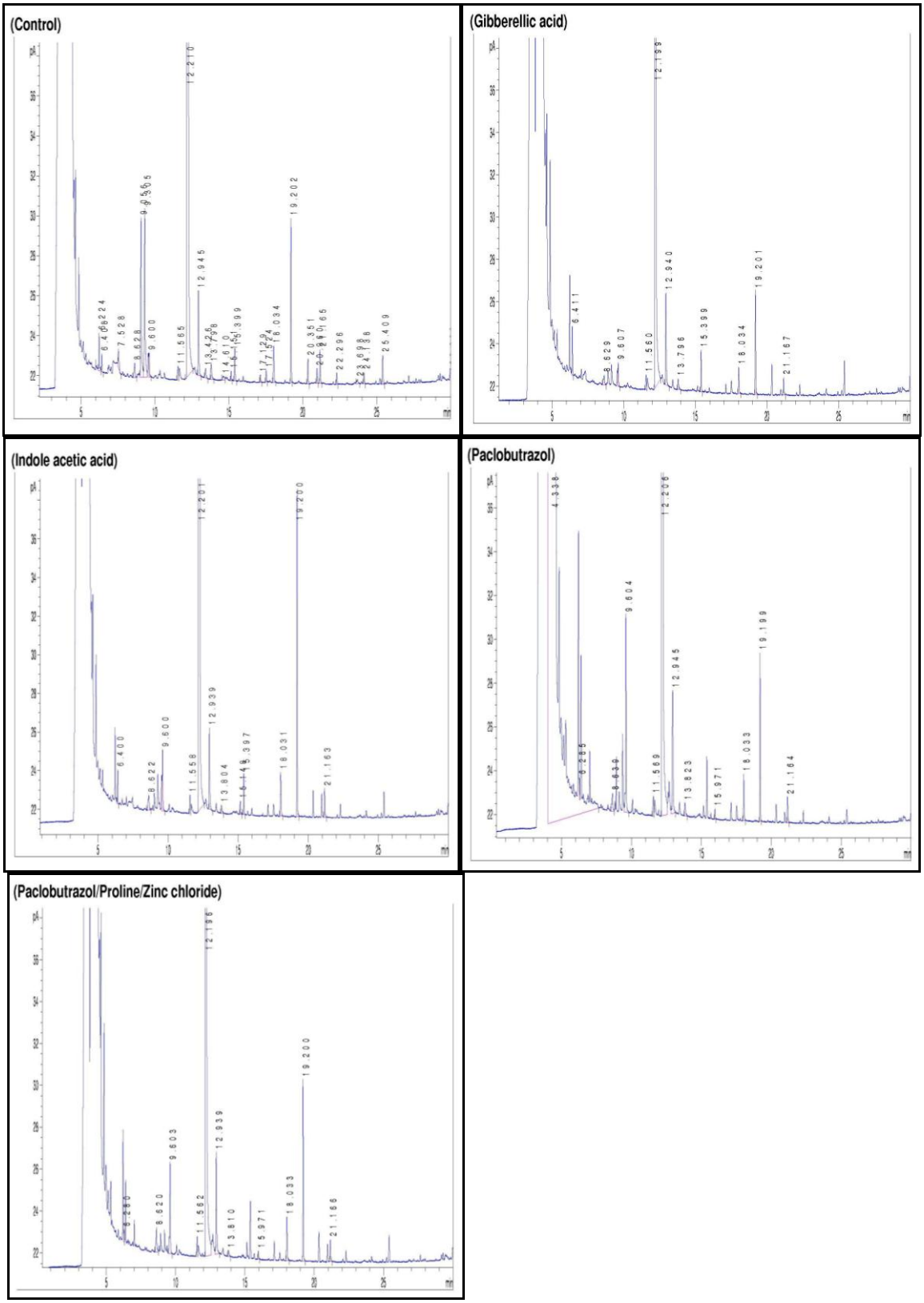




Appendix 9: Typical gas chromatographic profile of headspace volatiles in Guixiangzhan brown rice seeds after treatment with growth regulators



Appendix 10: Typical gas chromatographic profile of headspace volatiles in Peizaruanxiang brown rice seeds after treatment with growth regulators



List of tables

List of figures

List of appendix

Publication progress

Goufo P, Duan M, Wongpornchai S, Tang X R. 2010. Some Factors Affecting Concentration of the Aroma Compound 2-Acetyl-1-pyrroline in two Fragrant Rice Cultivars Grown in South China. *Frontiers of Agriculture in China* 4 (1): 1-9.

Goufo P, Wongpornchai S, Tang X R. 2010. Decrease in Rice Aroma after Application of Growth Regulators. *Agronomy for Sustainable Development* 30 (2):

Goufo P, Wongpornchai S, Tang X R. 2010. Appropriate Set of Pre- and Post-Harvest Treatments for Obtaining High Levels of 2-Acetyl-1-pyrroline in New Fragrant Rice Cultivars Developed in China. *Journal of Biotechnology* 148 (S1):

Abbreviations

2,4,6-TMP: 2,4,6-trimethylpyridine

2,6-DMP: 2,6-dimethylpyridine

2-AP: 2- acetyl-1-pyrroline

AEDA: aroma extract dilution analysis

ANOVA: analysis of variance

BSA: bovine serum albumin

CTR: control

DAH: days after heading

DAS: days after sowing

DAT: days after transplanting

DHS: dynamic headspace

EDTA: ethylene diamine tetraacetic acid

EI-MS: electron ionisation-mass spectrometer

EM: electromagnetic

FFA: free fatty acid

Fgr: fragrance

FID: flame ionization detector

GA3: gibberellic acid

GC: gas chromatography

GC-O: gas chromatography-olfactometry

HP-5MS: (5%-phenyl)-methylpolysiloxane

HPLC: High performance liquid chromatography

HS: headspace

HS-SPME: headspace solid-phase micro-extraction

IAA: 3-indole acetic acid

IR: infrared

IUPAC: International Union of Pure and Applied Chemistry

KDML 105: Jasmine Khao Dawk Mali 105

LOX-3: Lipoxygenase 3

LTPRI: linear temperature programme retention index

MDA: malondialdehyde

MS: mass spectrometry

MSE: mean square of error

MW: molecular weight

NBT: nitroblue tetrazolium

NIST-MSL: National Institute of Standards and Technology mass spectral library

NMR: nuclear magnetic resonance

NPD: nitrogen phosphorous detector

OAV: odor activity value

OD: optical density

PBZ: paclobutrazol

PDMS: polydimethylsioxane

POD: peroxidase

POX: proline oxidase

PPZ: combined paclobutrazol, proline, and zinc chloride

PRO: proline content

PTFE: Polytetrafluoroethylene

PVPP: polyvinylpolypyrrolidone

RFLP: Restriction fragment length polymorphism

RI: Kovats retention index

RT: retention time

SCAU: South China Agricultural University

SE: direct solvent extraction

SFE: supercritical fluid extraction

SHS: static headspace

SID: stable isotope dilution

SNP: single nucleotide polymorphism

SOD: superoxide dismutase

SPME: solid-phase micro-extraction

SSA: anhydrous sulfosalicylic acid

SSDE: simultaneous steam distillation and extraction

STD: standard

TBA: thiobarbituric acid

TCA: trichloroacetic acid

TIC: total ion chromatogram

TMS: tetramethylsilane

TOF-MS: time-of-flight-mass spectrometry

学位论文原创性声明

本人郑重声明：所呈交的论文是本人在导师的指导下独立进行研究所取得的研究成果。除了文中特别加以标注引用的内容外，本论文不包含任何其他个人或集体已经发表或撰写的成果作品。对本文的研究做出重要贡献的个人和集体，均已在文中以明确方式标明。本人完全意识到本声明的法律后果由本人承担。

作者签名：Philip Fong　　日期：2010 年 6 月 2 日

学位论文版权使用授权书

本学位论文作者完全了解学校有关保留、使用学位论文的规定，即：研究生在校攻读学位期间论文工作的知识产权单位属华南农业大学。学校有权保存并向国家有关部门或机构送交论文的复印件和电子版，允许学位论文被查阅(除在保密期内的保密论文外)；学校可以公布学位论文的全部或部分内容，可以允许采用影印、缩印或其它复制手段保存、汇编学位论文。本人电子文档的内容和纸质论文的内容相一致。

本学位论文属于：

□保密，在_____年解密后适用本授权书。

□不保密。

学位论文全文电子版提交后：

□同意在校园网上发布，供校内师生和与学校有共享协议的单位浏览。

(请在以上相应方框内打“√”)

本人签名：Philip Fong　　日期：2010/6/2

导师签名：_____　　日期　2010.6.8

华南农业大学

博士研究生毕业（学位）论文

答辩及学位授予审批材料

学　　号：	**2007100011**
申请人姓名：	**GOUFO PIEBIEP**
学院名称：	农　学　院
专业名称：	作物栽培学与耕作学
研究方向：	作物品质生理与化学调控
学科门类：	农　　学
指导教师姓名、职　　称：	唐湘如 教　　授

填表日期　　2010 年　04　月　26　日

填 表 注 意 事 项

1、审批材料各表均一式2份，均为原件，各一份成绩单附在第3、4页之间，审批材料一份入研究生本人人事档案，另一份送学校综合档案室，作为永久保存的材料。请本着认真负责的态度填写此材料，保持材料的完整、规范、整洁。

2、本表可用碳素、蓝黑墨水钢笔填写；也可计算机打印，但签名处须用碳素、蓝黑墨水钢笔书写。

3、封面及各表中的专业、研究方向均应与培养办所发成绩单上的专业及研究方向一致。

4、除第1页《博士研究生学位申请表》背面留空，其余页均用A4纸双面打印，装订成册。

博士研究生学位申请表

（着重概述在校期间政治思想表现、完成培养计划情况、成绩是否合格、论文工作完成情况等。）

Chinese political and economical reforms have led to an excellent and friendly environment for international students in China. It is in such a context that I conducted over 3 consecutive years my research in South China Agricultural University in Guangzhou, Guangdong province, P.R. China with the aim of obtaining a doctorate degree. Enrolled in 2007, I successfully fulfill all the requirements needed by the University for the Award of a PhD degree in Crop Science, option Plant Physiology and Biochemistry. Specifically I have:

1. Completed my coursework consisting of 6 subjects and 13 credits in the first and second terms of the academic year 2007-2008;
2. Conducted my research under field and laboratory conditions over a time period of two years that is during the second term of 2008, the first term of 2009, the second term of 2009 and the first term of 2010. The field work consisted of four distinct experiments (planting density, planting season with an emphasis on ripening temperature, harvesting time and application of growth regulators). The laboratory work included evaluation of physical and chemical characteristics of rice grains and leaves obtained from the four pre-harvest treatments, following it through three post-harvest treatments (storage time, storage temperature, milling degree);
3. Published two knowledgeable works in high impact journals from my research program, namely *Agronomy for Sustainable Development* and *Frontiers of Agriculture in China* and had my paper accepted for presentation during the 14th International Biotechnology Symposium and for publication in the *Journal of Biotechnology.*
4. Submitted my PhD thesis on March 2010, which was approved on April 2010 and defended on May 2010. The information related to my coursework and research including the relevant documents (transcript, research proposal, final dissertation, publications) is already in the archives of the postgraduate office concerned.

In witness whereof this letter is written to request for the grant of the diploma of Doctor of Philosophy (PhD).

申请人签名：

申请日期：2010 年 04 月 26 日

(注：本页背面请留空。)

姓名	GOUFO PIEBIEP	性别	男	出生日期	1980/03/23	身份证号	01101254
籍贯	CAMEROON	民族	CMR	入学年月	2007.9	政治面貌	无
专业名称	作物品质生理与化学调控			导师姓名、职称			唐湘如，教授
攻博方式（划“√”）	脱产（√ ）； 在职（ ）； 提前攻博（ ）						
工作单位及职务（在职）							
大学毕业院校	University of Yaoundé I, Cameroon	专业	Food Science and Technology	毕业年月	2001.6		
硕士毕业院校	University of Yaoundé I, Cameroon	专业	Plant Biochemistry and Pathology	毕业年月	2005.6		
攻博前获得最后学位	M.Sc in Biochemistry						

简历

起止日期	学习或工作单位	职称、职务
2007.9-2010.6	Department of Crop Science and Technology, SCAU, P.R. China	Doctoral Research Fellow
2008.9-2009.3	Department of Chemistry, Chiang Mai University, Thailand	Visiting Researcher
2004.6-2006.6	Department of Biochemistry, University of Yaoundé I, Cameroon	Graduate Teaching Assistant
2003.9-2005.6	Department of Biochemistry, University of Yaoundé I, Cameroon	Master Research Fellow
2003.12-2006.2	Department of Crop Protection, University of Dschang, Cameroon	Visiting Researcher
2001.9-2003.6	Department of Biochemistry, University of Yaoundé I, Cameroon	Research Fellow
1998.9-2001.6	Department of Biochemistry, University of Yaoundé I, Cameroon	Bachelor Student
1998.2-2006.5	Segning Ranch, Mbouda, Cameroon	Farm Input Manager

奖惩情况

2009: Best International Student of South China Agricultural University, Guangzhou, China (first position)
2008: Best International Student of South China Agricultural University, Guangzhou, China (second position)
2005: Cameroon Minister of Higher education' Excellence Prize for Master's Degree with Thesis (3rd in a class of 55 students)
2003: Cameroon Minister of Higher education' Excellence Prize for Master's Degree without thesis (3rd in a class of 194 students)
2001: Cameroon Minister of Higher education' Excellence Prize for Bachelor's Degree (2nd in a class of 282 students)

华南农业大学博士研究生成绩表（2007级）

姓名 piebiep　　性别 男　　学号 2007100011

专业 作物栽培学与耕作学　　方向 作物品质、生理与化学调控

课程名称	学时	学分	学位课	成绩	考核时间	备注
作物栽培学研究进展	40	2	√	90		
作物栽培学研究生班讨论	40	2	√	91		
耕作学研究进展	20	1		85		文献综述
高级作物生理学（2）	39	2		90		
中国概况	60	3		83		
作物栽培与生理研究法（2）	61	3		91		
合计		13				

学院教务员签名：刘春燕　　学院盖章：　　2010年 5月 20日

论文题目	收获前后调控香稻香气和米质的研究		论文字数	5.390 万
论文选题来源	13	项目名称及代码	Grant No. 30671221	
论文类型	1, 2	论文工作起止时间	2007.9-2010.6	

学位论文工作简介（主要是论文选题的意义和价值；研究目的；创造性成果等）

香稻是特种稻，以其芳香的气味和超优的米质被人们所熟知。随着香稻的不断发展，探明影响香稻香气成分含量和米质的有关因素，对香稻种植者和加工者的经济利益提高具有重要的作用。因此，本论文以华南地区两个香稻品种香稻桂香占和培杂软香为材料，研究了收获前后调控香稻香气和米质的作用，主要结果如下：

利用了顶空固相微萃取技术和静态顶部空间气质联用技术，在最佳的条件下定性及定量测定香稻桂香占和培杂软香的具有强烈爆米花气味的化合物 2 -乙酰- 1 -吡咯啉（2 -AP）含量，以桂香占 2-AP 含量为最高(3.86 μg/g)，高出泰国米 KDML105 5 倍；其他非 2-AP 化合物对促进培杂软香特有的香气形成具有一定的作用。

对桂香占和培杂软香两个品种进行了 4 个收获前处理：种植密度处理分别为 16、19、22、28 和 37 穴/m2；收获时期处理分别为抽穗后 10d、20d、30d、40d 和 50d 收割；种植季节处理分别为早季和晚季；植物生长调节剂处理分别为在抽穗期施用赤霉素、多效唑、 3 -吲哚乙酸和多效唑、脯氨酸、和氯化锌的混配剂等和 3 个收获后处理：贮藏时间处理分别为贮藏 3 个月和 6 个月；贮藏温度处理分别为-4℃、8℃、20℃和 30℃及碾磨程度处理分别为和常规碾磨和 85%的常规碾磨程度。测定了不同收获前处理的香稻样品的过氧化物酶、超氧化物歧化酶和脯氨酸氧化酶活性，有效穗、每穗总粒数、结实率、千粒重和产量等性状，糙米率、精米率、整精米率、垩白粒率、垩白度和直链淀粉含量等品质性状，以及丙二醛、脯氨酸和蛋白质含量。结果表明，桂香占和培杂软香的 2-AP 含量最高值分别出现在种植密度最稀的 16 穴/m2 处理分别为 3.73μg/g 和 0.69 μg/g；收获时间最早的处理即抽穗后 10d 收获的 2-AP 含量最高，桂香占为 5.24μg/g 和培杂软香为 0.72 μg/g；成熟期温度最低的 25℃处理的 2-AP 含量最高，桂香占为 7.12μg/g 和培杂软香为 2.42 μg/g；储藏时间最短的 3 个月处理的 2-AP 含量最高，桂香占为 2.40μg/g 和培杂软香为 0.45 μg/g；储存温度最低的-4℃处理 2-AP 含量最高，桂香占为 3.42μg/g 和培杂软香为 0.49 μg/g；碾磨后，桂香占和培杂软香的 2-AP 含量低出研磨前含量 1.5 倍；相对于对照，所植物生长调节剂处理，虽然提高了桂香占和培杂软香的谷粒产量和质量，增强了其清除和控制其产品中活性氧危害的能力，但是减少了香气物质的含量，降幅介于 9%至 24%，从而导致了在气味评估方面的消极影响。

上述结果表明，利用收获前后处理，可大大改善中国国产香米的具体特征。通过冷藏香米、低的碾磨程度和储存时间低于 6 个月是有利于维持香米优良特征可行办法，有利于防止香米 2-AP 水平的下降；利用改变播期使种子成熟的关键阶段与良好的田间环境相适应，即，使香稻灌浆熟期处于温度相对较低和干燥的时间段，以及在低密度和适期早收的种植方式等可以提高香稻香气含量和米质；香稻生产上应注意使用植物生长调节剂对香气含量的不利影响。

因此，通过应用收获前后对香稻香气和米质有利的调控措施可以在不同程度上改良香稻香气和品质性状，增加香米国内市场份额，甚至打入国际市场。

注：（1）选题来源分类：11 国家计委、科委项目；12 国家经贸委项目；13 国家自然科学基金项目；14 国务院其它部委项目；21 主管部门（部委级）项目；22 省级项目；31 学校级项目；41 国际合作项目；42 自选项目；43 其它项目（企业委托项目）

（2）论文类型分类：1、基础研究 2、应用研究 3、开发研究 4、其他

在学期间取得的科研成果（含论文、专著、专利等）（不够请另加页）

序号	成果鉴定、获专利、颁奖与采用部门或发表刊物及出版单位	年月（卷期号）	论文（成果）名称	第几作者	被索引收录情况	对应学位论文的哪一部分（章、节）
1.	Chiang Mai (Thailand) Center of Excellence for Innovation in Chemistry Notes	2009.3: 10pp (ISSN	Application of a Solid Phase Micro-Extraction and a Static Headspace Sampling System for the Extraction of Volatile Components from Rice.	1		Part 3.1.
2.	Frontiers of Agriculture in China	2010.2: 4(1) (ISSN 1673-7334)	Some Factors Affecting Concentration of the Aroma Compound 2-Acetyl-1-pyrroline in two Fragrant Rice Cultivars Grown in South China.	1	Covered by SCI	Part 3.1 Part 3.2
3.	Agronomy for Sustainable Development	2010.4: 30(3) (ISSN 1774-0746)	Decrease in Rice Aroma after Application of Growth Regulators.	1	SCI 1.649	Part 3.3
4	Accepted for the 14th International Biotechnology Symposium (Italy) and the Journal of Biotechnology	2010.9 (2pp) (ISSN 0168-1656)	Appropriate Set of Pre and Post-Harvest Treatments for Obtaining High Levels of 2-Acetyl-1-pyrroline in New Fragrant Rice Cultivars Developed in China.	1	SCI 2.748	Part 3.1 Part 3.2 Part 3.3

在学期间以本校名义发表论文共 3 篇，分类统计如下：

国内刊物	0	国内会议	0	国外刊物	3	国际会议	1	专利	0

被索引收录数分类统计：2

申请人确保以上所填内容属实。如有虚假，造成的一切法律后果由申请人承担。

申请人（签名） 2010 年 04 月 26 日

导师意见（包括对申请人的学习情况、思想表现及论文的学术评语，科研工作能力和完成科研工作情况以及是否同意申请学位论文答辩的意见）：

该生学习态度端正，勤奋刻苦，成绩优良。思想态度端正，积极进取；遵守纪律，遵守实验室规章制度，无违法乱纪行为。

科研上有创新意识，踏实肯干，实事求是，善于与他人合作。具有一定的综合、收集和正确利用各种信息及获取新知识的能力。能比较独立的查阅文献并较好地撰写毕业论文。

该论文实验设计合理、数据可靠，结论正确，条理清晰，研究结果有很好的生产应用前景和一定的学术意义。表明作者具有较扎实的基础理论知识和专业技能。

同意该生申请学位论文答辩。

导师签名：

2010 年 5月 20日

学院学位评定分委员会意见（是否同意申请学位论文答辩的意见）：

同意

学院学位评定分委员会主席签名：

2010 年 5 月 25日

博士学位论文答辩委员会组成审核表

学　　号	2007100011	专　　业	作物栽培学与耕作学
研究生姓名	**GOUFO PIEBIEP**	导师姓名、职称	唐湘如　教授
论文题目	收获前后调控香稻香气和米质的研究		

答辩委员会组成	姓　名		职　称	是否博导	所　在　单　位
	主席	官春云	院士	是	湖南农业大学
	委员	刘向东	教授	是	华南农业大学
		谭中文	教授	是	华南农业大学
		梁计南	教授	否	华南农业大学
		钟旭华	教授	否	广东省农业科学院水稻所
答辩委员会秘书		段美洋	实验师	华南农业大学农学院	
答辩时间及地点			2010年5月26日下午3：00，农学院楼323室		

是否同意以上名单组织论文答辩

学院学位评定分委员会审核意见：

同意

分会主席签名：

日期：2010.5.20

学校学位评定委员会审核意见：

同意

华南农业大学学位评定委员会公章

日期：2010.5.21

注：该表请填写一式二份，均为原件。

华南农业大学博士学位论文答辩委员会决议书

论文答辩	表决情况记录						
	通过	不通过	弃权	建议授予	同意	不同意	弃权
	5 票	0 票	0 票	农 学博士学位	5 票	0 票	0 票

答辩委员会决议

该论文以华南地区两个香稻品种桂香占和培杂软香为材料，研究了种植密度、收获时期、种植季节和植物生长调节剂等 4 个收获前处理和贮藏时间、贮藏温度和碾磨程度等 3 个收获后处理，对香稻香气和米质的调控作用。结果表明：桂香占 2-AP 含量为最高，高出泰国米 KDML105 5 倍；种植密度为 16 穴/m2，抽穗后 10d，成熟期温度 25℃，储藏时间 3 个月，储存温度-4℃各处理的桂香占和培杂软香的 2-AP 含量均达到最高；碾磨程度增加显著降低桂香占和培杂软香的 2-AP 含量；所有植物生长调节剂处理能提高桂香占和培杂软香的谷粒产量和质量，增强了其清除和控制其产品中活性氧危害的能力，但是减少了香气物质的含量，降幅介于 9%至 24%，从而导致了在气味评估方面的消极影响。研究确定了使香稻灌浆成熟期处于温度相对较低和干燥的时间段，以及在低密度和适期早收的种植方式等可以提高香稻香气含量和米质。研究结果对香稻高产优质浓香栽培与品种选育具有重要的参考价值和良好的生产应用前景。

论文试验设计合理，数据翔实，结果可信，具有创新性和实用价值；论文写作规范，条理清晰。在答辩过程中能正确回答问题，表明其具有扎实的基础理论知识和专业技能，以及较强的独立从事科研的能力。

答辩委员会一致同意通过论文答辩，并建议授予农学博士学位。

答辩委员会成员签名：（主席）

2016年 5月 26日

华南农业大学博士学位审批意见表

学院学位评定分委员会审批意见	决议： 经学院学位评定分委员会审核、无记名投票表决，建议授予农学博士学位。 全体委员 15 人；到会委员 13 人。 表决结果：建议授予学位 12 票；不建议授予学位 1 票； 弃权 0 票。 学院学位评定分委员会主席签名： 学院公章： 2010 年 6 月 7 日
学校学位评定委员会审批意见	经学校学位评定委员会审核、无记名投票表决，决定授予农学博士学位。 学校学位评定委员会主席： 学校学位评定委员会（公章） 授予学位日期：2010 年 6 月 23 日

论 文 答 辩 情 况 记 录

答辩中提出的主要问题及回答问题的简要情况（请如实、详细填写，不够请另加页）：

1. For how long have you been in China and how is your Chinese language level?

I have been in China for over 3 years. Although I can use Chinese language for daily communications, I am still not familiar with technical works used for research.

2. According to the content of your dissertation, most of the work was done in 2008. What were you doing in 2009 and 2010?

Most of the field work was done in 2008. Laboratory work was done in 2009 and 2010. It should be emphasized that work on aroma and flavor is quite difficult and time-consuming. We used almost three different methods for the analysis of 2-acetyl-1-pyrroline, with the first two methods giving us unsatisfactory results.

3. You studied four pre-harvest treatments. What was the field design of the test? How were the four treatments combined?

Treatments were not combined. Experiments were conducted simultaneously during the early and late seasons of 2008, but separately i.e. one field for planting density, one field for harvesting time, and one field for application of growth regulators.

4. Which factor affected the most rice aroma and flavor?

Genotype affected the most rice aroma and flavor with Guixiangzhan rice cultivar having five times more 2-acetyl-1-pyrroline compared to Peizaruanxiang rice cultivar.

5. How do you explain the decrease/increase of aroma following planting density, harvesting time, ripening temperature or application of growth regulators?

Fragrant rices are susceptible to lodging. It appears that with increased planting density, lodging is increased with the consequence of plants competing to absorb energy and micronutrients needed for biosynthesis purposes. Decrease in rice aroma after application of growth regulators can mostly be explained by the inhibition of metabolic processes associated with the formation of volatile compounds. In the case of ripening temperature and harvesting time, degradation either by enzymes or heat occurs.

6. How will you explain the decrease of 2-acetyl-1-pyrroline and lipid-derived volatiles during storage?

The major deteriorating mechanism occurring in rice during storage is lipid oxidation and decomposition. The high level observed for lipid-derived volatiles can be explained by the action of lipooxygenase and lipase activities. The same comment might hold true for 2-acetyl-1-pyrroline which is a highly volatile and lipophilic compound.

7. It seems that most of the factors increasing yield in normal rice will lead to decrease aroma in rice.

That is right. Fragrant rices compared to the leading varieties are low-yielding and most attempts made to increase their yield using agricultural chemicals have led to decrease aroma, henceforth the necessity to define an appropriate set of pre- and post-harvest treatments for their cultivation.

8. What can be the practical use for harvesting at 10 days after heading? Can it be used for production purposes?

Of course harvesting at 10 days after heading cannot be used for production purposes since most grains are still in their immature state. However, at 10 days after heading the content of 2-acetyl-1-pyrroline in very high and the compound can be extracted, purified and used as a flavoring agent for other food products.

9. In your opinion in which part of the seed is 2-acetyl-1-pyrroline found?

Zero percent, 15% and 25% milling of brown rice indicated the aleurone layer (bran) and the outer endosperm as the primary site of origin of 2-acetyl-1-pyrroline.

10. Which gene control 2-acetyl-1-pyrroline biosynthesis?

It is admitted that one gene on chromosome 8 encoding a putative betaine aldehyde dehydrogenase 2 controls 2-acetyl-1-pyrroline biosynthesis. An eight-base pair deletion in exon 7 of the gene will lead to 2-acetyl-1-pyrroline biosynthesis although some cultivars with no deletion have recently been found to contain 2-acetyl-1-pyrroline.

11. Can the fragrance gene be detected in all stages of rice growth?

Yes, the fragrance gene can be detected in all stages of rice growth.

12. Which way is the best to screen for 2-acetyl-1-pyrroline in rice, gene analysis or 2-acetyl-1-pyrroline content analysis?

If considering time as the most important factor, 2-acetyl-1-pyrroline content analysis will be the easier way, especially using methods like automated headspace which can analyze 50 samples a day. If considering the cost, DNA analysis will be the best choice.

13. Can you explain why in page 81 and table 3.2.12, the temperature affects grain yield in Peizaruanxiang rice cultivar, but not in Guixiangzhan rice cultivar?

That should be a genotype effect, different cultivars responding differently to different treatments.

14. If you are given a new cultivar of rice with the aim to increase its aroma, how will you proceed?

Following the results of my study, I will find a sowing day allowing seed ripening to coincide with the cool segment of the year, use lower planting densities, harvest early, avoid the use of agricultural chemical and when necessary, establish and optimum concentration of chemicals to be used and an optimum milling degree, and store harvested samples at a low temperature.

15. Do you think any regulator can be used to increase the aroma content of rice?

Growth regulators are generally classified into 5 groups, auxins, cytokinins, gibberellins, abscisic acid and ethylene, with some other new families like polyamines, paclobutrazol, brassinolides considered as sixth group. We studied only growth regulators belonging to three groups which were found to decrease the aroma content. It will be interesting to study as much as regulators as possible before drawing any general conclusion.

论文答辩记录员签名：

日期：2010 年 5月 26 日

博士研究生

毕业证书

GOUFO PIEBIEP 先生／女士，国籍　　喀麦隆，

一九八〇 年 三 月二十三 日生，于二〇〇七年 九 月至二〇一〇 年 六 月在 作物栽培学与耕作学 专业学习，学制 三 年，修完博士研究生培养计划规定的全部课程，成绩合格，毕业论文答辩通过，准予毕业。

培养单位：华南农业大学　　校(院、所)长：

证书编号：105649201001000004

二〇一〇 年 六 月二十一 日

查询网址：http://www.chsi.com.cn

中华人民共和国教育部监制

DOCTORAL CANDIDATE GRADUATION CERTIFICATE

(Translation)

(Photo of the holder with the embossed seal of South China Agricultural University)

Mr. GOUFO PIEBIEP, Cameroonian Nationality, born on March 23, 1980, majoring in Crop Science and Technology (3-year program) in this University from September 2007 to June 2010, has successfully completed all curriculums of doctoral postgraduate program and passed dissertation defence. Hereby he is allowed to graduate.

Chen Xiaoyang

President

(Stamped Signature)

Trained by: South China Agricultural University (Official Seal)

June 21, 2010

Certificate No.105649201001000004

Website: http://www.chsi.com.cn

Prepared under supervision of Ministry of Education of The People's Republic of China

公　证　书

(2010)粤穗广证字第48861号

兹证明前面的复印件与GOUFO PIEBIEP的证书编号：105649201001000004的《博士研究生毕业证书》原件相符。并证明前面的英文译本与中文本内容相符。

中华人民共和国广东省广州市广州公证处

公证员 林峰

二〇一〇年七月一日

广州公证处　林峰

XM60062160

CERTIFICATE

2010YSGZZi,No.48861

This is to certify that the duplicate copy attached hereto is in conformity with the original Certificate No.105649201001000004 DOCTORAL CANDIDATE GRADUATION CERTIFICATE held by GOUFO PIEBIEP, and that the English translated copy attached hereto is in conformity with the Chinese copy.

Notary:Lin Feng

Guangzhou Notary Public Office

Guangzhou City,Guangdong Province

The People's Republic of China

July 1, 2010

广州公证处

林峰

XW6C062181