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# Flavour qualities of new Australian fragrant rice cultivars

A report for the Rural Industries Research and Development Corporation

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# Foreword

This project was funded from industry revenue which is matched by funds provided by the Australian Government. This project was based at the University of NSW in the Department of food Science and Technology and run in collaboration with the rice breeding program at Yanco Agricultural Institute (YAI). The project aimed to facilitate the rice breeding program with the development and improvement of new Australian fragrant rice cultivars.

Fragrant rices constitute an important subgroup of rice. They are characterised by their unique aroma and can fetch up to four times the price of non-aromatic rice on the world market. There are essentially two major types of fragrant rice, which are based on the geographical region from which they originate, including Jasmine from Thailand and Basmati from India. At present Australia produces only one fragrant rice cultivar on a commercial scale, which is of the Thai Jasmine type. Fragrant rices are becoming increasingly popular in western countries including Australia and there is an opportunity for Australia to tap further into the international rice market. Therefore the development of an Australian grown Basmati cultivar is a high priority within the NSW rice breeding program.

This project will assist with the development of Australian fragrant rices by:

- investigating the aroma of new breeding lines,
- comparing these to imported varieties using both sensory studies and analysis of the volatile components,
- determining the effect of nitrogen fertiliser level on rice aroma,
- developing methods for early generation screening of rice fragrance,
- investigating the biosynthetic and other pathways of volatile component formation.

This report summarises investigation of the above research areas carried out during this project.

This report, an addition to RIRDC's diverse range of over 1000 research publications, forms part of our Rice R&D Program, which aims to improve the profitability and sustainability of the Australian rice industry.

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**Simon Hearn**
Managing Director
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# Abbreviations

YAI.....Yanco Agricultural Institute

GC/FID.....gas chromatography/flame ionisation detection

GC/MS .....gas chromatography mass spectrometry

SPME .....solid phase micro-extraction

RWR.....relative weight response

2-AP .....2-acetyl-1-pyrroline

LN/SDE.....Likens and Nickerson simultaneous distillation extraction apparatus

AJF .....Australian Jasmine fragrant

IJF.....imported Jasmine fragrant

NF.....non-fragrant

ABF .....Australian Basmati fragrant

IBF.....imported Basmati fragrant

PDMS .....Polydimethylsiloxane

PA.....Polyacrylate

PDMS/DVB .....Polydimethylsiloxane Divinylbenzene

PDMS/CAR.....Polydimethylsiloxane Carboxen

PDMS/CAR/DVB ...Polydimethylsiloxane Carboxen Divinylbenzene

SIM.....selected ion monitoring

HFBA .....heptafluorobutyric anhydride

Ho.....null hypothesis

# Contents

# Executive Summary

The research presented has investigated aspects of the flavour qualities of new Australian fragrant rice cultivars which are important in facilitating the NSW rice breeding program and presenting new knowledge as to the chemistry of rice aroma and its implications for the sensory properties and acceptability of rice.

Identification of the effects of nitrogen fertiliser on the aroma volatiles of rice has demonstrated the impact of nitrogen fertiliser level on the aroma of rice. From data over three seasons, it was concluded that nitrogen fertiliser level had a significant effect on the concentration of volatile compounds in rice plant material at several stages during growth. Plants fertilised at the lower nitrogen rate (50kg N/ha) had a lower concentration of volatile compounds compared to the plants fertilised at the normal nitrogen rate (150kg N/ha). In particular this was the case for the compound 2-AP, which contributes significantly to the aroma of rice. In addition, at tillering, compounds including, (E)pent-2-enal; (E)hex-2-enal; hexanal; (E)pent-2-en-1-ol; hexanol and methyl salicylate, which contribute green/grassy aromas, were present in lower concentrations in the plants fertilised at the lower nitrogen level. There were many significant effects on the concentration of individual volatile components in the rice grain. Over two seasons it was found that the majority of important volatile compounds were significantly higher in grain fertilised at the lower nitrogen level for both fragrant and one non-fragrant rice variety, but lower in another non-fragrant variety.

The volatile aroma components of Australian fragrant rices were compared with those of their imported counterparts. Australian Basmati breeding line did not contain several volatiles present in the imported samples, but contained others, including 2-AP, in higher amounts. Caution must be exercised in interpreting these results as the Australian sample was analysed soon after harvest while imported Basmati rice traditionally undergoes storage for up to 12 months. Of those compounds in higher amounts in the imported Basmati, several of these are probably derived from lipid breakdown and may be a reflection of this storage period, the conditions of which are unknown. Similarly, the higher 2-AP levels in the Australian samples may simply indicate that this compound had broken down during the storage encountered by the imported material.

Commercial Australia Jasmine rice was very similar to imported varieties both qualitatively and quantitatively with respect to volatile aroma compounds. However, there were some obvious quantitative differences, which distinguished the rices. The close resemblance of the Australian Jasmine rice to imported varieties suggests its acceptance in the international market. Some concern over the aroma potential of an Australian Jasmine breeding line YRF207 arose since it contained significantly less 2-AP than the commercial Kyeema variety over two seasons of testing.

Substantial changes in volatile components occurred during storage of Australian Basmati breeding lines with new compounds forming and existing ones decreasing. Most importantly, 66% of 2-AP was lost during a 3 month storage period. This underscores the importance off storage protocols for Basmati rices developed within Australia.

Sensory evaluation of rice aroma provided valuable insight into consumer attitudes and perceptions to the aroma of Australian and imported Jasmine and Basmati rices as well as Australian non-fragrant rice. Consumers, as a group, could distinguish between the aroma of fragrant and non-fragrant rice and between Australian and imported Jasmine and Basmati fragrant rice. There were differences with respect to age of subject and ability to distinguish between Australian fragrant Jasmine and non-fragrant rice and between Australian fragrant and imported fragrant Jasmine rice. In addition it was found that consumers as a group could distinguish between the aroma of Australian fragrant Basmati and imported fragrant Basmati rice. Age, gender and cultural background had no effect on the subject's ability to distinguish between the aroma of these two rices. Differences in consumer perception between the three rice samples and aroma descriptions could be explained in the context of differences in their volatile components.

Aroma descriptions of the Australian fragrant Jasmine rice compared to the imported counterpart revealed a greater number of positive descriptions of rice aroma and less negative aroma descriptions for the former. However, preference tests indicated no direct preferences for the aroma of either the Australian or imported Basmati fragrant rice.

An SPME technique was optimised for the adsorption of 2-AP using pandan leaves and these optimum extraction conditions were utilised for the adsorption of 2-AP in rice plants. It was concluded that the 3-leaf growth stage of rice plants was the best to extract 2-AP from rice plants. Therefore SPME can be used for the screening of fragrance in rice plants at the early growth stages without waiting for the mature grain allowing the rapid detection of fragrance. The technique can be used on-farm for collection of volatile components for later GC/MS analysis in the laboratory

Plants and grain fertilised at 150kg N/ha had a greater concentration of total amino acids compared to plants and grain fertilised at 50kg N/ha. In particular, levels of proline were significantly lower in rice plants and grain fertilised at the lower nitrogen rate. Proline was specifically targeted in this work because it has been implicated as a precursor of 2-AP. The levels of proline were greater in the non-fragrant plant compared to the fragrant plants throughout growth, but the levels of proline in the grain were similar. Since fragrant and non-fragrant plants had similar levels of free proline, level of free proline is not indicative of the fragrance potential of the plant.

# 1. Introduction

Fragrant rice is characterised by its particular aroma that is attributed mainly to the 2-acetyl-1-pyrroline (2-AP) (Buttery *et al.*, 1983). There are two major types of fragrant rice: Jasmine which is of Thai origin and Basmati which is of Indian origin. Australia at present produces one fragrant rice variety on a commercial scale, which is of the Thai Jasmine type. All the Basmati rice sold in Australia is presently imported. Therefore, development of Basmati rice for local production is a high priority within the rice breeding programs in Australia.

Rice is a staple food commodity in most Asian countries and is becoming increasingly popular in Australia. The Australian rice industry produced 1.4 million tonnes of rice (paddy) in 1999, with fragrant rice production being approximately 5% of the total crop. Rice is Australia's third largest cereal grain export. Exports in 1997 were 550,000 tonnes, which increased to 650,000 tonnes in 1999 (ABS, 1999). The Australian rice industry generates around $500 million dollars a year, of which $400 million is from export sales. The consumption patterns of rice in Australia indicate the increasing popularity of this commodity. Australians consumed 170,000 tonnes of rice (paddy) in 1990, which increased to 258,000 tonnes in 1998. The trend of increased rice production and consumption, and its export from Australia is promising for the rice industry. By developing successful fragrant rice varieties, Australia can take advantage of this highly priced, growing rice market.

To exploit this opportunity, it will be necessary to develop fragrant rice cultivars suited to Australian growing conditions and agronomic practices. Application of nitrogen fertiliser as of urea is widely practiced to improve yield, especially where soils may be nitrogen deficient. However there may well be some impact of nitrogen fertiliser levels on the aroma of aromatic rices. Therefore investigation of the effects of nitrogen fertiliser level on the aroma volatiles in rice plants and grain will help clarify nitrogen fertiliser requirements for aroma development.

For the successful development of fragrant rices, research regarding the quality of its aroma is necessary. Studies involving the isolation and identification of volatile aroma components of rice grain have been performed for decades. With the development of new fragrant varieties, particularly Basmati cultivars, it is necessary to determine their quality with respect to aroma and compare this with existing varieties. In particular knowledge of the volatile aroma components of rice is important in the understanding of human perception of rice flavour and may aid the breeding of rice varieties with specific aroma traits.

The pathways of formation of volatile aroma compounds of rice in the rice plant are important. Very little is known about the biosynthesis of 2-AP although amino acids including proline and ornithine have been implicated as precursors (Muench, Hoffman and Schieberle, 1997). Recently, Yoshihashi, Nguyen and Inatomi (2002) showed that proline is the nitrogen source of 2-AP. In addition, it was shown that 2-AP can form in rice plant seedling and callus, suggesting a biosynthetic mechanism for the formation of 2-AP in rice. Therefore, levels of total and free amino acids, in fragrant and non-fragrant rice plants, and the effect of nitrogen fertiliser levels on their concentration were studied.

Imported fragrant rices are seen as the benchmark for aroma quality and therefore the aroma of Australian fragrant rice needs to be compared to these and consumer discrimination and preferences between the aroma of fragrant and non-fragrant rice, and between newly developed and traditional fragrant rice varieties be investigated. Understanding human perception of specific rice aroma constituents is essential since the characteristic aroma of fragrant rice needs to be readily detectable by consumers, characteristic of fragrant type, distinguishable from that of non-fragrant rice, and acceptable to consumers. Also of great importance are eating and cooking qualities of fragrant rice especially Basmati types, but these are outside the scope of the present research, which is focussed on rice aroma.

Rice breeders have used various techniques to detect and evaluate aroma in order to study the inheritance of aroma and improve the effectiveness of rice breeding programs. Initially, aroma was evaluated by chewing grains (Dhulappanawar, 1976) and cooking a sample of seeds from individual plants and noting the aroma (Choudhury and Ghosh, 1978 and Ghose and Butany, 1952). The leaf tissues of aromatic plants also contain the characteristic aroma (Nagaraju *et al.*, 1975 and Sood and Siddiq, 1978). Heating leaf tissue in water and noting the aroma (Nagaraju *et al.*, 1975) and eluting aroma from leaves with dilute KOH (Sood and Siddiq, 1978) have been used to identify aromatic plants. However these methods are subjective. Important aroma compounds have been detected in young rice plant tissue (Leung *et al.*, 1998). This has great potential as an early indicator of fragrance in young rice plants. Techniques such as solid phase micro-extraction (SPME), especially if adaptable to use on-farm, could provide rapid early generation screening without the requirement for mature grain.

# 2. Objectives

1. To investigate the effect of nitrogen fertlilisation levels on the volatile compounds of rice plants and grain during growth and at maturity;
2. To expand knowledge of volatile components of new Australian fragrant rice cultivars to assess their aroma quality;
3. To compare the perceived odours of Australian fragrant rice and non-fragrant rice and between Australian and imported fragrant rice;
4. Investigation of methods and implications for the rapid detection of fragrance in rice plants;
5. To investigate the levels of total and free amino acids in rice plants and grain in relation to the biogenesis of 2-AP.

# 3. Methodology

## 3.1 Effect of nitrogen fertiliser level on volatile components of rice plants and grain

### 3.1.1 Samples

Breeders from the Department of Agriculture and Yanco Agricultural Institute in Yanco, NSW, performed all the rice plant trials. All trials were performed in the field and cultivated in the same way as commercial rice crops.

Trials were performed over three seasons, 1999-2000, 2000-2001 and 2001-2002. Each season 4 rice plant varieties were planted and fertilised at a normal nitrogen rate of 150kg N/ha and at a low nitrogen rate of 50kg N/ha. Nitrogen fertiliser was applied as urea before permanent flooding at approximately the 3-leaf stage of growth. In the 1999-2000 season, 3 fragrant and one non-fragrant rice cultivars were used. These were a commercial jasmine cultivar known as Kyeema, a jasmine breeding line YRF207, a Basmati breeding line YRF203 and a commercial non-fragrant cultivar Millin. In the 2000-2001 season the varieties remained the same except a non-fragrant long grain breeding line (L203) was planted instead of the Basmati breeding line YRF203. In the 2001-2002 season the plant varieties utilised were the same as the 1999-2000 season.

Rice plant samples were taken at the growth stages of tillering, panicle initiation, flowering and maturity for all seasons. In addition, rice grain samples were collected from the 2000-2001 and 2001-2002 seasons only.

### 3.1.2 Extraction of volatile aroma compounds from rice plants and grain

For method details please refer to Wilkie, Craske and Wootton (2002).

### 3.1.3 GC/FID parameters

For method details please refer to Wilkie, Craske and Wootton (2002).

### 3.1.4 Identification of components by GC/FID

For method details please refer to Wilkie, Craske and Wootton (2002).

### 3.1.5 Quantitation - Internal Standardisation (GC/FID)

For method details please refer to Wilkie, Craske and Wootton (2002).

### 3.1.6 GC/MS parameters

For method details please refer to Wilkie, Craske and Wootton (2002).

### 3.1.7 Identification of components by GC/MS

For method details please refer to Wilkie, Craske and Wootton (2002).

### 3.1.8 Moisture content of rice plants and grain

The moisture contents of rice plants at the growth stages of tillering, panicle initiation, flowering and maturity and in the grain were determined. Approximately 2g of rice plant tissue or rice grain was

accurately weighed using an analytical balance into a moisture dish. The sample was dried in a vacuum oven at 70°C until a constant weight was reached. The moisture content was calculated as a percentage with the weight lost corresponding to moisture lost. The equation used:

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

### 3.1.9 Nitrogen content of rice plants and grain

Samples used to obtain the moisture content were ground with a mortar and pestle. Approximately 0.100g (rice plants) and 0.25g (rice grain) were used for nitrogen analysis using a LECO nitrogen analyser.

## 3.2 Comparison of the aroma of Australian and imported fragrant rice

### 3.2.1 Samples

Samples were either commercial rice samples purchased from a local supermarket or were grown by breeders from NSW agriculture at Yanco Agricultural Institute (YAI), Leeton, NSW. Australian rice grain samples included the commercial Jasmine Koala and Sunrice brands, a Jasmine breeding line YRF207 and a Basmati breeding line YRF203. Imported rice grain samples included Jasmine varieties of different brand, such as Kumarnthong, Tilda, Lion and Green Earth. Basmati varieties included the brands of Kumarnthong, Riviana and Tilda.
A total of 14 Basmati breeding lines developed and grown by the breeders at NSW agriculture at YAI, Leeton, NSW were also analysed, Table 3.1.

**Table 3.1 Basmati breeding lines analysed**

| Breeding line | Breeding line |
|---|---|
| Entry 19- Cross 95200-34-6 Pelde/G'bhog(4)/D.10//YRL101 (4:2) | Entry 29- Cross 95200-47-4 Pelde/G'bhog(4)/D.10//YRL101 (4:2) |
| Entry 21- Cross 95200-47-1 Pelde/G'bhog(4)/D.10//YRL101 (4:2) | Entry 30- Cross 95200-47-4 Pelde/G'bhog(4)/D.10//YRL101 (4:2) |
| Entry 22- Cross 95200-47-1 Pelde/G'bhog(4)/D.10//YRL101 (4:2) | Entry 31- Cross 95200-47-4 Pelde/G'bhog(4)/D.10//YRL101 (4:2) |
| Entry 23- Cross 95200-47-1 Pelde/G'bhog(4)/D.10//YRL101 (4:2) | Entry 32- Cross 95200-47-6 Pelde/G'bhog(4)/D.10//YRL101 (4:2) |
| Entry 25- Cross 95200-47-2 Pelde/G'bhog(4)/D.10//YRL101 (4:2) | Entry 38- Cross 95139-15-1 YRL101//Pelde/G'bhog(4)/D.10 (3:2) |
| Entry 27- Cross 95200-47-3 Pelde/G'bhog(4)/D.10//YRL101 (4:2) | Entry 53- Cross 95139-17-8 YRL101//Pelde/G'bhog(4)/D.10 (3:2) |
| Entry 28- Cross 95200-47-3 Pelde/G'bhog(4)/D.10//YRL101 (4:2) | Entry 71- Cross 95200-34-9 Pelde/G'bhog(4)/D.10//YRL101 (4:2) |

All samples were genetically unique. However, some of the samples were grouped based on breeding characteristics. These included:

Entry 21, 22, 23 (set 1)
Entry 27, 28 (set 2)
Entry 29, 30, 31 (set 3)

### 3.2.2 Analysis of volatile aroma compounds in rice grain

This was performed as detailed in section 3.1.2 to 3.1.7.

## 3.3 Sensory responses to the aroma of Australian and imported fragrant rice and non-fragrant rice

### 3.3.1 Trial 1

The rice samples used in the study were all commercial rice varieties purchased from a local supermarket. Subjects participated in two tasks, each involving a triangle test procedure. Task 1 involved comparing Australian Jasmine fragrant (AJF) Koala brand Jasmine and non-fragrant (NF) Sunrice brand rice. Task 2 involved comparing Australian Jasmine fragrant (AJF) Koala brand Jasmine and Imported Jasmine fragrant (IJF) Kumarnthong brand Jasmine rice. The odd sample for the two triangle tests was varied throughout testing (refer to Table 3.2).

**Table 3.2 Trial 1 sensory set up design for triangle tests performed by participants**

| | **Station 1** | | | **Station 2** | | |
|---|---|---|---|---|---|---|
| | **Sample set 1 (Task 1)** | | | **Sample set 1 (Task 1)** | | |
| **Samples** | AJF[a] | NF[b] | AJF | NF | AJF | NF |
| **Codes** | 738 | 841 | 999 | 515 | 347 | 333 |
| | **Set 2 (task 2)** | | | **Set 2 (task 2)** | | |
| **Samples** | IJF[c] | AJF | IJF | AJF | IJF | AJF |
| **Codes** | 652 | 967 | 414 | 558 | 429 | 794 |

[a]AJF = Australian Jasmine fragrant (Koala brand Jasmine rice), [b]NF = non-fragrant (Australian sun rice), [c]IJF = Imported Jasmine fragrant (Kumarnthong brand Jasmine rice)

Cooking was standardised using rice cookers. A 1-1.5 ratio of rice/water was used with an approximate cooking time of 16 min. The warm cooked rice was filled into 300ml sniffing bottles to ¼ of the volume at a temperature of 70°C. Sniffing bottles containing the rice were labelled with a three-digit code. Samples were presented to the subjects who were required to sniff the samples and to select the odd sample.

The sensory trial was run at an annual Science Expo in Sydney, 2001, held at the Australian technology park, which attracted students from the age of 6-18, teachers, parents and other members of the public. This venue was an opportunity to utilise a large consumer panel, consisting of various ages, gender and cultural backgrounds. The sensory test was performed in a controlled manner such that one subject at a time performed the test. Subjects were required to complete a questionnaire as shown below.

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**FOOD SCIENCE AND TECHNOLOGY "SNIFFING" TEST**

If you would like to take part in our sniffing test please fill in the following and complete the two tasks.

Gender: Male ☐ Female ☐

Age: _____

Cultural Background: _____

You will be asked to sniff various rice samples. These rice samples are commercially available products and have not been treated in any way nor any additives added.
Sniff the bottles by doing the following:

1. Raise the bottle until about 3cm from your nose.
2. Flip the lid of the bottle and give the bottle a firm squeeze.
3. Sniff the air expressed from the bottle.

**Task 1.**
You have been presented with 3 bottles labelled set 1.
The content of one bottle is different (odd) from the other two bottles.
Sniff the samples in the order presented and CIRCLE the number of the ODD sample.

Example: 123 456 (789)

Samples 738 841 999 **or** 515 347 333

Please describe why you thought this sample was different:

_____

The two triangle tests followed the Australian standard for triangle tests (SAA, 1983). The number of correct responses from subjects was compared to the minimum numbers of correct judgements to establish significant differentiation between the samples (O'Mahony, 1986 Table G.5.c). Chi-square analyses were performed on data relating to age, gender, and cultural background. The volatile aroma compounds of the rice samples tested were determined by simultaneous distillation extraction (Wilkie, Wootton, and Craske, 2000).

### 3.3.2 Trial 2

This sensory trial was run at a Science Expo in Sydney ("Science in the City", 2002), held at the Australian museum, which attracted a similar group of participants as trial 1. The triangle test was performed in the same controlled manner as trial 1.

The rice samples used in the study were Riviana brand (product of Pakistan) Basmati rice, purchased from a local supermarket, and an Australian Basmati fragrant breeding line YRF203 obtained from NSW Agriculture at Yanco Agricultural Institute (Y.A.I), Leeton NSW from the 2001/2002 rice season. Subjects participated in a triangle test, which involved comparing the aroma of these two rice samples. The odd sample for the triangle test was different for each of the two sniffing stations as shown in Table 3.3.

**Table 3.3 Trial 2 sensory set up design for triangle tests performed by participants**

| | **Station 1** | | | **Station 2** | | |
|---|---|---|---|---|---|---|
| | **Sample set 1 (Task 1)** | | | **Sample set 1 (Task 1)** | | |
| | ABF[a] | IBF[b] | ABF | IBF | ABF | IBF |
| *Samples* | | | | | | |
| **Codes** | 515 | 347 | 333 | 738 | 841 | 999 |

[a]ABF = Australian Basmati fragrant [ Basmati breeding line (YRF203)].
[b]IBF = Imported Basmati fragrant (Riviana brand Basmati rice).

Samples were prepared and maintained as in trial 1. Subjects were required to complete a questionnaire as shown in trial 1. In addition, the subjects were asked to select the preferred sample and describe the odours of the odd and duplicate samples.

The test procedure followed the Australian standard for triangle tests (SAA, 1983). Statistical analysis of results and analysis of volatile aroma compounds of the rice samples tested was performed as in trial 1.

## 3.4 The use of Solid Phase Micro-Extraction for the analysis of the volatile components of rice

### 3.4.1 SPME optimisation

Optimisation of SPME methodology was performed with pandan leaves that were purchased from a local oriental supermarket. Pandan leaves were placed in an 8mL vial fitted with a screw cap with hole and PTFE/Silicone septa and equilibrated at the adsorption temperature for 10 min before adsorption by the SPME fibre. 2-AP was adsorbed from the pandan leaves under various combinations of time (5, 15, 30, 60 and 90min) and temperature (25, 40, 60 and 80ºC). Five SPME fibres were evaluated including 100µm Polydimethylsiloxane (PDMS), 85µm Polyacrylate (PA), 65µm PDMS/Divinylbenzene (DVB), 75µm PDMS/Carboxen (CAR) and 50/30µm stableflex PDMS/CAR/DVB (Supelco, Bellefonte, PA). All extractions were performed in duplicate.

Samples were injected into a Hewlett Packard (HP) 5890 Gas Chromatograph fitted with a HP 5972 Mass Spectrometer (GC/MS). A 30m, 0.25mm diameter, 0.25µm film thickness DB-WAX capillary column (J&W scientific, Folsom, CA) was used. The GC/MS was operated in scan mode from m/z 15-350 and in selected ion monitoring (SIM) mode with target ion 111. 2AP was detected using HP Chemstation software with mass spectral library. The abundance of 2AP was recorded as the area under the peak.

### 3.4.2 Analysis of rice plants by SPME

Rice plants (fragrant Kyeema, YRF207 and YRF203 and non-fragrant Millin) were grown in field trials (2001-2002 season) at YAI, Yanco NSW. Samples from all varieties were collected at the three leaf (pre nitrogen fertiliser application), tillering, panicle initiation and flowering growth stages. All samples were analysed in duplicate by the PDMS/CAR and PDMS/CAR/DVB SPME fibres with an equilibrium time of 10min and extraction time of 15min at 60°C. All samples were injected into a Hewlett Packard (HP) 5890 Gas Chromatograph fitted with a HP 5972 Mass Spectrometer (GC/MS) under the conditions detailed above with the MS operating in the scan mode from m/z 15-350. Volatile aroma compounds were detected by the GC/MS and the abundance of 2-AP was recorded as the area under the peak. The compounds detected were identified using the HP chemstation software with mass spectral library.

## 3.5 Levels of amino acids in rice plants and grain

### 3.5.1 Amino acid derivatisation and determination of relative weight response (RWR)

The method used for amino acid derivatisation closely follows the method developed by Zumwalt *et al.* (1987).

1. A stock solution of amino acids standards (0.1mg each amino acid per mL in 0.1N HCl) was prepared, and 200µL was pipetted into a sample tube.
2. An internal standard solution (0.1mg/mL norleucine in 0.1N HCl) was prepared and 200µL of this solution was added to the sample tube.
3. The sample was evaporated to dryness under a stream of nitrogen at 55°C.
4. To the dried sample, 200µL of isobutanol.3N HCl was added and the sample was capped and heated at 110°C for 45min in a heating block.
5. After cooling, isobutanol.3N HCl was evaporated to dryness under nitrogen at 40°C and then 80µL of ethyl acetate and 20µL of HFBA were added.
6. The mixture was heated at 110°C for 20min and after cooling 200µL of ethyl acetate was added to dilute the sample before GC analysis.

7. A sample (1µL) was injected into the GC/FID under split (40/1) conditions onto a DB-1 column at a initial temperature of 80°C. The temperature was ramped at 5°C/min to 265°C and held for 30min at 265°C. The injector and detector temperatures were 250°C. This was replicated 4 times.

The relative weight responses of each amino acid, relative to the internal standard, norleucine, were determined using the standard reference solution of amino acids.

### 3.5.2 Total amino acid analysis

A C18 Sep-Pak cartridge (Waters) was used for sample cleanup. The recovery of amino acid standards was determined by filtering them through the C18 Sep-Pak cartridge as follows:

(a) Cartridge activated with 2x10mL of methanol,
(b) Washed with 2x10mL of 0.1% TFA in water,
(c) Washed with 10mL of 0.1% TFA in water/methanol (80/20).
(d) 200µL of amino acid standard solution mixed with 2mL of 0.1% TFA in water/methanol (70/30) and passes through Sep-Pak cartridge.
(e) All the eluent and 200µL and 200µL of norleucine (IS) were derivatised as N-HFB iso-butyl esters and 1µL of the derivatised sample was injected into the GC/FID as detailed in section 3.5.1, steps 3-7.

This was performed in duplicate.

#### *3.5.2.1 Analysis of rice plants and grain*

Samples: Rice plants were grown in field trials at Yanco Agricultural Institute in the 2001/2002 rice season. The varieties tested included fragrant Kyeema, YRF207 and YRF203 and non-fragrant Millin which were grown with normal (150gn N/ha) and low (50kgN/ha) levels of nitrogen fertiliser. Rice plant samples were tested at tillering, panicle initiation, flowering and maturity and mature rice grain samples were also tested. All samples were dried to constant weight under vacuum at 70ºC before analysis which involved:

1. Samples of rice plant (0.5g) or rice grain (1g) were weighed into a screw cap test tube and 15mL of 6N HCl was added.
2. The sample was flushed with nitrogen for 1min and the tube closed.
3. Samples were hydrolysed in an oven at 110°C for 24 hours and then removed to cool.
4. Samples were quantitatively transferred from the tubes and filtered through GF/A filter paper into a rotary evaporator flask washing with de-ionised water.
5. Samples were concentrated almost to dryness under vacuum at 65°C using a rotary evaporator.
6. Sample was quantitatively transferred from the rotary flask to a 25mL volumetric flask and made up to volume with de-ionised water.
7. Sample was filtered through C18 Sep-Pak as follows:
   (a) Cartridge activated with 2x10mL of methanol,
   (b) washed with 2x10mL of 0.1% TFA in water,
   (c) washed with 10mL of 0.1% TFA in water/methanol (80/20).
   (d) Sample (1mL) was mixed with 2mL of 0.1% TFA in water/methanol (70/30) and passed through the SEPPAK cartridge. Sample should be water or acid solubilised and free of particulate matter.
   (e) The first 1mL of eluent was discarded and the next 2mL collected. This fraction contained all amino acids. Lipids and high molecular weight proteins were retained on the Sep-Pak cartridge.
   (f) Eluent (1mL) and 200µL of norleucine (IS) were derivatised as N-HFB iso-butyl esters and1µL of the derivatised sample was injected into the GC/FID as detailed in section 3.5.1, steps 3-7.

### 3.5.3 Free amino acid analysis

A SCX cation exchange column (Alltech) was used to clean up samples analysed for free amino acids. Firstly the recovery of amino acid standards was determined by passing them through the SCX ion exchange column as follows:

1. Column conditioned with 5mL of de-ionised water
2. 200µL of amino acid standard solution added to SCX column.
3. Column washed with 5mL of de-ionised water
4. 2mL of 1M Ammonium hydroxide added to the column as the eluate
5. All eluent collected and 200µL of norleucine (IS) were derivatised as N-HFB isobutyl esters and 1µL of the derivatised sample was injected into the GC/FID as detailed in section 3.5.1, steps 3-7.

#### *3.5.3.1 Analysis of rice plants and grain*

Sample: the same samples as outlined for total amino acid analysis were used.
Rice plant material or grain (0.5g) was weighed into a Schott bottle and 10mL of 0.1N HCL added.

1. The sample was placed in a shaking water bath at 80ºC for 30min.
2. The sample was filtered through GF/A filter paper (Whatman) into a 10mL volumetric flask and made up to volume with de-ionised water.
3. The sample was passed through an SCX ion-exchange column as follows:
   (a) Column conditioned with 5mL of de-ionised water
   (b) 4mL of sample passed through the column
   (c) Column washed with 5mL of de-ionised water
   (d) Ammonium hydroxide (3mL, 1M) was added as the eluate
   (e) 1mL of the eluent and 200µL of norleucine (IS) were derivatised as N-HFB isobutyl esters and 1µL of the derivatised sample injected into the GC/FID as detailed in section 3.5.1, steps 3-7.

# 4. Results and discussion

## 4.1 Effect of nitrogen fertiliser level on volatile components of rice plants and grain

### 4.1.1 Volatile compounds identified in rice plants and grain

The volatile compounds identified in rice plants and grain with their aroma descriptions can be found in the Appendix, Table 7.1. The majority of compounds have been identified by GC/MS software through matching the compounds spectra with mass spectra in the NBS 75K library. The compounds identified in rice plants are from all growth stages, including tillering, panicle initiation, flowering and maturity, and grain, over three seasons.

Some compounds were unique to grain only. These included, 3-methylbut-3-en-2-one; decane; 2-methylbut-3-en-2-ol; dimethyl disulphide; undecane; 2-n-butylfuran; pent-4-en-1-ol; dimethyl trisulphide; oct-3-en-2-one; 1-(2-propenyloxy) pentane; (E)dec-2-enal; 3-methylhexane; (E,Z)nona-2,4-dienal; dodec-2-enal; 1-methyl-4-(1-methylethyl)cyclohexa-1,3-diene; (E)2-methylbut-2-enal; octanoic acid; decanoic acid; 3,7,11-trimethyldodeca-1,6,10-trien-1-ol; 2-methyl1H-indole and 2,5-dimethylhexa-1,3-diene.

There were also compounds present in rice plants but not in the grain. These included, (Z)hept-4-enal; 1-hydroxypropan-2-one; trans-2-(1-pentenyl)furan; (E)pent-2-en-1-ol; (Z)pent-2-en-1-ol; (Z)hex-3-en-1-ol; caryophyllene; 2,6-dimethylcyclohexanol; 2,6,6-trimethyl,1-cyclohexene-1-carboxaldehyde; methylsalicylate; 4-methylphenol and phytol. Many of the compounds unique to rice plants commonly contributed green, grassy aromas.

The formation and origin of volatile aroma compounds will not be discussed here but has been comprehensively explored by Leung (2000).

### 4.1.2 Effect of nitrogen fertiliser level on the concentration of volatiles present in rice plants during growth and in the grain

The effect of nitrogen fertiliser level on the aroma quality of rice is important since nitrogenous compounds are likely to have an effect on 2-AP formation.

Nitrogen fertiliser was applied prior to permanent flooding at approximately the 3- leaf growth stage. The first samples analysed were at the tillering stage that was approximately 2-3 weeks after permanent flooding. The fragrant varieties fertilised at the low nitrogen level had slight yellowing of the foliage and were generally smaller plants with fewer leaves. These characteristics of the rice plants were consistent with nitrogen deficiency symptoms including stunted plants with a limited number of tillers, narrow and short leaves which are erect and become yellowish green as they age (De Datta, 1981). The non-fragrant Millin plants did not show visual signs of nitrogen deficiency to the same extent as the fragrant varieties. The tillering stage plants generally had a fresh grassy smell.

Data including the concentration of volatile compounds present in fragrant and non-fragrant rice plants fertilised at 150 and 50 kg N/ha at the tillering growth stage for the 1999-2000, 2000-2001 and 2001-2002 seasons were subjected to two way ANOVA to determine if there was an overall effect of nitrogen fertiliser level on the concentration of volatile compounds in the rice plant tissue and grain, and if there was a significant difference between the varieties tested. The effect of nitrogen on rice plants was determined over three seasons, 1999-2000, 2000-2001 and 2001-2002.

For plants at the tillering stage higher nitrogen application resulted in significantly levels of total volatile compounds, with no significant difference ($p=0.05$) between rice varieties in the 1999-2000

and 2001-2002 seasons. However, for the 2000-2001 season, there was a highly significant difference ($p=0.05$) between varieties. This may be due to the fact that less volatile compounds were identified and quantified in this season and reflects seasonal variations. For the tillering growth stage over the three seasons, even though there was an overall significant effect of nitrogen fertiliser level on the concentration of volatile compounds, the levels of most individual compounds were not affected by fertiliser level.

The concentration of some compounds was lower in plants fertilised at the low nitrogen rate compared to the normal rate. These compounds included but-3-en-2-one, 2-ethylfuran, pentan-2-one, butane-2,3-dione, pent-1-en-3-one, pent-1-en-3-ol, (E)pent-2-enal, heptanal, (E)hex-2-enal, hexanal, pentan-1-ol, 1,1-dimethylcyclopropane, (Z)pent-2-en-1-ol, 2-acetyl-1-pyrroline, hexan-1-ol, methyl salicylate, benzyl alcohol, phenylethyl alcohol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)(E)but-3-en-2-one and 4-methylphenol. Only a few compounds were present in higher concentrations when fertilised at the low nitrogen rate compared to the normal nitrogen rate. These compounds included propan-1-ol and methyl isobutyl ketone.

The majority of the volatile compounds affected by nitrogen fertiliser rate were lower boiling point compounds that are more important for aroma contribution than higher boiling point compounds. It is not surprising that the concentration of 2-AP was less in the plants fertilised at the low nitrogen rate. Nitrogen fertiliser has been shown to influence protein concentration therefore it is logical that nitrogen containing compounds such as 2-AP would be affected by nitrogen fertilisation levels also, as their precursors are amino acids. In particular proline has been implicated as the nitrogen source of 2-AP (Yoshihashi, Nguyen and Inatomi, 2002). Thus increased 2-AP concentration may be attributed to increased fertiliser level.

Compounds including (E)pent-2-enal; (E)hex-2-enal; hexanal; (Z)pent-2-en-1-ol; hexan-1-ol and methyl salicylate were also present in lower concentrations in the plants fertilised at the low nitrogen rate. These compounds commonly contribute green, grassy aromas and their reduction in the plants fertilised at the low N rate may be attributed to the fact that the plants were depleted of essential nutrients and were therefore less healthy with less lush green foliage that is typical of healthy plants.

It is important to note the differences in the concentrations of volatile compounds between the rice plant varieties at the tillering growth stage. The trends associated between the rice varieties with respect to their concentration of volatile compounds is based on levels in plants fertilised at the normal nitrogen rate of 150kg N/ha. The most important difference to note was that the fragrant Kyeema, YRF207 and YRF203 had significantly more 2-AP than the non-fragrant L203 and Millin. The concentration of 2-AP detected in the fragrant rice plants was approximately 20 times that detected in the non-fragrant rice plants.

Another noticeable difference between fragrant and non-fragrant rice plant varieties was that the non-fragrant Millin plant contained significantly less methyl salicylate than the fragrant rice varieties. The Kyeema plant had significantly less 2-ethylfuran compared to the other varieties. Differences in volatile aroma compounds may assist in identifying varieties.

The next growth stage of the rice plants where samples were taken was panicle initiation, defined as the emergence of panicles. At this stage the fragrant varieties were beginning to emit their characteristic aroma and were still quite high in moisture (average water content 73%). Data collected including the concentration of volatile compounds present in fragrant and non-fragrant rice plants fertilised at 150 and 50 kg N/ha at panicle initiation for the 1999-2000, 2000-2001 and 2001-2002 seasons. Results from the 2000-2001 and 2001-2002 seasons were subjected to two way ANOVA to determine if nitrogen fertiliser level had an overall effect on the concentration of compounds present in the rice plants. There were insufficient data collected in the 1999-2000 season for statistical analysis to be performed.

At the panicle initiation stage of growth for seasons 2000-2001 and 2001-2002, there was no significant effect of nitrogen fertiliser level (p=0.05) on the concentration of aroma compounds and there was no significant difference (p=0.05) between varieties.

The next growth stage at which samples were taken was flowering. Again at this stage the fragrant rice plants omitted an aroma characteristic of fragrant rice grain Data collected included the concentration of volatile compounds present in fragrant and non-fragrant rice plants fertilised at 150 and 50 kg N/ha at flowering for the 1999-2000, 2000-2001 and 2001-2002 seasons. Again there were insufficient data from the 1999-2000 season for statistical analysis to be performed. Results from ANOVA for the flowering growth stage demonstrate that there is no significant difference (p=0.05) in the 2000-2001 and 2001-2002 seasons for the effect of nitrogen fertiliser level.

The final stage at which rice plants were sampled was maturity. At this stage, plants were very dry, average moisture content of 54 %, and straw like and in the process of senescence. The plant material no longer exhibited its fresh, green grassy aroma, but was reminiscent of straw or hay, and the concentration of volatile compounds was greatly diminished.

At the maturity stage, nitrogen fertiliser level had no significant effect (p=0.05) on the aroma volatiles over both the 2000-2001 and 2001-2002 seasons. In addition, there was no significant difference between varieties.

Data was also collected for the concentration of volatile compounds present in rice grain fertilised at 150 and 50kg N/ha for the 2000-2001 and 2001-2002 seasons. Statistical analysis (two way ANOVA) of the data demonstrated that there was no significant effect (p=0.05) of nitrogen fertiliser level on the overall concentration of aroma volatiles in the rice grain for 2000-2001 and 2001-2002 seasons and no significant difference between varieties for both seasons. For both seasons the concentration of most volatile compounds was significantly higher in grain fertilised at the low nitrogen level for all the fragrant varieties and non-fragrant L203 variety, but was lower in the non-fragrant Millin variety. For Millin, the compounds included pentanal; butane-2,3-dione; (E)but-2-enal; pentane-2,3-dione; hexanal; 2-n-butylfuran; butan-1-ol; heptan-2-one; heptanal; (E)hex-2-enal; 2-pentyl furan; pentan-1-ol; 3-hydroxybutan-2-one; octanal; (E)hept-2-enal; 6-methylhept-5-en-2-one; hexan-1-ol; nonanal; (E)oct-3-en-2-one; (E)oct-2-enal; methylcyclobutane; oct-1-en-3-ol; heptan-1-ol; 2-furancarboxaldehyde; (E,E)hepta-2,4-dienal; decanal; benzaldehyde; (E)non-2-enal; octan-1-ol; (E)dec-2-enal; (E,E)deca-2,4-dienal; tridecan-2-one; indole and tetradecanoic acid. These compounds are significant contributors to the aroma of rice grain. This may be a trait of the non-fragrant Millin variety. There were few volatile compounds that were present in lower concentrations in the rice plants fertilised at the low nitrogen level for all varieties over the two seasons. These included 2-ethyl furan, pyridine and undecane. There were also compounds that were not affected by the nitrogen fertiliser rate.

The concentration of 2-AP present in the rice grain fertilised at the normal and low nitrogen rates was very similar for both the 2000-2001 and 2001-2002 seasons. These results may be compared with those of Suwanarit *et al*. (1996) cited by Rohilla *et al*. (2000), who reported that aroma, softness, whiteness, stickiness and glossiness of cooked milled rice of Khao Dawk Mali 105 were adversely related to applied doses of nitrogen.

### 4.1.3 Effect of the level of nitrogen fertiliser on the concentration of 2-Acetyl-1-pyrroline in rice plants and grain

**Table 4.1 Concentration of 2-AP in rice plants fertilised at 150 and 50kg N/ha (1999-2000 season)**

| | Concentration of 2-AP (ppb) in the 1999-2000 season | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Kyeema | | YRF207 | | YRF203 | | Millin | |
| **Growth stage** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** |
| **Tillering** | 463 | 442 | 511 | 56 | 344 | 561 | 26 | 5 |
| **Panicle Initiation** | 489 | 272 | 161 | 353 | 184 | 39 | - | 8 |
| **Flowering** | 525 | 208 | 584 | 320 | 380 | 329 | 7 | 1 |
| **Maturity** | 79 | 3.8 | 249 | 43 | 124 | 26 | 2 | 1 |

Table 4.1 presents the concentration of 2-AP in rice plants fertilised at 150 and 50kg N/ha for the 1999-2000 season in Kyeema, YRF207, YRF203 and Millin rice varieties respectively. Generally all varieties fertilised at the normal nitrogen level had a higher concentration of 2-AP than plants fertilised at the low nitrogen rate.

**Table 4.2 Concentration of 2-AP in rice plants fertilised at 150 and 50kg N/ha (2000-2001 season)**

| | Concentration of 2-AP (ppb) in the 2000-2001 season | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Kyeema | | YRF207 | | L203 | | Millin | |
| **Growth stage** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** |
| **Tillering** | 93 | 71 | 136 | 79 | 0 | 2 | 0 | 6 |
| **Panicle Initiation** | 1508 | 1292 | 1747 | 1811 | 0 | 0 | 0 | 0 |
| **Flowering** | 276 | 300 | 501 | 487 | 7 | 0 | 2 | 0 |
| **Maturity** | 169 | 188 | 309 | 288 | 0 | 8 | 0 | 11 |
| **Grain** | 295 | 251 | 70 | 99 | 1 | 5 | 2 | 3 |

Table 4.2 presents the concentration of 2-AP in rice plants and grain fertilised at 150 and 50kg N/ha for the 2000-2001 season in Kyeema, YRF207, L203 and Millin rice varieties respectively. The results indicate that plants fertilised at the normal and low nitrogen rates have similar concentrations of 2-AP in the rice plant tissue samples and in the mature grain. The effect of nitrogen seems to be greater in the non-fragrant rice varieties L203 and Millin, however, the concentrations are so low that the differences are not significant. The nitrogen content of the rice plants and grain at the various growth stages was determined to see if there was a difference in nitrogen content between samples fertilised at the two nitrogen rates.

**Table 4.3 Nitrogen (%) in rice plants and grain fertilised at 150 and 50kgN/ha (2000-2001 season)**

| | % Concentration of nitrogen | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Kyeema | | YRF207 | | L203 | | Millin | |
| **Growth stage** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** |
| Panicle initiation | 2.67 | 2.47 | 3.12 | 2.45 | 2.93 | 3.17 | 3.86 | 2.75 |
| Flowering | 1.66 | 1.44 | 1.48 | 1.69 | 1.81 | 1.42 | 0.96 | - |
| Maturity | 0.74 | 0.72 | 0.85 | 0.62 | 0.92 | - | 0.64 | 0.72 |
| Grain | 1.54 | 1.34 | 1.44 | 1.45 | 1.48 | 1.39 | 1.62 | 1.50 |

Table 4.3 shows the % nitrogen present in rice plant and grain samples fertilised at the two nitrogen rates for the 2000-2001 season. The results demonstrate that the plants and grain fertilised at both rates had similar concentrations of nitrogen. Therefore this may have been the reason why the concentration of 2-AP in the rice plants and grain were not significantly affected by the nitrogen fertiliser rates. This may indicate that the cultivation area of the rice was naturally fertile and compensated for the low fertiliser rate.

**Table 4.4 Concentration of 2-AP in rice plants fertilised at 150 and 50kg N/ha (2001-2002 season)**

| | Concentration of 2-AP (ppb) in the 2000-2001 season | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Kyeema | | YRF207 | | YRF203 | | Millin | |
| **Growth stage** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** |
| **Tillering** | 843 | 744 | 639 | 325 | 932 | 440 | 0 | 8 |
| **Panicle Initiation** | 284 | 216 | 281 | 250 | 297 | 387 | 0 | 0 |
| **Flowering** | 311 | 335 | 298 | 328 | 350 | 326 | 0 | 0 |
| **Maturity** | 384 | 526 | 307 | 406 | 410 | 490 | 13 | 17 |
| **Grain** | 361 | 379 | 143 | 133 | 303 | 296 | 2 | 3 |

Table 4.4 presents the effect of nitrogen fertiliser level on the concentration of 2-AP in rice plants and grain in different rice varieties for the 2001-2002 season. Generally the effects of nitrogen fertiliser level are seen only at the earlier growth stages of tillering and panicle initiation where the plants fertilised at the low nitrogen level contain less 2-AP compared to the plants fertilised at the normal nitrogen rate.

Table 4.5 presents the concentration of nitrogen in rice plants and grain fertilised at 150 and 50kg N/ha in the different rice varieties for the 2001-2002 season. Plants fertilised at the low nitrogen rate had less nitrogen compared to the plants fertilised at the normal nitrogen rate. The greatest differences in nitrogen concentration are seen at the earlier growth stages of tillering panicle initiation and flowering where the nitrogen concentration is the highest compared to rice plants at maturity and the rice grain.

**Table 4.5 Nitrogen (%) in rice plants and grain fertilised at 150 and 50kgN/ha (2001-2002 season)**

| | % Concentration of nitrogen | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Kyeema | | YRF207 | | YRF203 | | Millin | |
| **Growth stage** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** | **150kg N/ha** | **50kg N/ha** |
| Tillering | 2.80 | 1.78 | 3.10 | 2.38 | 3.22 | 2.40 | 3.01 | 2.08 |
| Panicle initiation | 1.94 | 1.69 | 2.02 | 1.67 | 2.30 | 1.45 | 2.09 | 1.20 |
| Flowering | 2.64 | 1.33 | 2.74 | 1.59 | 2.44 | 1.67 | 2.62 | 1.22 |
| Maturity | 0.47 | 0.42 | 0.60 | 0.47 | 0.45 | 0.47 | 0.52 | 0.49 |
| Grain | 1.66 | 1.34 | 1.61 | 1.33 | 1.42 | 1.29 | 1.32 | 1.44 |

## 4.2 Comparison of the aroma of Australian and imported fragrant rice

Table 7.2 (Appendix) shows volatile compounds and their concentrations in the five Basmati rice samples. There was a great difference in the concentration of volatile compounds between the Australian Basmati breeding line YRF203 and the imported Basmati varieties. These findings of were consistent with sensory analysis performed on YRF203 and Riviana samples where consumers detected a significant difference between the aroma of these two rices, but neither was preferred over the other (see section 4.3). Table 7.2 (Appendix) demonstrates that YRF203 has much higher total concentration of volatile aroma compounds (approximately 5800 ppb) compared to most of the imported varieties (2000-2400ppb). One exception was Kumarnthong imported Basmati rice with a total volatile aroma concentration of approximately 4900ppb. Other major differences of the Australian YRF203 sample compared to the imported samples were the absence of some compounds and the higher concentrations of others. These differences will be discussed later. It should be noted that one reason for these differences may be the different storage conditions that the rice samples experienced. The YRF203 sample was freshly harvested and had undergone very limited storage, while the storage history of the imported rice varieties is unknown. It is most likely that they were stored for 12 months in jute bags as this is a traditional practice for Basmati rice. This storage period probably results in many changes to the volatile aroma compounds, especially in association with lipid

reactions. The effects of storage will be investigated later in the chapter. YRF203 did not contain some compounds that were found in the imported samples. These included (E)hex-2-enal; octan-2-one; dimethyl trisulphide; 2-furancarboxaldehyde; heptan-1-ol; (E,E)octa-3,5-dien-2-one; glycocyanidine; undecan-1-ol; (E,E)nona-2,4-dienal; (E)undec-2-enal; benzyl alcohol; phenyl ethyl alcohol; 9-octadecenoic acid [Z] methyl ester, and 9,12-octadecadienoic acid [Z,Z] methyl ester. The majority of these compounds, including the aldehydes, alcohols and ketones, would most likely be formed by lipid oxidation of unsaturated fatty acids (Whitfield, 1992). However, compounds such as dimethyl trisulphide may arise from the degradation of sulphur containing amino acids (cysteine and methionine) that give rise to precursors, such as hydrogen sulphide and methanethiol (Schutte, 1974).

There were compounds in YRF203 present at higher levels compared than in imported samples. These included butane-2,3-dione; 2-AP; decanal; (E)non-2-enal; (E,E)deca-2,4-dienal; 4-vinylguaiacol; decanoic acid; tetradecanoic acid; hexadecanoic acid and 9,12-octadecadienoic acid [Z, Z]. These compounds may have been degraded or lost during storage, or were not present at all, in the imported rice. The most important compound to consider is 2-AP, that was considerably lower in the imported rice varieties compared to the YRF203 sample. This does not necessarily indicate that YRF203 has much greater fragrance, but may be attributed to loss during storage of the imported samples. Loss of 2-AP during storage will be discussed later.

Kumarnthong brand Basmati had more total volatile compounds than the other imported rice, but less than in the YRF203 sample, although it had similar levels of hexanal; heptanal; 2-pentylfuran; pentan-1-ol; octanal; (E)hept-2-enal; nonanal; (E)oct-3-en-2-one; (E)oct-2-enal; oct-1-en-3-ol; heptan-1-ol; benzaldehyde; (E)non-2-enal; octan-1-ol; (E)dec-2-enal; (E,E)deca-2,4-dienal; benzyl alcohol; phenylethyl alcohol; octanoic acid; indole; (Z)9-octadecenoic acid methyl ester and (Z,Z)9,12-octadecadienoic acid methyl ester.

The results indicate that the Australian Basmati breeding line YRF203 has great fragrance potential. However, the major point to focus on with the development of the Basmati rice with respect to aroma will involve storage protocols. Storage conditions of Basmati rice will not only play a vital role in texture development of the rice, but also may affect changes in volatile aroma compounds and aroma development. Therefore, future work in this area will be essential.

Table 7.3 (Appendix) presents volatile compounds and their levels found in Australian and imported Jasmine rice samples. Kumarnthong, Green Earth, Lion and Tilda are imported Jasmine rice brands that are products of Thailand. The Koala and Sunrice Jasmine brands are the commercial Australian Jasmine rice that is the Kyeema variety and YRF207 is an Australian Jasmine breeding line, both of which were grain samples from the 2001-2002 rice season grown at YAI, Leeton NSW.

There were both similarities and differences between the various imported Jasmine samples tested. The Tilda and Lion brands had similar total concentration of volatile compounds (4200-4300 ppb) and also showed remarkable similarities in the concentration of individual aroma compounds. Since these two imported samples are both products of Thailand, it is possible that they are the same or related varieties. The Kumarnthong and Green Earth brands had significantly lower concentrations of total volatile aroma compounds (2200-2600 ppb) compared to the Tilda and Lion brands. A major contributing factor to this lower level of total volatile compounds was the reduced concentration of acids including tetradecanoic acid; hexadecanoic acid; oleic acid and 9,12-octadecadienoic acid [Z,Z] in the Kumarnthong and Green Earth brand samples. The Kumarnthong and Green Earth samples, however, had a number of individual aroma compounds that were present at significantly different concentrations and therefore did not possess the remarkable similarities that were seen between the Tilda and Lion brand samples. The Green earth brand sample contained significantly higher concentrations of volatiles including, 2-pentylfuran; 2-AP; nonanal; (E)oct-2-enal; (E)non-2-enal; (E)dec-2-enal and (E,E)deca-2,4-dienal. These compounds are known to contribute significantly to the aroma of rice due to their low aroma thresholds. The type of aroma that they would contribute would include, nutty, popcorn, green, floral and fatty notes. On the other hand the Kumarnthong brand sample contained significantly more 4-vinylguaicol and indole, which generally possess unpleasant

odours at higher concentrations. Therefore these results would suggest that the Green Earth Brand would have a more desirable aroma qualities.

The most important comparisons for the Australian rice industry are between the imported and Australian rice varieties. Firstly the Kumarnthong brand imported Jasmine rice and the Koala brand Australian Jasmine rice will be compared. Sensory analysis of the Kumarnthong and Koala brand discussed in section 4.3 showed that the aroma of these two rices could be distinguished. Koala brand and Kumarnthong brand have similar levels of total volatile compounds of approximately 2400-2600 ppb (Table 7.3). Many of the compounds are present in similar proportions, however there are significant differences between a number of compounds which would explain the different aroma detected by consumers. The Kumarnthong brand had a higher concentration of (E)but-2-enal and indole. Indole contributes faecal and floral aromas on high dilution. The Koala brand had a higher concentration of compounds including (E)hept-2-enal; (E)dec-2-enal and (E,E)deca-2,4-dienal. These compounds have low aroma thresholds and will generally contribute green, fatty aromas that are desirable for rice aroma. The sensory differences that could be detected by consumers are also reflected by the different concentrations of volatile aroma compounds.

Koala and Sunrice brand samples are the Australian variety Kyeema. However, Kyeema that is seen as lower in quality is packaged under the Koala brand, while the premium quality Kyeema grain produced is packaged under the Sunrice brand. The Sunrice sample contained a concentration of total volatile compounds similar to those of the imported Tilda and Lion brand samples, compared to the significantly lower concentration in the Koala brand sample. Like the imported Tilda and Lion samples, the major volatiles contributing to this increased total volatile concentration in Sunrice samples were tetradecanoic acid, hexadecanoic acid, oleic acid and 9,12-octadecadienoic acid.

The results demonstrate that the concentration of individual volatile compounds in the Sunrice sample closely resembles those in the imported Tilda and Lion brand samples. Again these similarities to imported rice varieties are promising for the future potential of Australian fragrant rice.

Freshly harvested Kyeema and YRF207 contained up to 3 times the concentration of 2-AP, tetradecanoic acid, hexadecanoic acid and 9,12-octadecadienoic acids compared to all other samples tested. In addition some volatile compounds including octan-2-one; nonan-2-one; 2-furancarboxaldehyde; heptan-1-ol; glycocyanidine; (E,E)nona-2,4-dienal; methyl salicylate; 2-undecenal; (E,E)deca-2,4-dienal; benzyl alcohol; phenylethyl alcohol; phenol; (Z)9-octadecanoic acid methyl ester and (Z,Z)9,12-octadecadienoic acid methyl ester were present in all other samples but not present in Kyeema and YRF207. These major differences may probably due to the fact that these samples were tested when they were freshly harvested or could reflect varietal differences.

The Kyeema and YRF207 samples had very similar concentrations for most of their volatile aroma compounds. However, the YRF207 sample contained significantly less 2-AP than the Kyeema sample (Table 7.3, Appendix). This finding was consistent with the results from the 2000-2001 season, where the YRF207 sample was found to have a concentration of 70.3 ppb of 2-AP whereas Kyeema and YRF203 (Basmati breeding line) were found to have 294.4 and 354.5 ppb of 2-AP respectively. These findings raise some concern over the aroma potential or quality of the YRF207 breeding line.

For Australian fragrant rice to be competitive its aroma needs to resemble that of the imported varieties. The results of this research indicate that Australian Jasmine and Basmati varieties meet this criterion.

Basmati and Jasmine type rices have their own distinct aroma. For Jasmine, the aroma is generally known to Orientals as ‘pandan’ like due to similarities to pandan leaves which contain high levels of 2-AP (Buttery, Juliano and Ling, 1983). Western consumers generally describe the aroma of fragrant rices as ‘popcorn’ like.

The identity and concentration of individual aroma compounds from Basmati and Jasmine type rices can be compared (Tables 7.2 and 7.3). Most volatile compounds are common to both fragrant rice types and are generally present in similar proportions. The significant differences are that Jasmine type rices had greater concentrations of methyl salicylate; (E,E)deca-2,4-dienal; (E,Z)deca-2,4-dienal; (E)hept-2-enal; 2-pentylfuran; (E)but-2-enal and hexanal compared to the Basmati type rices. These compounds commonly contribute, green, fatty and sweet aromas. Therefore Jasmine rice may be described as having a fresher, greener, slightly fatty aroma compared to Basmati fragrant rice. This was supported by sensory data with major aroma descriptions of Jasmine rice including sweet and fresh. The main aroma descriptors for the Basmati rice included popcorn, corn cornchips, old, smoke and rice. These descriptors were mainly describing the Australian Basmati breeding line YRF203 and therefore would mainly be attributed to the significantly high concentration of 2-AP.

A selection of 14 Basmati breeding lines developed by breeders from NSW Agriculture YAI was analysed to determine the identity and concentration of volatile aroma compounds present and to investigate the fragrance potential of these lines. A storage trial was also performed where a selection of the breeding lines underwent a 3-month storage period at 20ºC. These breeding lines were from the 2000-2001 rice season.

Tables 7.4 and 7.5 (Appendix) demonstrate the concentration of volatile aroma compounds present in fresh and stored Basmati breeding line samples respectively.

There were many significant differences in the concentration of individual aroma compounds between the stored and fresh samples. Compounds including butan-1-ol; thiazole; 6-methylhept-5-en-2-one; dimethyl trisulphide; nonan-2-one; methylcyclobutane; 2-furancarboxaldehyde; (E,E)deca-2,4-dienal; dodecan-2-one; octanoic; 4-vinyl guaiacol; 4-vinylphenol; dodecanoic acid and dibutyl phthalate were present at significantly higher concentrations in the stored Basmati rice samples. These compounds were formed during storage from the breakdown of other compounds or grain metabolism. On the other hand compounds such as (E)but-2-enal; pentane-2,3-dione; hexanal; heptan-2-one; heptanal; (E)hex-2-enal; 2-pentylfuran; pentan-1-ol; 3-hydroxybutan-2-one; 2-AP; hexan-1-ol; heptan-1-ol and oleic acid were present in significantly higher concentrations in the fresh samples. These compounds are mostly of low boiling point and may be lost by volatilisation from the rice. Others may be lost by chemical reaction. The effects of storage on the concentration of 2-AP were quite severe with losses of 50-66% occurring. It is important to note that these significant changes have occurred in the rice in a very short storage period under very mild conditions. Basmati rice is traditionally stored for up to a year before it is consumed under storage conditions that may involve the rice being subject to temperatures much greater than 20ºC. This demonstrates the importance of storage with respect to the aroma quality of fragrant rice. A similar situation was seen when the aroma of cooked rice between a commercial Basmati rice and cultivated Italian line B5-3 were compared (Tava and Bocchi, 1999). The commercial Basmati had undergone a longer storage time compared with B5-3, which was analysed just after harvesting.. 2-AP was present at 570 and 2350ppb in the commercial Basmati and B5-3 respectively. It is suggested that the higher 2-AP content in B5-3 was most likely due to differences in genotype and environmental conditions. However, it is also highly probable that the difference in 2-AP levels was also due to storage time since it has been found (Widjaja *et al.*, 1996b) that losses of 2-AP during storage can be quite significant.

The aroma potential of the breeding lines was also investigated. The results indicate that generally the stored Basmati breeding lines analysed had similar levels of volatile compounds. However, there were some significant differences. The most striking difference was the concentration of 2-AP in Entry 71. Entry 71 contained twice the level of 2-AP compared to all other breeding lines. This suggests that this breeding line had greater fragrance potential than the other breeding lines did. Some breeding lines (Entry 38, 53 and 71) had higher levels of alcohols, such as butan-1ol, pentan-1-ol and hexan-1-ol. This could be a varietal characteristic. Therefore, these results for the Basmati breeding lines are promising. Except for entry 71, all lines had similarly high levels of 2-AP, which would indicate potential fragrance. These results are promising for the Australian rice industry because they demonstrate that it is possible to grow Basmati type rices that will express fragrance characteristics

under Australian growing conditions. However, effects of storage conditions are an essential area for future research.

## 4.3 Sensory responses to the aroma of Australian and imported fragrant rice and non-fragrant rice

### 4.3.1 Trial 1

Raw data relating to the triangle sensory tests was recorded by listing each participant's gender, age and cultural background and then recording their result for each task as correct or incorrect (This raw data is not included but is available by request). Any comments were also recorded. Plotting the minimum numbers of correct judgements to establish significance at the 0.05 probability level against the number of trials or test subjects produced a standard curve (O'Mahony, 1986 Table G.5.c, appendix G). A regression equation was produced from the data. This equation was used to determine the minimum numbers of correct responses to establish significant differentiation from the triangle tests performed in the study.

The two hypotheses devised for analysis of the complete set of raw data included:

- Null hypothesis (Ho) 1: There is no significant difference between the aroma of fragrant and non-fragrant rice (Set 1).
- Alternate hypothesis 1: There is a significant difference between the aroma of fragrant and non-fragrant rice (Set 1).
- Null hypothesis (Ho) 2: There is no significant difference between the aroma of Australian Jasmine fragrant and imported Jasmine fragrant rice (Set 2).
- Alternate hypothesis 2: There is a significant difference between the aroma of Australian Jasmine fragrant and imported Jasmine fragrant rice (Set 2).

To test the hypotheses the number of correct responses from subjects is compared to the minimum number correct required. Subjects accept the null hypothesis (Ho) when the minimum number correct is less than the recorded number correct.

#### *4.3.1.1 Overall panel responses*

For the 254 subjects participating in the triangle test comparing Australian fragrant and non-fragrant rice aroma, the minimum number of correct judgements required to establish significant differentiation between them was 103. A total of 139 subjects recorded a correct result. Therefore the Null hypothesis (Ho) 1 was rejected and the consumer group as a whole found a significant difference ($p<0.05$) between the aroma of AJF and NF samples.

For the 226 subjects participating in the second task involving Australian Jasmine fragrant and imported Jasmine fragrant rice aroma, the minimum number of correct judgements required to establish significant differentiation between them was 92. A total of 115 recorded a correct result, establishing that the consumer group as whole found a significant difference ($p<0.05$) between the aromas of the two fragrant rices.

The imported varieties are seen as the benchmark and the Australian rices must meet or even exceed their standards of fragrance. The ability of consumers to differentiate between the two will be discussed later when the comments recorded from the consumers are analysed.

#### *4.3.1.2 Effects of age, gender and cultural background*

The raw data obtained from the sniffing test were also broken up into non-Australian versus Australian background, male versus female and 6-12, 13-18 and $19^{+}$ age groups to investigate the effect of

cultural background, gender and age, respectively, on the outcome of the two hypotheses outlined previously.

Cultural backgrounds were categorised into Australian (135 and 127 subjects for tasks 1 and 2, respectively), non-Australian (105 and 83) and Asian (49 and 40) subjects. Non-Australians were those from cultural backgrounds other than Australian and a further subset of these were 'Asian' subjects, who were from Thailand, Taiwan, Burma, Korea, China, Sri Lanka, India, Malaysia, Hong Kong, Japan, Singapore and Vietnam. These were categorised separately because of the greater likelihood that they would be familiar with fragrant rices.

There was a significant difference detected by males and females and individuals of all cultural backgrounds, with one exception, for the aroma of fragrant versus non-fragrant rice and Australian Jasmine fragrant versus imported Jasmine fragrant rice. The exception was that Asian subjects could not distinguish between Australian and imported Jasmine fragrant rice.

In addition, all age groups could distinguish between Australian and imported Jasmine rice, but the $19^{+}$ group could not distinguish between fragrant and non-fragrant rice aroma. The low numbers of participants in this age category may explain this result.

#### *4.3.1.3 Chi-square statistics*

With the use of chi-square statistics the following hypotheses were tested:

- Null hypothesis (Ho) 1: There is no significant difference between two specific target groups in their ability to distinguish between the aroma of fragrant and non-fragrant rice (Set 1).
- Alternate hypothesis 1: There is a significant difference between two specific target groups in their ability to distinguish between the aroma of fragrant and non-fragrant rice (Set 1).
- Null hypothesis (Ho) 2: There is no significant difference between two specific target groups in their ability to distinguish between the aroma of Australian Jasmine fragrant and imported Jasmine fragrant rice (Set 2).
- Alternate hypothesis 2: There is a significant difference between two specific target groups in their ability to distinguish between the aroma of Australian Jasmine fragrant and imported Jasmine fragrant rice (Set 2).

The calculated Chi-square value is then compared to a tabulated value at the 0.05 probability level (Table G.7 O'Mahony, 1986) with the degrees of freedom (d.f) calculated from the equation: d.f = (no. rows-1) (no. columns-1). If the calculated $\chi^2$ value is > Tabulated $\chi^2$ value, Ho (set 1or set 2) is rejected.

Chi Square statistics indicated that there was no significant difference ($\chi^2$=1.02, $p<0.05$), in the ability of Australians versus non-Australians and Asians, or Males versus Females, at perceiving differences between the aromas from the Australian fragrant and non-fragrant rices. In addition, there was no significant difference ($\chi^2$= 0.44, $p<0.05$) in the ability of Australians versus non-Australians and Asians, or males versus females in distinguishing between the aroma of Australian and imported fragrant Jasmine rice. It was expected that Asians would be better at distinguishing between the rices but this finding may reflect the cultural diversity of Australia cuisines, with widespread consumption of traditional Asian dishes containing fragrant rices.

However, there is a significant difference between subjects of different ages being able to determine the difference between AJF and NF rice. In this case subjects 13-18 years were better then the other age groups. In addition, there was a significant difference between subjects' age of being able to determine the difference between AF and IF rice where subjects$19^{+}$ years were better.

#### *4.3.1.4 Aroma descriptions of Australian and imported Jasmine fragrant rice and non-fragrant rice*

Comments for Australian fragrant rice versus non-fragrant rice and those relating to Australian fragrant rice versus imported fragrant rice were also recorded. The most commonly used descriptor for describing the odd samples for both the Australian fragrant and non-fragrant was “stronger”. Negative comments used to describe the non-fragrant rice included rotten, off smell, worse, disgusting, unpleasant, bad smell, rotten eggs, revolting, rancid and stinks more. Positive comments to describe the non-fragrant rice included hot chips and nicer. On the other hand negative comments used to describe the aroma of the fragrant rice included, rotten, off smell, worse and disgusting. Positive comments for the fragrant rice were, nicer, didn’t stink and fresher.

Similarly, the most commonly used descriptor for describing the odd samples for both the Australian fragrant and imported fragrant rice samples was “stronger”. There were many negative comments towards the imported fragrant rice including, off smell, rotten, not as nice, sulphur, off faint, disgusting faint, worse and yucky. The imported fragrant rice had few positive comments such as better and cornier. The Australian fragrant rice had very few negative comments, including off smelling, yucky, and more positive comments, such as, nicer, less stinky and better. This would suggest that the aroma of the Australian fragrant rice was preferred over the imported fragrant rice.

#### *4.3.1.5 Volatile components of the rice samples*

Figures 7.1, 7.2 and 7.3 (Appendix) present the chromatograms of volatile components isolated from Australian fragrant, imported fragrant and non-fragrant rice respectively. The two chromatograms of fragrant rice (Fig 7.1 and 7.2) are similar, with that of non-fragrant rice (Fig 7.3) being different in several respects.

The compounds used in relation to consumer odour perception were selected based on findings from Yajima (1978, 1979), Buttery *et al.,* (1988), Paule and Powers (1989), Widjaja, Craske and Wootton (1996a), Petrov *et al.* (1996) and Grosch and Schieberle (1997). Table 4.6 lists the selected aroma compounds, their odour descriptions, odour thresholds, concentrations and odour units.

**Table 4.6 Concentration, thresholds, odour unit and odour description of significant volatile aroma compounds in Australian and imported Jasmine fragrant rice and non-fragrant rice**

| | | | Rice Grain Samples | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Imported Jasmine | | Australian Jasmine | | Non-fragrant | |
| **Significant aroma Compounds in rice** | **Thresh (ppb)[h]** | **Odour description** | **Conc (ppb)** | **Odour Unit[i]** | **Conc (ppb)** | **Odour Unit** | **Conc (ppb)** | **Odour Unit** |
| Hexanal[a,b,c,e,f,g] | 5 | Green,grass[e,j] | 853 | 171 | 998 | 199.6 | 1960 | 301.4 |
| Butanol[a] | 500 | Medicinal[j] | 5 | 0.01 | 6 | 0.008 | 9 | 0.02 |
| Heptan-2-one[e,f] | 140 | Fruit,spicy[e,j] | 23 | 0.16 | 25 | 0.18 | 40 | 0.3 |
| Heptanal[c,e,f] | 3 | Fruity,fatty[e,f] | 25 | 8.3 | 20 | 6.8 | 26 | 8.6 |
| (E)hex-2-enal[e] | 17 | Green,fruity[e] | 7 | 0.4 | 7 | 0.4 | 15 | 0.9 |
| 2-Pentyl furan[c,e] | - | Nutty,beany[e] | 35 | - | 47 | - | 78 | - |
| Pentan-1-ol[a,e,f] | 4000 | Sweet,strong[j] | 84 | 0.02 | 102 | 0.03 | 104 | 0.03 |
| Octanal[c,f] | 0.7 | Citrus, fatty[f,j] | 26 | 37.3 | 24 | 34.1 | 29 | 41.4 |
| (E)hept-2-enal[c,e,f] | 13 | Fatty[f],green[j] | 45 | 3.5 | 93 | 7.1 | 80 | 6.2 |
| 2-acetyl-1-pyrroline[c,d,e,f,g] | 0.1 | Sweet[e], popcorn[c,d,f,g] | 49 | 490 | 34 | 338 | 3 | 30 |
| 6-methylhept-5-en-2-one[e] | 50 | Herby, green[j] | 11 | 0.2 | 7 | 0.14 | 3 | 0.07 |
| Hexan-1-ol[b,c,d,f] | 2500 | Sweet,green[f,j] | 51 | 0.02 | 77 | 0.03 | 59 | 0.02 |
| Nonanal[a,c,e,g] | 1 | Floral,fatty[j] | 28 | 28.2 | 29 | 28.5 | 42 | 41.9 |
| (E)oct-2-enal[a,e,f] | 3 | Green,herby[e,j] | 47 | 15.8 | 59 | 19.7 | 95 | 31.5 |
| Oct-1-en-3-ol[e,f] | 1 | Herby,earthy[f,j] | 34 | 33.6 | 40 | 39.9 | 58 | 51.5 |
| 2-ethyl hexanol[a] | - | Oily,sweet[j] | 0 | - | 0 | - | 44 | - |
| Benzaldehyde[c,e,f] | 350 | Nutty,sweet[e,f,j] | 36 | 0.1 | 23 | 0.06 | 49 | 0.14 |
| (E)non-2-enal[c,g] | 0.08 | Fatty,waxy[f,j] | 14 | 175 | 14 | 175 | 28 | 350 |
| (E)dec-2-enal[c] | 0.4 | Sl.fatty,green[j] | 11 | 31.1 | 38 | 95.0 | 15 | 38.3 |
| (E,E)deca-2,4-dienal[c,e,f,g] | 0.07 | Fatty, citrus, powerful[e,f,j] | 13 | 191 | 36 | 518.6 | 31 | 437.1 |
| 4-vinylguaicol[a,c,e] | 3 | Spicy,fruity[j] | 15 | 5.0 | 14 | 4.6 | 42 | 14.1 |
| Indole[b,e] | 140 | Faecal,floral[e,j] | 12 | 0.09 | 2 | 0.02 | 17 | 0.12 |

a- Yajima *et al.* (1978)
b- Yajima *et al.* (1979)
c- Buttery *et al.* (1988)
d- Paule and Powers (1989)
e- Widjaja, Craske and Wootton (1996)
f- Petrov *et al.* (1996)
g- Grosch and Schieberle (1997)
h- Threshold data from Buttery *et al.* (1988)
i- Odour unit (Uo) calculated by conc (ppb)/threshold (ppb)
j- Aldrich (1998-99)

The characteristic aroma of fragrant rice is attributed mainly to 2AP. The concentration of 2AP which varies from 40-90 ppb in fragrant rice varieties to 8 ppb or less in non-fragrant rice (Buttery *et al.*, 1983).

The significance of a volatile compound to the total aroma is determined by calculation of the odour unit (Uo) for that compound. The odour unit is calculated by dividing the concentration (C) of the compound in food by the odour threshold (T) of the compound in water (Uo = C/T). Assuming that T is the same for the food, a compound is considered to have a significant contribution to the total odour when Uo is greater than one. For example, Petrov *et al.*, (1996), determined the odour unit of 2AP in a fragrant rice to be 250. From Table 18, compounds expected to contribute to the aroma of both fragrant and non-fragrant rice included 2-AP, hexanal; heptanal; octanal; (E)hept-2-enal; nonanal; (E)oct-2-enal; oct-1-en-3-ol; (E)non-2-enal; (E)dec-2-enal; (E,E)deca-2,4-dienal and 4-vinylguaicol.

2-Ethylhexanol was present only in non-fragrant rice, therefore would contribute to the characteristic aroma of the non-fragrant rice. In addition, non-fragrant rice also contained approximately twice the amount of (E)hex-2-enal; nonanal; 2-pentylfuran; (E)oct-2-enal; (E)non-2-enal; hexanal and 4-vinylguaicol than the fragrant rices.

The Australian fragrant and imported fragrant rice had the same significant volatile aroma compounds but at differing concentrations.. It is such differences that give the characteristic aromas to each rice. The Australian fragrant rice was found to contain twice the amount of (E)hept-2-enal and three times the amount of (E)dec-2-enal and (E,E)deca-2,4-dienal compared to the imported fragrant rice. In addition the imported fragrant rice contained significantly more indole than the Australian fragrant rice.

#### *4.3.1.6 Correlation of sensory descriptions with individual aroma compounds*

The sensory findings on aroma differences and descriptions were compared with the concentrations of significant volatile aroma compounds in the rice samples (Table 4.6). The concentration of the volatile aroma compounds, including, (E)hept-2-enal; (E)dec-2-enal and (E,E)deca-2,4-dienal were much greater in the Australian fragrant rice compared to the imported fragrant rice. The common odours contributed by these compounds include green and fatty odours. Therefore comments such as nicer, better and fresher, for the Australian fragrant rice, may be attributed to the higher concentrations of these compounds compared to the imported fragrant rice. The imported fragrant rice contained extensively more indole compared to the Australian fragrant rice. Indole contributes faecal, putrid, musty, unpleasant odours, but exhibits floral odours at low concentrations. Therefore aroma descriptions of the imported rice such as, off smell, rotten, sulphur, disgusting and pungent may be due to the high indole levels.

The most significant difference in volatile components between fragrant and non-fragrant rice was the concentration of 2AP. Its contribution to the aroma of Australian and imported fragrant rice included odours that were sweeter, cornier and popcorn-like. Another notable difference between fragrant and non-fragrant rice was that non-fragrant rice contained 2-ethylhexanol while the fragrant rice did not. This compound contributes oily, sweet and slightly rosy odours. In addition non-fragrant rice also contained significantly more (E)hex-2-enal, nonanal, 2-pentylfuran, (E)oct-2-enal, (E)non-2-enal and hexanal compared to the fragrant rices. These volatile compounds contribute odours such as green, grassy and fatty. And may explain aroma descriptions like rancid and hot chips. The non-fragrant rice also had a significant number of negative odour descriptions such as unpleasant, bad smell, rotten eggs, unlike rice and revolting. This may have been due to the high level of 4-vinylguaiacol that can contribute an‘unpleasant’ odour.

### 4.3.2 Trial 2

Raw data relating to the triangle sensory test for trial 2 included participant’s gender, age and cultural background and their result for the task as a correct or incorrect answer were recorded. A preference and odour description for the duplicate and odd samples is also recorded (raw data is not listed but is available on request). A standard curve was produced and was used to determine the minimum numbers of correct responses to establish significant differentiation from the triangle tests performed in the study as in trial 1.

The hypothesis devised for analysis of the complete set of raw data included:

- Null hypothesis (Ho) 1: There is no significant difference between the aroma of Australian Basmati fragrant and Imported Basmati fragrant rice.
- Alternate hypothesis 1: There is a significant difference between the aroma of Australian Basmati fragrant and Imported Basmati fragrant rice.

#### *4.3.2.1 Overall panel responses*

For the 127 subjects participating in the triangle test comparing Australian Basmati and Imported Basmati rice aroma, the minimum number of correct judgements required to establish significant differentiation between them was 53. A total of 65 subjects recorded a correct result, establishing that the consumer group as a whole found a significant difference ($p<0.05$) between the aromas of the two fragrant rice samples.

#### *4.3.2.2 Effects of age, gender and cultural background*

Differences in the ability of subjects to distinguish between the aroma of Australian fragrant and imported fragrant rice based on age, gender and cultural background was investigated. Cultural backgrounds were categorised into Australian (67 subjects), non-Australian (60) and Asian (40) subjects. Non-Australians were those from cultural backgrounds other than Australian and a further subset of these were 'Asian' subjects, who were from Thailand, China, Sri Lanka, India, Malaysia, Japan, Philippines, Indonesia, Laos, Bangladesh and Vietnam. These were categorised separately because of the greater likelihood that they would be familiar with fragrant rices. The ages were categorised into ranges of 6-12 years (63 subjects), 13-18 years (44) and 19+ years (20). The influence of gender was also investigated with male (40) and female (87 subjects) participating.

The Null hypothesis (Ho) was rejected for both Australian, non-Australian and Asian subjects, establishing that these subjects can all distinguish between the aroma of Australian and imported Basmati rice aroma. However consumers of female gender can distinguish between the aroma of Australian and imported Basmati fragrant rice while male subjects cannot. In addition subjects aged 6-18 years were able to distinguish between the aroma of Australian and Basmati fragrant rice whereas the subjects aged $19^{+}$ years could not.

#### *4.3.2.3 Chi-square ($\chi^2$) statistics*

Results from the triangle test from trial 2 were used to test the following hypothesis:

- Null hypothesis (Ho): There is no significant difference between two specific target groups in their ability to distinguish between the aroma of Australian Basmati fragrant and imported Basmati fragrant rice.
- Alternate hypothesis: There is a significant difference between two specific target groups in their ability to distinguish between the aroma of Australian Basmati fragrant and imported Basmati fragrant rice.

Chi-square statistics were applied to the data as they were in trial 1and indicated that there was no significant difference ($\chi^2=1.16$, $p<0.05$), in the ability of Australians versus non-Australians and Asians to perceive differences between the aromas from the Australian and Imported Basmati fragrant rices. There was also no significant difference, ($\chi^2= 1.76$, $p<0.05$) between male and female or between subjects of all ages ($\chi^2= 3.21$, $p<0.05$) in their ability to distinguish between the aroma of Australian and imported Basmati fragrant rice.

### *4.3.2.4 Aroma descriptions and preferences of Australian and imported Basmati fragrant rice*

The preference data collected from the trial 2 were used to test the following hypothesis.

- Null hypothesis (Ho): There is no significant preference for the aroma between the Australian Basmati fragrant and imported Basmati fragrant rice.
- Alternate hypothesis: There is a significant preference for the aroma between the Australian Basmati fragrant and imported Basmati fragrant rice.

The results indicated that there was no preference for the aroma of Australian or imported Basmati rice.

The aroma descriptions for Australian and imported Basmati rice associated with correct responses from the triangle test were also recorded. The most commonly used descriptions for rice aroma included, stronger, popcorn and rice. Both rices had negative and positive aroma description. Some positive aroma descriptions included, nicer, good, better, corn chips and Basmati rice. Negative descriptions of rice aroma included, stinky, bad, weird, disgusting, off, yuck, rotten egg and old. Therefore, it is no surprise that neither was preferred over the other.

### *4.3.2.5 Volatile components of the rice samples*

Figures 7.4 and 7.5 (Appendix) present the chromatograms of volatile components isolated from Australian and imported Basmati fragrant rice respectively. Each variety has an individual pattern of volatile aroma compounds.

It is important to note that the Australian Basmati rice sample was analysed as a freshly harvested sample. The traditional practice for Basmati rice is storage for at least one year before consumption. This is to allow physiochemical changes that result in a more favourable texture of the rice. It is also expected that there would be significant changes in the volatile aroma compounds in this storage period due to loss of some volatile components to the atmosphere or chemical change, and generation of new ones, especially via lipid oxidation.

Table 4.7 lists selected aroma compounds, their odour descriptions, odour thresholds, concentrations and odour units for the two Basmati rice samples andshows many differences between the concentration of volatile compounds in Australian and imported fragrant Basmati rice. Many of the significant would be due to different storage conditions and time. The compounds hexanal, butanol, heptanal, 2-pentylfuran, 2-AP, nonanal, (E)oct-2-enal, oct-1-en-3-ol, (E)non-2-enal, (E,E)deca-2,4-dienal, 4-vinylguaicol and indole are present in significantly greater concentrations in the Australian Basmati rice compared to the imported Basmati variety. The most important to note of these compounds would be that of 2-AP. The significantly lower concentration of 2-AP in the imported Basmati rice may reflect loss of this compound during storage.

Compounds including (E)hex-2-enal and (E)dec-2-enal are present in much lower concentrations in the Australian Basmati compared to the imported Basmati rice. These compounds collectively contribute green and fatty aroma to the rice. The most likely pathway would be lipid degradation, which would explain the resulting fatty aroma. The extent of the differences probably reflects the changes to the volatiles of rice over time.

**Table 4.7 Concentration, thresholds, odour unit and odour description of significant volatile aroma compounds in Australian and imported fragrant Basmati rice**

| | | | Rice grain samples | | | |
|---|---|---|---|---|---|---|
| | | | Imported Basmati | | Australian Basmati | |
| Aroma compounds | Threshold (ppb)[h] | Odour description | Conc (ppb) | Odour unit[i] | Conc (ppb) | Odour unit |
| Hexanal[a,b,c,e,f,g] | 5 | Green,grass[e,j] | 751 | 150 | 1420 | 284 |
| Butanol[a] | 500 | Medicinal[j] | 1 | 0.002 | 7 | 0.01 |
| Heptan-2-one[e,f] | 140 | Fruit,spicy[e,j] | 22 | 0.2 | 23 | 0.2 |
| Heptanal[c,e,f] | 3 | Fruity,fatty[e,f] | 34 | 11.3 | 112 | 37.3 |
| (E)hex-2-enal[e] | 17 | Green,fruity[e] | 5 | 0.3 | 0 | - |
| 2-pentyl furan[c,e] | - | Nutty,beany[e] | 21 | - | 144 | - |
| Pentan-1-ol[a,e,f] | 4000 | Sweet,strong[j] | 139 | 0.03 | 106 | 0.03 |
| Octanal[c,f] | 0.7 | Citrus, fatty[f,j] | 40 | 57.1 | 62 | 88.6 |
| (E)hept-2-enal[c,e,f] | 13 | Fatty[f],green[j] | 22 | 1.7 | 130 | 10 |
| 2-acetyl-1-pyrroline[c,d,e,f,g] | 0.1 | Sweet[e], popcorn[c,d,f,g] | 7 | 70 | 355 | 3550 |
| 6-methylhept-5-en-2-one[e] | 50 | Herby, green[j] | 3 | 0.06 | 9 | 0.2 |
| Hexan-1-ol[b,c,d,f] | 2500 | Sweet,green[f,j] | 45 | 0.02 | 46 | 0.02 |
| Nonanal[a,c,e,g] | 1 | Floral,fatty[j] | 25 | 25 | 368 | 368 |
| (E)oct-2-enal[a,e,f] | 3 | Green,herby[e,j] | 27 | 9 | 103 | 34.3 |
| Oct-1-en-3-ol[e,f] | 1 | Herby,earthy[f,j] | 25 | 25 | 90 | 90 |
| Benzaldehyde[c,e,f] | 350 | Nutty,sweet[e,f,j] | 27 | 0.08 | 39 | 0.1 |
| (E)non-2-enal[c,g] | 0.08 | Fatty,waxy[f,j] | 6 | 75 | 47 | 587.5 |
| (E)dec-2-enal[c] | 0.4 | Sl.fatty,green[j] | 9 | 22.5 | 0 | - |
| (E,E)deca-2,4dienal[c,e,f,g] | 0.07 | Fatty, citrus, powerful[e,f,j] | 8 | 114.3 | 114 | 1628.6 |
| 4-vinylguaicol[a,c,e] | 3 | Spicy,fruity[j] | 23 | 7.7 | 69 | 23 |
| Indole[b,e] | 140 | Faecal,floral[e,j] | 3 | 0.02 | 14 | 0.1 |

k- Yajima *et al.* (1978)
l- Yajima *et al.* (1979)
m- Buttery *et al.* (1988)
n- Paule and Powers (1989)
o- Widjaja, Craske and Wootton (1996)
p- Petrov *et al.* (1996)
q- Grosch and Schieberle (1997)
r- Threshold data from Buttery *et al.* (1988)
s- Odour unit (Uo) calculated by conc (ppb)/threshold (ppb)
t- Aldrich (1998-99)

### *4.3.2.6 Correlation of sensory descriptions with individual aroma compounds*

Most subjects described the aroma of the Australian Basmati rice as popcorn, corn and corn chips. This was probably due to the fact that the Australian Basmati rice had a significantly greater concentration of 2-AP. In addition, most subjects described the Australian Basmati rice aroma as stronger than that of the imported Basmati rice. This could be attributed to the fact that the Australian Basmati rice had many other compounds present in greater proportions than the imported Basmati rice.

Many aroma descriptions of the imported Basmati rice related to old/rancid/stored type aromas such as, smoke, mud, off, butter, old/stored, rotten egg, bad, weirder, stinky. This most likely was due to the higher concentration of compounds including (E)hex-2-enal and (E)dec-2-enal but also the fact that many other compounds had decreased in concentration and there was no distinct aroma from any one compound.

## 4.4 The use of solid phase micro-extraction for the analysis of volatile components of rice

### 4.4.1 Effect of fibre type and extraction time on the adsorption of 2-AP from pandan leaves

**Table 4.8 Effect of fibre type and extraction time on the adsorption of SPME from pandan leaves**

| | 2-AP abundance | | | | |
|---|---|---|---|---|---|
| **Extraction time (min)** | **PDMS** | **PA** | **PDMS/DVB** | **PDMS/CAR** | **PDMS/CAR/DVB** |
| **5** | 0 | $1.9x10^5$ | $7.8x10^5$ | $1.5x10^7$ | $9.8x10^7$ |
| **15** | $2.8x10^5$ | $9.1x10^5$ | $1.7x10^6$ | $1.8x10^7$ | $2.3x10^8$ |
| **30** | $2.6x10^5$ | $7.2x10^5$ | $1.6x10^6$ | $2.6x10^7$ | $2.7x10^8$ |
| **60** | $2.2x10^5$ | $6.4x10^5$ | $9x10^5$ | $1.5x10^7$ | $2.4x10^8$ |
| **90** | 0 | $5x10^5$ | $9.7x10^5$ | $1x10^7$ | $2.1x10^8$ |

Five different SPME fibres were evaluated for their ability to adsorb 2-AP from pandan leaves. Table 4.8 presents the relative amounts of 2-AP adsorbed by the fibres: PDMS/CAR/DVB > PDMS/CAR > PDMS/DVB > PA > PDMS. The PDMS fibre was the least efficient and this was attributed to its non-polar nature. On the other extreme the polar PA fibre is a polar was more efficient in adsorbing 2-AP than the PDMS fibre. This may be because the 2-AP molecule that both polar and non-polar sites. However the PA fibre was inferior to the other fibre types in its ability to adsorb 2-AP. The PDMS/DVB fibre was significantly better than the PDMS and PA fibres in adsorbing 2-AP. This is expected because the fibre is suitable for more volatile and nitroaromatic compounds and the DVB is designed to reduce molecular weight discrimination. The two fibres demonstrating the greatest adsorption efficiency of 2-AP were PDMS/CAR and PDMS/CAR/DVB. These are suitable for low molecular weight compounds. The carbowax coating itself results in different selectivity towards alcohols and ketones compared to the PDMS fibre. The PDMS/CAR/DVB fibre was the most effective in adsorbing 2-AP absorbing up to 10 times more than the PDMS/CAR fibre and 1000 times more than the PDMS fibre.

In general an extraction time of 15min for SPME was found to be adequate to efficiently adsorb the large number of volatile aroma compounds from the headspace of a sample matrix (Yang and Peppard, 1994). However this research aimed to optimise only the adsorption time of 2-AP from the headspace of pandan leaves and not all volatile aroma compounds. The results demonstrated that the extraction time had a noticeable effect on the amount of 2-AP adsorbed by the fibres. All fibres demonstrated the poorest adsorption at the shortest (5min) and longest (90min) extraction times. The PDMS, PA and PDMS/DVB fibres had an optimum extraction time of 15min while the PDMS/CAR and PDMS/CAR/DVB fibres had longer optimum extraction times of 30min. The longer optimum extraction time for these fibres may be attributed to them favouring adsorption of compounds which were more volatile and therefore needed extra time to pick up 2-AP more effectively which is of medium volatility.

### 4.4.2 Effect of GC/MS Scanning mode on the adsorption of 2AP from pandan leaves

Operating of the GC/MS in the selected ion monitoring (SIM) mode allows scanning only for target and or qualifying ions compared to the general scanning mode that scans for all ions. This may increase sensitivity for 2-AP detection and quantification.

Firstly the absorption of all 5 fibre types was tested using the SIM scanning mode of the GC/MS. The molecular weight ion (111) of 2-AP was selected as the single target ion to monitor. Extraction of 2-

AP from the pandan leaves was performed at 25 and 60°C with a 15min extraction time. Table 4.9 demonstrates the results.

**Table 4.9 Comparison of the SCAN and SIM GC/MS modes for the detection of 2-AP adsorbed from pandan leaves at 25 and 60°C for 15min with various SPME fibres**

| | Fibre Type | | | | |
|---|---|---|---|---|---|
| **Extraction conditions** | **PDMS** | **PA** | **PDMS/DVB** | **PDMS/CAR** | **PDMS/CAR/DVB** |
| 25°C, 15min, SCAN | 0 | 0 | 0 | 821816 | 695702 |
| 25°C, 15min, SIM | 48578 | 60385 | 87849 | 1212267 | 662237 |
| 60°C, 15min, SCAN | 319112 | 665516 | 1223200 | 20754709 | 16978980 |
| 60°C, 15min, SIM | 251825 | 781704 | 2205046 | 16028463 | 22830947 |

When 2-AP was extracted at a temperature of 25°C, all fibre types demonstrated more 2-AP when the GC/MS was operating in the SIM mode compared to scan mode. For the PDMS, PA and PDMS/DVB fibres no 2-AP was detected in the Scan mode at 25°C but 2-AP was detected in the SIM mode at this temperature. However at the adsorption temperature of 60°C more 2-AP was detected in the SIM mode compared to the scan mode for the PA, PDMS/DVB and PDMS/CAR/DVB fibres, but less was detected for the PDMS and PDMS/CAR fibres. The results at 60ºC may be due to the fact that, overall, more 2-AP was absorbed by all fibres therefore the GC/MS scanning mode did not seem to make a difference for all fibres.

### 4.4.3 Effect of extraction temperature and sample size on the adsorption of 2-AP from pandan leaves

**Table 4.10 Effect of extraction temperature on the adsorption of 2-AP from pandan leaves with the PDMS/CAR and PDMS/CAR/DVB fibres**

| | 2-AP abundance | |
|---|---|---|
| **Extraction Temperature (°C)** | **PDMS/CAR** | **PDMS/CAR/DVB** |
| 25 | $1.3x10^6$ | $7.5x10^5$ |
| 40 | $5.3x10^6$ | $4.6x10^6$ |
| 60 | $1.8x10^7$ | $2.1x10^7$ |
| 80 | $1.9x10^7$ | $1.3x10^7$ |

Temperature affects both the sensitivity and extraction kinetics. In general the highest temperature which provides satisfactory sensitivity should be used. Results demonstrated that extraction temperature has a profound effect on 2-AP adsorption (Table 4.10). As the extraction temperature is increased from 25 to 60°C the adsorption of 2-AP increases but at 80°C, less 2-AP was collected on the PDMS/CAR DVB fibre, but slightly more was collected on the PDMS/CAR fibre was used. Therefore, temperatures between 60 and 80°C provided the optimum conditions for absorption of 2-AP by these SPME fibre types.

**Table 4.11 Effect of sample size on levels of 2-AP detected inpandan leaves using the PDMS/CAR and PDMS/CAR/DVB SPME fibres at various extraction times**

| | 2-AP abundance | | | |
|---|---|---|---|---|
| | **PDMS/CAR** | | **PDMS/CAR/DVB** | |
| **Extraction time (min)** | **1g sample** | **2g sample** | **1g sample** | **2g sample** |
| **5** | $1.9x10^7$ | $1.5x10^7$ | $8.2x10^5$ | $1.3x10^6$ |
| **15** | $2x10^7$ | $1.8x10^7$ | $7.8x10^6$ | $5.3x10^6$ |
| **30** | $2.5x10^7$ | $2.6x10^7$ | $2.1x10^7$ | $1.8x10^7$ |
| **60** | $1.8x10^7$ | $1.5x10^7$ | $2.2x10^7$ | $1.9x10^7$ |

Sample size will also have an impact on the amount of analyte adsorbed onto the SPME fibre. The nature of the SPME method allows the use of relative small sample sizes. This is a great advantage of the technique since, in some circumstances, there is only limited sample size available. Therefore sample sizes of 1 and 2 g of the pandan leaves were compared to see if this had an effect on adsorption

of 2-AP and in turn the relative abundance detected. Results indicated that a 1g sample generally showed an equivalent or slightly increased abundance of 2-AP compared to the 2g sample Table 4.11). This may reflect a larger headspace in the vial for volatile compounds for the former and less compaction of the sample in the vial. Therefore, the 1g sample size was utilised for analysis of rice plant samples.

Optimum operating parameters for the adsorption of 2-AP using SPME were taken as: extraction temperature of between 60 and 80ºC, extraction time of between 15 and 30min, sample size of 1-2g, SIM or SCAN GC/MS operating modes and the PDMS/CAR and PDMS/CAR/DVB fibres.

### 4.4.4 Detection of 2-AP in rice plants using SPME

The optimised SPME extraction conditions for 2-AP from pandan leaves were used to detect 2-AP in fragrant and non-fragrant rice plants at various growth stages. Table 7.6 (appendix) lists the volatile aroma compounds isolated from Kyeema plants, by the PDMS/CAR SPME fibre, at the 3-leaf , tillering, panicle initiation and flowering growth stages.

From Table 7.6 (Appendix) it can be seen that there were significantly more volatile aroma compounds detected in the Kyeema plants at the tillering, panicle initiation and flowering growth stage in comparison to the 3-leaf growth stage.

In comparison to other volatile aroma compounds, 2-AP was detected at a high abundance at the 3-leaf growth stage than at tillering, panicle initiation and flowering. This is in contrast with the fact that 2-AP was found in rice plants at high concentrations at all growth stages when isolated by SDE. Grimm *et al.* (2001) found that SPME analysis of rice grain only had a 0.3% recovery of 2-AP compared to a solvent extraction method. In any event, the 3-leaf growth stage of rice plants chosen for isolation of 2-AP from rice plants.

**Table 4.12 Abundance of 2-AP extracted from fragrant and non-fragrant rice plants at the 3-leaf growth stage using the PDMS/CAR and PDMS/CAR/DVB SPME fibres**

| | **2-AP abundance** | |
|---|---|---|
| **Variety** | **PDMS/CAR** | **PDMS/CAR/DVB** |
| **Kyeema** | 3.2x106 | 2.9x106 |
| **YRF207** | 3x106 | 1.8x106 |
| **YRF203** | 2x106 | 2.4x106 |
| **Millin** | 0 | 0 |

Volatile aroma compounds from fragrant Kyeema, YRF207, YRF203 and non-fragrant Millin plants at the 3-leaf growth stage were extracted using the PDMS/CAR and PDMS/CAR/DVB SPME fibres. All these varieties had the same pattern of volatile aroma compounds with the exception of Millin which contained no 2-AP. Table 4.12 presents the abundance of 2-AP detected from fragrant and non-fragrant rice plants at the 3-leaf growth stage by the PDMS/CAR and PDMS/CAR/DVB fibres respectively.

The different ability of fibres to adsorb 2-AP is demonstrated by the different amounts isolated by them. Results from the PDMS/CAR fibre indicated that the YRF207 plant had a higher abundance of 2-AP than the YRF203 plant, whereas the PDMS/CAR/DVB fibre indicated the reverse. However both fibre types demonstrated that the Kyeema plant had more 2-AP than other fragrant rice varieties. Therefore, SPME could be utilised to distinguish between plants that will produce fragrant and non-fragrant rice grain. SPME could be utilised for screening for fragrance in rice plants at the very early stages of growth.

## 4.5 Levels of amino acids in rice plants and grain

### 4.5.1 Total amino acids in rice plants and grain

Tables 7.7 to 7.11 (Appendix) present the total amino acid levels in fragrant and non-fragrant rice plants fertilised at 150 and 50kg N/ha at tillering, panicle initiation, flowering, maturity and rice grain. Glutamic and aspartic acids were present in the highest proportions in rice plants at all growth stages and in the mature grain. In addition, leucine was present in high proportions at tillering, leucine, isoleucine and valine at panicle initiation and flowering and hydroxyproline and valine at maturity. In the rice grain, glutamic acid, aspartic acid and arginine were present in the highest proportions. This is consistent with Juliano (1985) and Mosse, Huet and Baudet (1988), who also found these amino acids to be present in the highest proportions in milled rice grain.

Statistical analysis involving 2-way ANOVA was performed to determine if fertiliser level had a significant effect on the concentration of total amino acids in the rice plants at tillering, panicle initiation, flowering and maturity and in the grain. In addition, this statistical analysis also determined if there was a significant difference between the concentration of total amino acids between varieties and the interaction between nitrogen fertilisation level and variety.

Two way ANOVA showed that there is a significant ($p<0.05$) lower level of total amino acids in rice plants and grain fertilised at 150kgN/ha compared to 50kg N/ha except in the rice plants at maturity. When consulting Tables 7.7-7.11 (Appendix) it can be seen that the concentration of the majority of amino acids was significantly lower when the rice plants were fertilised at the lower nitrogen rate. Rice grain from plants fertilised at the lower nitrogen rate was lower in lower nitrogen implying lower concentration of protein and amino acids. However, hydroxyproline levels in all rice varieties and methionine levels in YRF203 and Millin were higher in rice grain fertilised at the lower nitrogen rate. This is consistent Mosse, Huet and Baudet (1988), who increases in amino acids including valine, leucine, isoleucine, serine, proline and tyrosine, little variation for glycine, alanine, threonine, phenylalanine, histidine and arginine and decreases in lysine, methionine and cystine at lower nitrogen fertiliser levels.

**Table 4.13 Concentration (%) of total proline present in fragrant and non-fragrant rice plants fertilised at 150 and 50kg N/ha at various growth stages and in the mature grain**

| | % Concentration of total proline | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Growth Stages** | **Kyeema 150kg N2/ha** | **Kyeema 50kg N2/ha** | **YRF207 150kg N2/ha** | **YRF207 50kg N2/ha** | **YRF203 150kg N2/ha** | **YRF203 50kg N2/ha** | **Millin 150kg N2/ha** | **Millin 50kg N2/ha** |
| **Tillering** | 0.77 | 0.56 | 0.66 | 0.83 | 0.84 | 0.55 | 1.07 | 0.56 |
| **Panicle Initiation** | 0.72 | 0.58 | 0.63 | 0.50 | 0.62 | 0.73 | 1.01 | 0.53 |
| **Flowering** | 0.63 | 0.45 | 0.60 | 0.58 | 0.57 | 0.43 | 0.63 | 0.47 |
| **Maturity** | 0.14 | 0.16 | 0.18 | 0.15 | 0.14 | 0.17 | 0.39 | 0.39 |
| **Grain** | 0.36 | 0.21 | 0.30 | 0.26 | 0.27 | 0.19 | 0.31 | 0.17 |

The effect of nitrogen fertiliser level on the concentration of total proline in rice plants and grain needs attention because of its potential involvement in the biosynthesis 2-AP.. From table 4.13, it can been seen that generally rice plants and mature grain fertilsed at the lower rate had lower levels of total proline. In rice plants fertilised at the normal nitrogen rate of 150kg N/ha, the level of total proline is highest at the tillering growth stage and then decreased gradually to maturity. Total proline is highest in the non-fragrant Millin plants at all growth stages although total proline levels were similar in grain from fragrant and non-fragrant varieties.

### 4.5.2 Free amino acids in rice plants and grain

Tables 7.12 to 7.16 (Appendix) present the free amino acid levels in fragrant and non-fragrant rice plants fertilised at 150 and 50kg N/ha at tillering, panicle initiation, flowering and maturity and in the rice grain. Free amino acids were present in the highest proportions in the rice plants fertilised at 150kg N/ha at all growth stages. Phenylalanine, glutamic acid and proline were present in the highest proportions at tillering, valine and proline at panicle initiation, glutamic acid and proline at flowering and isoleucine, glutamic acid and valine at maturity. In the rice grain the free amino acids present in the highest proportions included aspartic acid, glutamic acid, alanine and isoleucine. These results were consistent with those of Saikusa, Horino and Mori (1994) who found that free aspartic acid, glutamic acid and alanine were present in the highest proportions in milled rice grain.

2-Way ANOVA demonstrated that there were significant differences only in the concentration of free amino acids between rice plants fertilised at 150 and 50kg N/ha at tillering and flowering growth stages. Notably, free amino acids in rice grain were not significantly effected by nitrogen fertiliser rate. Because proline was implicated as a precursor for 2-AP, it will be discussed in more detail. Table 4.14, presents the concentration of free proline present in fragrant and non-fragrant rice plants fertilised at 150 and 50kgN/ha at tillering, panicle initiation, flowering and maturity and in the mature grain.

**Table 4.14 Concentration (%) of free proline present in fragrant and non-fragrant rice plants fertilised at 150 and 50kgN/ha at tillering, P.I, flowering and maturity**

| | % Concentration of free proline | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Growth Stages** | **Kyeema 150kg N2/ha** | **Kyeema 50kg N2/ha** | **YRF207 150kg N2/ha** | **YRF207 50kg N2/ha** | **YRF203 150kg N2/ha** | **YRF203 50kg N2/ha** | **Millin 150kg N2/ha** | **Millin 50kg N2/ha** |
| **Tillering** | 0.0121 | 0.0036 | 0.0136 | 0.0047 | 0.0125 | 0.0079 | 0.0055 | 0.0059 |
| **Panicle Initiation** | 0.0090 | 0.0096 | 0.0075 | 0.0081 | 0.0076 | 0.0087 | 0.0292 | 0.0184 |
| **Flowering** | 0.0233 | 0.0169 | 0.0345 | 0.0177 | 0.0259 | 0.0220 | 0.0135 | 0.0095 |
| **Maturity** | 0.0017 | 0.0024 | 0.0029 | 0.0041 | 0.0013 | 0.0024 | 0.0020 | 0.0020 |
| **Grain** | 0.0005 | 0.0003 | 0.0008 | 0.0004 | 0.0004 | 0.0003 | 0.0006 | 0.0004 |

At tillering and flowering, rice plants fertilised at the lower nitrogen rate of 50kg N/ha generally had lower concentrations of free proline. However, at panicle initiation and maturity, fragrant rice plants fertilised at the lower nitrogen rate had higher concentrations of free proline than those fertilised at the normal nitrogen rate.

Free proline was present at similar levels at the early growth stages of tillering and panicle initiation. It reached the highest concentration at the flowering stage of growth in the fragrant rice plants and at panicle initiation in the non-fragrant Millin plant and was present at the lowest concentration at maturity. The amount of free proline present in the rice grain was approximately 0.0006%, which was lower than that present at in the mature rice plants and was more than 100 times lower than total proline levels. Grain from varieties fertilised at the lower nitrogen rate contained lower less free proline than grain from plants fertilised at the normal nitrogen rate. Yoshihashi, Nguyen and Inatomi (2002) found that proline was the nitrogen source for 2-AP. Results from this study show that both fragrant and non-fragrant rice plants contain similar levels of free proline and therefore, free proline levels are not an indicator of fragrance. It seems that it is a biosynthetic pathway in fragrant rice plants which enables the formation of 2-AP from proline.

# 5. Implications

This research has demonstrated that nitrogen fertiliser level impacts on the aroma quality of rice. From data over three seasons, plants, at the tillering stage of growth, fertilised at the lower nitrogen rate (50kg N/ha), had a lower concentration of volatile compounds compared to the plants fertilised at the normal nitrogen rate (150kg N/ha), in particular for the 2-AP. Nitrogen fertiliser level had no significant effect on levels of volatile compounds in rice plants at panicle initiation, flowering and maturity or in the mature grain. Over two seasons, many important volatile compounds were significantly higher in grain fertilised at the lower nitrogen level for all fragrant and the non-fragrant L203 rice variety, but lower in the non-fragrant Millin variety. However, this effect was not reflected on 2-AP the grain. Therefore, aroma quality is independent of nitrogen fertiliser at the levels investigated and should be monitored if nitrogen fertiliser levels above the normal levels are applied.

Research performed on the volatile aroma components of Australian versus imported rices and Australian breeding lines has facilitated the N.S.W rice breeding program with the development of new Australian fragrant rice cultivars. The Australian Basmati breeding line (YRF203) displayed many differences in concentration of volatile compounds compared to imported varieties. The YRF203 sample did not contain many volatiles that were present in the imported varieties, but contained some compounds in higher amounts, most importantly was that of 2-AP. These differences may be attributed to either or both longer storage of the imported sample or varietal differences. In addition, YRF203 sample had higher concentrations of 2-AP being quantified. However, research into fragrant rice development of should include effects of storage conditions on volatile components.

The volatile components of commercial Australia Jasmine rice were very similar to those of imported varieties both qualitatively and quantitatively. However, there were quantitative differences, which distinguished the rices on a chemical basis and, as a result, on a sensory basis. Some concern was raised over the aroma potential of an Australian Jasmine breeding line YRF207. Over two seasons, this sample contained significantly less 2-AP than the commercial Kyeema variety.

During storage of some Australian Basmati breeding lines there were changes, with compounds being formed and others being reduced in concentration over time. Importantly, 2-AP decreased by 66% during a 3 month storage period. This highlighted the fact that storage protocols will be of great importance for the successful development of Basmati rices developed within Australia. The aroma potential of the Basmati breeding lines was promising for Australia, since such varieties can be produced under Australian growing conditions.

Consumers, of various ages, gender and cultural background, could distinguish between the aroma of fragrant and non-fragrant rice and between Australian and imported Jasmine fragrant rice and between Australian and imported Basmati fragrant rice. These differences in consumer perception between the rice samples and aroma descriptions could be explained by differences in volatile components. Aroma descriptions of the Australian and imported Jasmine rice revealed a greater number of positive descriptions of rice aroma and less negative aroma descriptions for the former.

Preference testing for Australian versus imported Basmati fragrant rice indicated no direct preferences for the aroma of either. These results are encouraging for the likely acceptance of Australian fragrant rice in local and export markets. Australian Jasmine and Basmati fragrant rice varieties have their own characteristic aroma, perceived as significantly different from imported counterparts.

An SPME technique was optimised for the adsorption of 2-AP using pandan leaves and these optimum extraction conditions were utilised for the adsorption of 2-AP in rice plants. It was concluded that the 3-leaf growth stage of rice plants was the best to extract 2-AP from rice plants using SPME. This rapid, simple technique can be used for the screening of fragrance in rice plants at early plant growth stages and, importantly, can be used on-farm for collection of volatile components for later GC/MS in

the laboratory. This allows the rapid detection of fragrance, and can replace current subjective aroma detection.

Nitrogen fertiliser level affected the concentration of total amino acids in rice plants at the growth stages of tillering, panicle initiation and flowering and in the mature grain, levels being higher at higher nitrogen application. In particular levels of total proline were significantly lower in rice plants and grain fertilised at the lower nitrogen rate. The levels of total proline were greater in the non-fragrant plant compared to the fragrant plants throughout growth, but the levels of total proline in the grain were similar.

Nitrogen fertiliser level had a significant effect on the concentration of free amino acids only in rice plants at tillering and flowering but not in the grain; plants fertilised at the lower nitrogen rate had lower concentrations of free amino acids. Plants at tillering and flowering, fertilised at the lower nitrogen rate, were lower in free proline. In addition, both fragrant and non-fragrant rice grain fertilised at the lower nitrogen rate had lower concentrations of free proline, which was similar for both. This suggests that the free proline is not indicative of the fragrance potential of the plant.

# 6. Recommendations

From the results of the project involving application of nitrogen fertiliser level it is recommended that normal fertiliser rates are adhered to. If higher than normal levels of nitrogen fertiliser are used for purposes such as improved yield then the aroma quality needs to be carefully monitored.

Preliminary storage trials and analysis of Australian Basmati breeding lines suggests that for further successful development of an Australian grown Basmati rice extensive storage trials and the development of storage protocols is needed.

The SPME technique developed for the screening of fragrance in rice plants at early plant growth stages can be used on-farm for collection of volatile components for later GC/MS in the laboratory. This allows the rapid detection of fragrance, and may replace current subjective aroma detection.

# 7. Appendix

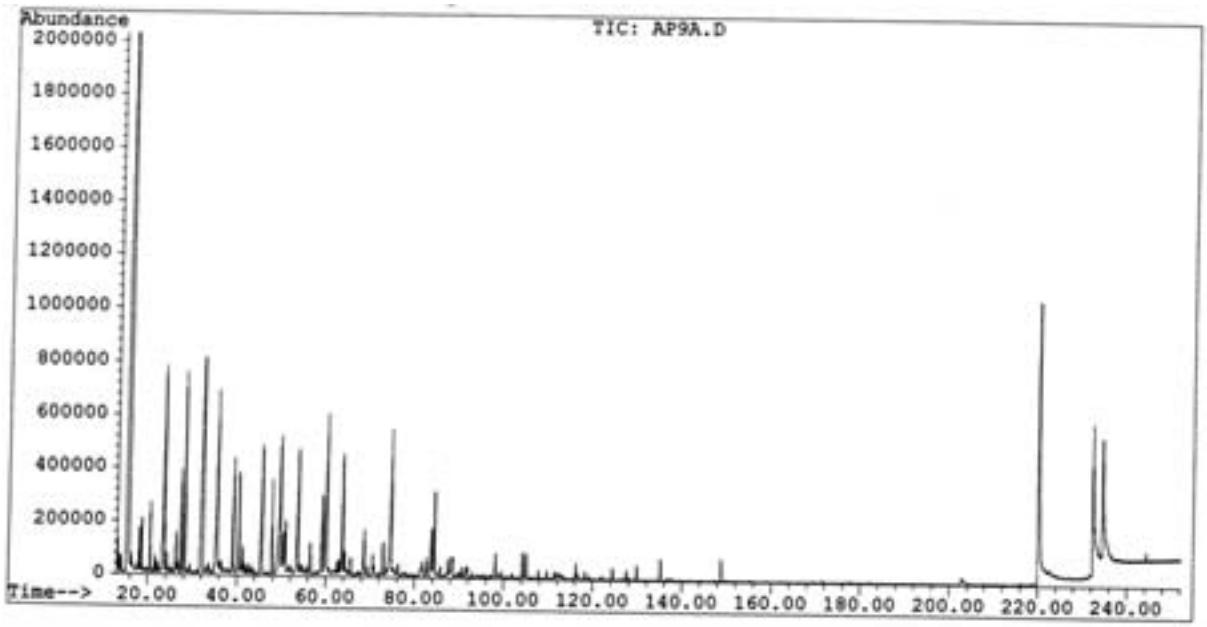


**Figure 7.4 Chromatogram of Imported Basmati fragrant Rice (Riviana brand)**

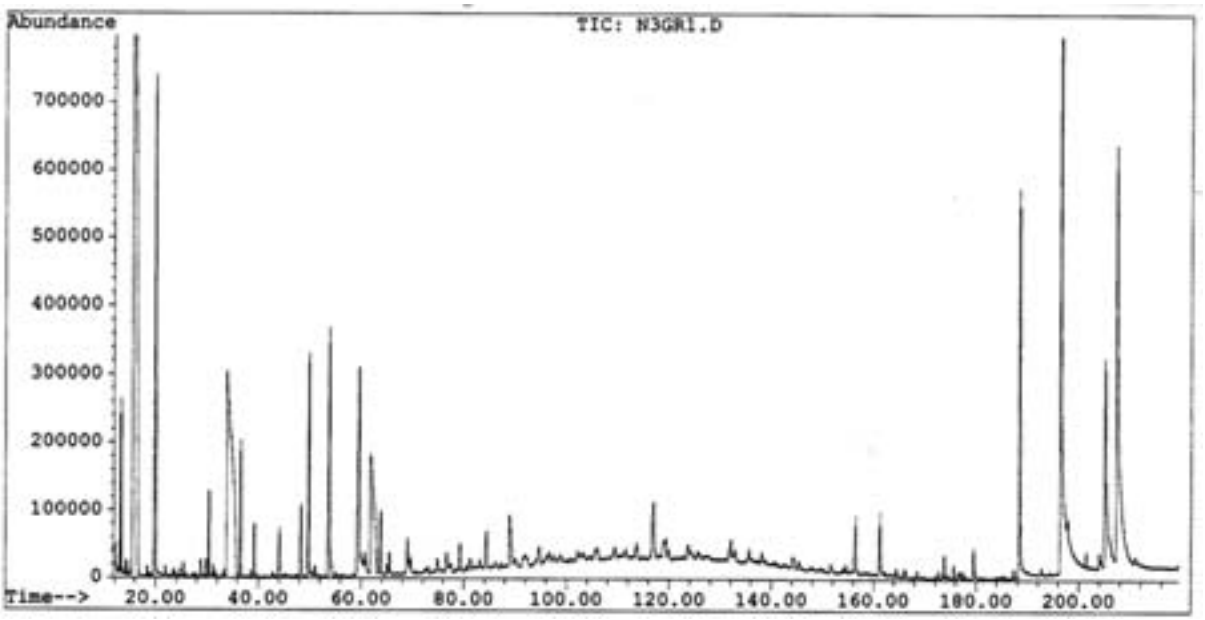


**Figure 7.5 Chromatogram of Australian Basmati breeding line (YRF203)**

**Table 7.6 Volatile aroma compounds isolated by SPME from Kyeema plants at the 3-leaf, tillering, panicle initiation and flowering growth stages**

| Volatile Compounds | Growth stage | | | |
|---|---|---|---|---|
| | 3-leaf | Tillering | Panicle Initiation | Flowering |
| Dimethyl sulphide | + | + | + | + |
| Propanal | | + | + | + |
| 2-methylfuran | + | | | |
| Butanal | | + | + | + |
| 3-methylfuran | + | + | + | + |
| 3-methylbutanal, | + | + | + | + |
| 2-ethylfuran | + | + | + | + |
| Butane-2,3-dione | | + | + | + |
| Pentan-2-one | + | | | |
| Pentanal | + | + | + | + |
| Methyl isobutyl ketone | | + | + | + |
| Pent-1-en-3-one | + | + | + | + |
| Hexanal | + | + | + | + |
| Ethylbenzene | | + | + | + |
| 1,2-dimethylbenzene | | + | + | + |
| 1,3-dimethylbenzene | | + | + | + |
| Pent-1-en-3-ol | + | + | + | + |
| p-xylene | | + | + | + |
| Hexanoic acid methyl ester | | + | + | + |
| Heptan-2-one | + | + | + | + |
| Heptanal | + | + | + | + |
| Limonene | | + | + | + |
| (E)hex-3-enal | | + | + | + |
| (E)hex-2-enal | + | + | + | + |
| 2-pentylfuran | + | + | + | + |
| Hexanoic acid ethyl ester | | + | + | + |
| pentan-1-ol | + | | | |
| rans-2-(1-pentenylfuran) | | + | + | + |
| Octanal | | + | + | + |
| (Z)penten-1-ol | + | + | + | + |
| Octane-2,3-dione | | + | + | + |
| Heptan-2-ol | + | + | + | + |
| 6-methylhept-5-en-2-one | + | + | + | + |
| 2-acetyl-1-pyrroline | + | + | + | + |
| Hexan-1-ol | + | + | + | + |
| (Z)hex-3-en-1-ol | + | + | + | + |
| (E)hex-2-en-1-ol | | + | + | + |
| Nonanal | + | + | + | + |
| (E,E)hexa-2,4-dienal | | + | + | + |
| Octanoic acid ethyl ester | | + | + | + |
| Acetic acid | + | | | |
| Oct-1-en-3-ol | + | + | + | + |
| (E,E)hepta-2,4-dienal | | + | + | + |
| 2-ethylhexan-1-ol | + | + | + | + |
| (E,Z)hepta-2,4-dienal | | + | + | + |
| Benzaldehyde | | + | + | + |
| (E,E)octa-3,5-dien-2-one | | + | + | + |
| 3,7-dimethylocta-1,6-dien-3-ol | + | + | + | |
| Pentadecane | + | | | |
| Octan-1-ol | + | + | + | + |
| (E,Z)octa-3,5-dien-2-one | | + | + | + |
| (E,Z)nona-2,6-dienal | | + | | |
| 4-methyl-1-(1-methylethyl)cyclohex-3-en-1-ol | | + | + | + |
| 2,6,6-trimethyl cyclohex-1-ene-1-carboxaldehyde, | + | + | + | + |
| Acetophenone | | + | + | + |

| | | | | |
|---|---|---|---|---|
| Nonan-1-ol | + | | | |
| Heptadecane | + | + | + | + |
| Cyclohex-2-en-1-one | | + | + | + |
| Methyl salicylate | | + | + | + |
| Benzyl alcohol | | + | + | + |
| Butylated hydroxytoluene | | + | + | + |
| 4(2,6,6-trimethyl-1-cyclohexen-1-yl)[E] but-3-en-2-one | | + | + | + |
| 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hep-1-yl)but-3-en-2-one | | + | + | + |
| Pentadecanal | | + | | |
| 2-methylcyclopent-2-en-1-one | | + | + | + |
| 5,6,7,7a-tetrahydro,4,4,7a-trimethyl 2(4H) benzofuranone | | + | + | + |
| 6,10,14-trimethyl pentadecan-2-one | + | | | |
| Dibutyl phthalate | + | | | |

**Table 7.7 Concentration (%) of total amino acids present in rice plants fertilised at 150 and 50kg N/ha at the tillering growth stage**

| | Concentration (%) of total amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Rice Variety** | **Kyeema** | | **YRF207** | | **YRF203** | | **Millin** | |
| **Amino acid** | **150** | **50** | **150** | **50** | **150** | **50** | **150** | **50** |
| **Alanine** | 0.52 | 0.32 | 0.45 | 0.48 | 0.68 | 0.32 | 0.64 | 0.30 |
| **Glycine** | 1.13 | 0.49 | 0.94 | 0.87 | 1.62 | 0.57 | 1.08 | 0.52 |
| **Valine** | 0.86 | 0.84 | 0.96 | 0.91 | 1.03 | 1.32 | 1.10 | 1.71 |
| **Threonine** | 0.62 | 0.47 | 0.72 | 0.63 | 0.83 | 0.76 | 0.51 | 0.79 |
| **Serine** | 0.76 | 0.38 | 0.74 | 0.60 | 0.99 | 0.56 | 0.52 | 0.52 |
| **Leucine** | 1.31 | 0.98 | 1.25 | 1.14 | 1.64 | 0.97 | 1.71 | 1.07 |
| **Isoleucine** | 0.93 | 0.87 | 1.00 | 0.82 | 1.11 | 1.53 | 1.17 | 2.10 |
| **Proline** | 0.77 | 0.56 | 0.66 | 0.83 | 0.84 | 0.55 | 1.07 | 0.56 |
| **Hydroxy proline** | 0.39 | 0.59 | 0.38 | 0.79 | 0.41 | 0.40 | 0.73 | 0.64 |
| **Ornithine** | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Methionine** | 0.38 | 0.16 | 0.27 | 0.23 | 0.66 | 0.25 | 0.47 | 0.17 |
| **Aspartic acid** | 1.73 | 0.95 | 1.70 | 1.40 | 2.13 | 1.46 | 1.52 | 1.43 |
| **Phenylalanine** | 0.64 | 0.49 | 0.64 | 0.49 | 0.89 | 0.41 | 0.84 | 0.50 |
| **Histidine (DA)** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Glutamic acid** | 1.99 | 1.09 | 1.81 | 1.69 | 2.57 | 1.34 | 2.03 | 1.34 |
| **Lysine** | 0.50 | 0.50 | 0.81 | 0.93 | 0.51 | 0.86 | 0.45 | 0.67 |
| **Tyrosine** | 0.29 | 0.37 | 0.37 | 0.44 | 0.23 | 0.33 | 0.36 | 0.39 |
| **Arginine** | 0.00 | 0.55 | 0.74 | 0.39 | 0.00 | 0.40 | 0.11 | 0.40 |
| **Histidine (MA)** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Cystine** | 0.31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

**Table 7.8 Concentration (%) of total amino acids present in rice plants fertilised at 150 and 50kg N/ha at the panicle initiation growth stage**

| | Concentration (%) of total amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Rice variety** | **Kyeema** | | **YRF207** | | **YRF203** | | **Millin** | |
| **Amino acid** | **150** | **50** | **150** | **50** | **150** | **50** | **150** | **50** |
| **Alanine** | 0.419 | 0.324 | 0.361 | 0.274 | 0.349 | 0.408 | 0.534 | 0.283 |
| **Glycine** | 0.743 | 0.587 | 0.656 | 0.485 | 0.641 | 0.758 | 0.842 | 0.507 |
| **Valine** | 1.045 | 1.025 | 1.666 | 1.477 | 1.976 | 0.938 | 0.884 | 1.022 |
| **Threonine** | 0.728 | 0.636 | 0.934 | 0.733 | 1.002 | 0.641 | 0.557 | 0.557 |
| **Serine** | 0.638 | 0.520 | 0.722 | 0.510 | 0.685 | 0.585 | 0.501 | 0.431 |
| **Leucine** | 1.140 | 0.914 | 1.084 | 0.894 | 1.079 | 1.102 | 1.237 | 0.766 |
| **Isoleucine** | 0.997 | 1.100 | 1.950 | 1.849 | 2.487 | 0.869 | 0.771 | 1.186 |
| **Proline** | 0.718 | 0.575 | 0.632 | 0.500 | 0.624 | 0.725 | 1.009 | 0.529 |
| **Hydroxy proline** | 0.419 | 0.386 | 0.374 | 0.442 | 0.324 | 0.307 | 0.721 | 0.454 |
| **Ornithine** | 0.040 | 0.034 | 0.052 | 0.018 | 0.023 | 0.000 | 0.000 | 0.012 |
| **Methionine** | 0.284 | 0.171 | 0.229 | 0.175 | 0.278 | 0.293 | 0.153 | 0.170 |
| **Aspartic acid** | 1.545 | 1.287 | 1.789 | 1.385 | 1.802 | 1.352 | 1.150 | 1.073 |
| **Phenylalanine** | 0.516 | 0.411 | 0.479 | 0.401 | 0.455 | 0.539 | 0.585 | 0.363 |
| **Histidine (DA)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Glutamic acid** | 1.633 | 1.265 | 1.594 | 1.179 | 1.572 | 1.543 | 1.559 | 1.035 |
| **Lysine** | 0.806 | 0.785 | 1.225 | 0.737 | 0.896 | 0.846 | 0.478 | 0.645 |
| **Tyrosine** | 0.438 | 0.332 | 0.388 | 0.322 | 0.371 | 0.419 | 0.466 | 0.285 |
| **Arginine** | 0.851 | 0.611 | 0.968 | 0.445 | 0.372 | 0.297 | 0.232 | 0.317 |
| **Histidine (MA)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Cystine** | 0.000 | 0.000 | 0.248 | 0.000 | 0.156 | 0.000 | 0.000 | 0.000 |

**Table 7.9 Concentration (%) of total amino acids present in rice plants fertilised at 150 and 50kg N/ha at the flowering growth stage**

| | Concentration (%) of total amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Rice variety** | **Kyeema** | | **YRF207** | | **YRF203** | | **Millin** | |
| **Amino acid** | **150** | **50** | **150** | **50** | **150** | **50** | **150** | **50** |
| **Alanine** | 0.371 | 0.261 | 0.367 | 0.307 | 0.349 | 0.233 | 0.349 | 0.254 |
| **Glycine** | 0.574 | 0.410 | 0.595 | 0.474 | 0.524 | 0.387 | 0.585 | 0.418 |
| **Valine** | 0.992 | 0.863 | 1.380 | 0.485 | 1.015 | 0.521 | 0.687 | 0.921 |
| **Threonine** | 0.617 | 0.459 | 0.737 | 0.266 | 0.560 | 0.303 | 0.406 | 0.477 |
| **Serine** | 0.478 | 0.367 | 0.546 | 0.281 | 0.428 | 0.314 | 0.389 | 0.362 |
| **Leucine** | 1.019 | 0.606 | 0.948 | 0.552 | 0.842 | 0.479 | 0.771 | 0.596 |
| **Isoleucine** | 1.012 | 0.905 | 1.830 | 0.388 | 1.090 | 0.379 | 0.606 | 0.904 |
| **Proline** | 0.627 | 0.452 | 0.603 | 0.583 | 0.572 | 0.433 | 0.627 | 0.466 |
| **Hydroxy proline** | 0.262 | 0.313 | 0.280 | 0.533 | 0.309 | 0.613 | 0.701 | 0.835 |
| **Ornithine** | 0.000 | 0.019 | 0.015 | 0.000 | 0.000 | 0.000 | 0.000 | 0.013 |
| **Methionine** | 0.258 | 0.165 | 0.186 | 0.148 | 0.209 | 0.131 | 0.146 | 0.120 |
| **Aspartic acid** | 1.385 | 0.947 | 1.394 | 0.582 | 1.172 | 0.732 | 0.956 | 0.892 |
| **Phenylalanine** | 0.474 | 0.275 | 0.420 | 0.249 | 0.373 | 0.224 | 0.382 | 0.279 |
| **Histidine (DA)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Glutamic acid** | 1.420 | 0.960 | 1.442 | 0.840 | 1.203 | 0.835 | 1.147 | 0.901 |
| **Lysine** | 0.682 | 0.725 | 1.049 | 0.447 | 0.633 | 0.557 | 0.588 | 0.647 |
| **Tyrosine** | 0.379 | 0.234 | 0.342 | 0.205 | 0.310 | 0.186 | 0.300 | 0.225 |
| **Arginine** | 0.228 | 0.455 | 0.303 | 0.222 | 0.382 | 0.329 | 0.644 | 0.682 |
| **Histidine (MA)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Cystine** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

**Table 7.10 Concentration (%) of total amino acids present in rice plants fertilised at 150 and 50kg N/ha at the maturity growth stage**

| | Concentration (%) of total amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Rice variety** | **Kyeema** | | **YRF207** | | **YRF203** | | **Millin** | |
| **Amino acid** | **150** | **50** | **150** | **50** | **150** | **50** | **150** | **50** |
| **Alanine** | 0.048 | 0.071 | 0.073 | 0.061 | 0.056 | 0.081 | 0.176 | 0.144 |
| **Glycine** | 0.087 | 0.109 | 0.127 | 0.104 | 0.099 | 0.124 | 0.329 | 0.276 |
| **Valine** | 0.390 | 0.291 | 0.369 | 0.361 | 0.254 | 0.309 | 0.545 | 0.494 |
| **Threonine** | 0.149 | 0.071 | 0.156 | 0.122 | 0.101 | 0.088 | 0.255 | 0.202 |
| **Serine** | 0.132 | 0.081 | 0.145 | 0.111 | 0.104 | 0.094 | 0.279 | 0.216 |
| **Leucine** | 0.128 | 0.137 | 0.185 | 0.137 | 0.131 | 0.160 | 0.302 | 0.260 |
| **Isoleucine** | 0.330 | 0.163 | 0.268 | 0.218 | 0.175 | 0.196 | 0.457 | 0.376 |
| **Proline** | 0.139 | 0.164 | 0.177 | 0.150 | 0.140 | 0.175 | 0.393 | 0.390 |
| **Hydroxy proline** | 0.150 | 0.624 | 0.314 | 0.408 | 0.301 | 0.421 | 1.858 | 2.530 |
| **Ornithine** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Methionine** | 0.035 | 0.039 | 0.045 | 0.038 | 0.024 | 0.049 | 0.090 | 0.090 |
| **Aspartic acid** | 0.297 | 0.220 | 0.342 | 0.289 | 0.244 | 0.245 | 0.588 | 0.506 |
| **Phenylalanine** | 0.062 | 0.053 | 0.083 | 0.059 | 0.056 | 0.066 | 0.119 | 0.101 |
| **Histidine (DA)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Glutamic acid** | 0.233 | 0.213 | 0.305 | 0.197 | 0.225 | 0.251 | 0.591 | 0.490 |
| **Lysine** | 0.158 | 0.128 | 0.138 | 0.165 | 0.115 | 0.132 | 0.435 | 0.375 |
| **Tyrosine** | 0.049 | 0.045 | 0.069 | 0.053 | 0.049 | 0.056 | 0.107 | 0.099 |
| **Arginine** | 0.217 | 0.105 | 0.218 | 0.178 | 0.093 | 0.097 | 0.279 | 0.206 |
| **Histidine (MA)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Cystine** | 0.037 | 0.000 | 0.355 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

**Table 7.11 Concentration (%) of total amino acids present in rice grain fertilised at 150 and 50kg N/ha.**

| | Concentration (%) of total amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Rice variety** | **Kyeema** | | **YRF207** | | **YRF203** | | **Millin** | |
| **Amino acid** | **150** | **50** | **150** | **50** | **150** | **50** | **150** | **50** |
| **Alanine** | 0.135 | 0.153 | 0.000 | 0.116 | 0.000 | 0.118 | 0.122 | 0.119 |
| **Glycine** | 0.337 | 0.212 | 0.282 | 0.250 | 0.264 | 0.217 | 0.297 | 0.227 |
| **Valine** | 0.755 | 0.394 | 0.588 | 0.600 | 0.729 | 0.651 | 0.419 | 0.468 |
| **Threonine** | 0.383 | 0.142 | 0.301 | 0.175 | 0.346 | 0.236 | 0.221 | 0.136 |
| **Serine** | 0.493 | 0.205 | 0.410 | 0.272 | 0.396 | 0.279 | 0.336 | 0.229 |
| **Leucine** | 0.703 | 0.423 | 0.531 | 0.411 | 0.490 | 0.445 | 0.444 | 0.559 |
| **Isoleucine** | 0.791 | 0.392 | 0.582 | 0.636 | 0.802 | 0.703 | 0.391 | 0.463 |
| **Proline** | 0.356 | 0.209 | 0.299 | 0.257 | 0.270 | 0.194 | 0.314 | 0.173 |
| **Hydroxy proline** | 0.018 | 0.058 | 0.018 | 0.104 | 0.013 | 0.142 | 0.015 | 0.258 |
| **Ornithine** | 0.020 | 0.018 | 0.016 | 0.000 | 0.015 | 0.000 | 0.016 | 0.070 |
| **Methionine** | 0.201 | 0.111 | 0.170 | 0.100 | 0.158 | 0.167 | 0.111 | 0.238 |
| **Aspartic acid** | 1.072 | 0.597 | 0.911 | 0.658 | 0.900 | 0.740 | 0.749 | 0.713 |
| **Phenylalanine** | 0.334 | 0.185 | 0.243 | 0.167 | 0.218 | 0.186 | 0.176 | 0.340 |
| **Histidine (DA)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Glutamic acid** | 1.702 | 0.854 | 1.292 | 0.897 | 1.228 | 0.774 | 1.146 | 0.616 |
| **Lysine** | 0.507 | 0.151 | 0.465 | 0.094 | 0.529 | 0.192 | 0.338 | 0.043 |
| **Tyrosine** | 0.144 | 0.041 | 0.123 | 0.084 | 0.099 | 0.084 | 0.103 | 0.000 |
| **Arginine** | 0.331 | 0.203 | 0.716 | 0.119 | 0.652 | 0.082 | 0.461 | 0.000 |
| **Histidine (MA)** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| **Cystine** | 0.152 | 0.000 | 0.173 | 0.000 | 0.216 | 0.000 | 0.000 | 0.000 |

**Table 7.12 Concentration (%) of free amino acids present in rice plants fertilised at 150 and 50kg N/ha at the tillering growth stage**

| | Concentration (%) of free amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Rice variety** | **Kyeema** | | **YRF207** | | **YRF203** | | **Millin** | |
| **Amino acid** | **150** | **50** | **150** | **50** | **150** | **50** | **150** | **50** |
| **Alanine** | 0.0453 | 0.0133 | 0.0362 | 0.0204 | 0.0396 | 0.0338 | 0.0232 | 0.0242 |
| **Glycine** | 0.0085 | 0.0025 | 0.0056 | 0.0048 | 0.0069 | 0.0062 | 0.0049 | 0.0045 |
| **Valine** | 0.0113 | 0.0045 | 0.0114 | 0.0099 | 0.0086 | 0.0095 | 0.0066 | 0.0063 |
| **Threonine** | 0.0025 | 0.0020 | 0.0047 | 0.0027 | 0.0047 | 0.0079 | 0.0038 | 0.0031 |
| **Serine** | 0.0101 | 0.0042 | 0.0090 | 0.0067 | 0.0095 | 0.0075 | 0.0064 | 0.0075 |
| **Leucine** | 0.0017 | 0.0006 | 0.0017 | 0.0008 | 0.0013 | 0.0011 | 0.0007 | 0.0009 |
| **Isoleucine** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Proline** | 0.0121 | 0.0036 | 0.0136 | 0.0047 | 0.0125 | 0.0079 | 0.0055 | 0.0059 |
| **Hydroxy proline** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Ornithine** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Methionine** | 0.0041 | 0.0010 | 0.0029 | 0.0060 | 0.0025 | 0.0034 | 0.0013 | 0.0017 |
| **Aspartic acid** | 0.0349 | 0.0073 | 0.0222 | 0.0210 | 0.0386 | 0.0109 | 0.0197 | 0.0158 |
| **Phenylalanine** | 0.0311 | 0.0059 | 0.0130 | 0.0200 | 0.0290 | 0.0304 | 0.0148 | 0.0142 |
| **Histidine (DA)** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Glutamic acid** | 0.0117 | 0.0058 | 0.0137 | 0.0134 | 0.0161 | 0.0200 | 0.0123 | 0.0139 |
| **Lysine** | 0.0091 | 0.0036 | 0.0084 | 0.0049 | 0.0081 | 0.0091 | 0.0045 | 0.0079 |
| **Tyrosine** | 0.0077 | 0.0030 | 0.0069 | 0.0035 | 0.0062 | 0.0054 | 0.0036 | 0.0046 |
| **Arginine** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Histidine (MA)** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Cystine** | 0.0000 | 0.0000 | 0.0000 | 0.0015 | 0.0000 | 0.0021 | 0.0000 | 0.0030 |

**Table 7.13 Concentration (%) of free amino acids present in rice plants fertilised at 150 and 50kg N/ha at the panicle Initiation growth stage**

| Growth Stage | Concentration (%) of free amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rice variety | Kyeema | | YRF207 | | YRF203 | | Millin | |
| Amino acid | 150 | 50 | 150 | 50 | 150 | 50 | 150 | 50 |
| Alanine | 0.0303 | 0.0303 | 0.0300 | 0.0257 | 0.0311 | 0.0330 | 0.0370 | 0.0293 |
| Glycine | 0.0042 | 0.0057 | 0.0054 | 0.0043 | 0.0039 | 0.0057 | 0.0050 | 0.0048 |
| Valine | 0.0133 | 0.0101 | 0.0094 | 0.0069 | 0.0106 | 0.0101 | 0.0142 | 0.0117 |
| Threonine | 0.0053 | 0.0047 | 0.0035 | 0.0028 | 0.0050 | 0.0042 | 0.0058 | 0.0050 |
| Serine | 0.0059 | 0.0060 | 0.0043 | 0.0047 | 0.0047 | 0.0063 | 0.0093 | 0.0100 |
| Leucine | 0.0013 | 0.0015 | 0.0010 | 0.0011 | 0.0010 | 0.0015 | 0.0016 | 0.0016 |
| Isoleucine | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Proline | 0.0090 | 0.0096 | 0.0075 | 0.0081 | 0.0076 | 0.0087 | 0.0292 | 0.0184 |
| Hydroxy proline | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Ornithine | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Methionine | 0.0014 | 0.0028 | 0.0007 | 0.0019 | 0.0027 | 0.0033 | 0.0027 | 0.0020 |
| Aspartic acid | 0.0130 | 0.0106 | 0.0107 | 0.0083 | 0.0149 | 0.0106 | 0.0246 | 0.0176 |
| Phenylalanine | 0.0051 | 0.0071 | 0.0147 | 0.0056 | 0.0038 | 0.0070 | 0.0076 | 0.0068 |
| Histidine (DA) | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Glutamic acid | 0.0045 | 0.0040 | 0.0053 | 0.0054 | 0.0052 | 0.0061 | 0.0073 | 0.0090 |
| Lysine | 0.0052 | 0.0076 | 0.0033 | 0.0061 | 0.0030 | 0.0075 | 0.0071 | 0.0094 |
| Tyrosine | 0.0053 | 0.0085 | 0.0039 | 0.0058 | 0.0047 | 0.0077 | 0.0064 | 0.0068 |
| Arginine | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Histidine (MA) | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Cystine | 0.0000 | 0.0047 | 0.0000 | 0.0000 | 0.0000 | 0.0024 | 0.0000 | 0.0031 |

**Table 7.14 Concentration (%) of free amino acids present in rice plants fertilised at 150 and 50kg N/ha at the flowering growth stage**

| | Concentration (%) of free amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rice variety | Kyeema | | YRF207 | | YRF203 | | Millin | |
| Amino acid | 150 | 50 | 150 | 50 | 150 | 50 | 150 | 50 |
| Alanine | 0.0636 | 0.0432 | 0.0905 | 0.0485 | 0.0724 | 0.0578 | 0.0387 | 0.0343 |
| Glycine | 0.0055 | 0.0025 | 0.0054 | 0.0031 | 0.0041 | 0.0041 | 0.0030 | 0.0030 |
| Valine | 0.0247 | 0.0136 | 0.0236 | 0.0148 | 0.0224 | 0.0164 | 0.0163 | 0.0083 |
| Threonine | 0.0076 | 0.0062 | 0.0132 | 0.0077 | 0.0108 | 0.0088 | 0.0093 | 0.0054 |
| Serine | 0.0149 | 0.0070 | 0.0101 | 0.0067 | 0.0102 | 0.0085 | 0.0079 | 0.0041 |
| Leucine | 0.0023 | 0.0124 | 0.0027 | 0.0150 | 0.0019 | 0.0154 | 0.0012 | 0.0077 |
| Isoleucine | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Proline | 0.0233 | 0.0169 | 0.0345 | 0.0177 | 0.0259 | 0.0220 | 0.0135 | 0.0095 |
| Hydroxy proline | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Ornithine | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Methionine | 0.0035 | 0.0012 | 0.0038 | 0.0017 | 0.0026 | 0.0026 | 0.0020 | 0.0000 |
| Aspartic acid | 0.0745 | 0.0164 | 0.0435 | 0.0183 | 0.0453 | 0.0246 | 0.0294 | 0.0038 |
| Phenylalanine | 0.0152 | 0.0081 | 0.0169 | 0.0099 | 0.0126 | 0.0123 | 0.0073 | 0.0051 |
| Histidine (DA) | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Glutamic acid | 0.0324 | 0.0189 | 0.0350 | 0.0181 | 0.0293 | 0.0314 | 0.0203 | 0.0202 |
| Lysine | 0.0098 | 0.0066 | 0.0154 | 0.0046 | 0.0119 | 0.0084 | 0.0066 | 0.0052 |
| Tyrosine | 0.0061 | 0.0080 | 0.0148 | 0.0088 | 0.0107 | 0.0098 | 0.0056 | 0.0030 |
| Arginine | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Histidine (MA) | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Cystine | 0.0078 | 0.0034 | 0.0083 | 0.0054 | 0.0075 | 0.0033 | 0.0040 | 0.0043 |

**Table 7.15 Concentration (%) of free amino acids present in rice plants fertilised at 150 and 50kg N/ha at the maturity growth stage**

| | Concentration (%) of free amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Rice variety** | **Kyeema** | | **YRF207** | | **YRF203** | | **Millin** | |
| **Amino acid** | **150** | **50** | **150** | **50** | **150** | **50** | **150** | **50** |
| **Alanine** | 0.0039 | 0.0062 | 0.0066 | 0.0093 | 0.0038 | 0.0057 | 0.0057 | 0.0049 |
| **Glycine** | 0.0009 | 0.0015 | 0.0015 | 0.0020 | 0.0010 | 0.0013 | 0.0013 | 0.0012 |
| **Valine** | 0.0039 | 0.0051 | 0.0068 | 0.0060 | 0.0017 | 0.0072 | 0.0066 | 0.0056 |
| **Threonine** | 0.0021 | 0.0025 | 0.0031 | 0.0028 | 0.0009 | 0.0026 | 0.0029 | 0.0019 |
| **Serine** | 0.0028 | 0.0030 | 0.0035 | 0.0030 | 0.0017 | 0.0028 | 0.0036 | 0.0027 |
| **Leucine** | 0.0032 | 0.0050 | 0.0044 | 0.0069 | 0.0021 | 0.0044 | 0.0045 | 0.0031 |
| **Isoleucine** | 0.0060 | 0.0085 | 0.0120 | 0.0083 | 0.0007 | 0.0117 | 0.0107 | 0.0118 |
| **Proline** | 0.0017 | 0.0024 | 0.0029 | 0.0041 | 0.0013 | 0.0024 | 0.0020 | 0.0020 |
| **Hydroxy proline** | 0.0004 | 0.0005 | 0.0005 | 0.0007 | 0.0004 | 0.0003 | 0.0004 | 0.0002 |
| **Ornithine** | 0.0000 | 0.0000 | 0.0006 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Methionine** | 0.0005 | 0.0008 | 0.0008 | 0.0012 | 0.0003 | 0.0007 | 0.0008 | 0.0000 |
| **Aspartic acid** | 0.0080 | 0.0064 | 0.0086 | 0.0077 | 0.0043 | 0.0071 | 0.0089 | 0.0054 |
| **Phenylalanine** | 0.0014 | 0.0022 | 0.0017 | 0.0034 | 0.0008 | 0.0020 | 0.0017 | 0.0013 |
| **Histidine (DA)** | 0.0000 | 0.0000 | 0.0034 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Glutamic acid** | 0.0062 | 0.0048 | 0.0059 | 0.0077 | 0.0028 | 0.0050 | 0.0056 | 0.0041 |
| **Lysine** | 0.0020 | 0.0027 | 0.0030 | 0.0034 | 0.0015 | 0.0027 | 0.0034 | 0.0013 |
| **Tyrosine** | 0.0009 | 0.0018 | 0.0011 | 0.0025 | 0.0007 | 0.0017 | 0.0012 | 0.0012 |
| **Arginine** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Histidine (MA)** | 0.0000 | 0.0000 | 0.0017 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Cystine** | 0.0008 | 0.0007 | 0.0010 | 0.0009 | 0.0010 | 0.0000 | 0.0008 | 0.0000 |

**Table 7.16 Concentration (%) of free amino acids present in rice grain fertilised at 150 and 50kg N/ha**

| | Concentration (%) of free amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Rice variety** | **Kyeema** | | **YRF207** | | **YRF203** | | **Millin** | |
| **Amino acid** | **150** | **50** | **150** | **50** | **150** | **50** | **150** | **50** |
| **Alanine** | 0.0015 | 0.0023 | 0.0024 | 0.0021 | 0.0020 | 0.0017 | 0.0017 | 0.0019 |
| **Glycine** | 0.0006 | 0.0007 | 0.0006 | 0.0005 | 0.0005 | 0.0003 | 0.0004 | 0.0004 |
| **Valine** | 0.0008 | 0.0005 | 0.0008 | 0.0004 | 0.0008 | 0.0006 | 0.0011 | 0.0010 |
| **Threonine** | 0.0008 | 0.0004 | 0.0006 | 0.0004 | 0.0009 | 0.0004 | 0.0000 | 0.0004 |
| **Serine** | 0.0007 | 0.0009 | 0.0011 | 0.0008 | 0.0012 | 0.0005 | 0.0007 | 0.0009 |
| **Leucine** | 0.0002 | 0.0002 | 0.0002 | 0.0002 | 0.0004 | 0.0000 | 0.0000 | 0.0000 |
| **Isoleucine** | 0.0029 | 0.0016 | 0.0021 | 0.0016 | 0.0033 | 0.0020 | 0.0027 | 0.0016 |
| **Proline** | 0.0005 | 0.0003 | 0.0008 | 0.0004 | 0.0004 | 0.0003 | 0.0006 | 0.0004 |
| **Hydroxy proline** | 0.0004 | 0.0000 | 0.0006 | 0.0000 | 0.0004 | 0.0000 | 0.0001 | 0.0002 |
| **Ornithine** | 0.0006 | 0.0010 | 0.0023 | 0.0010 | 0.0013 | 0.0010 | 0.0007 | 0.0016 |
| **Methionine** | 0.0000 | 0.0034 | 0.0003 | 0.0060 | 0.0000 | 0.0034 | 0.0000 | 0.0049 |
| **Aspartic acid** | 0.0022 | 0.0027 | 0.0066 | 0.0011 | 0.0086 | 0.0013 | 0.0032 | 0.0003 |
| **Phenylalanine** | 0.0007 | 0.0011 | 0.0009 | 0.0000 | 0.0008 | 0.0000 | 0.0002 | 0.0000 |
| **Histidine (DA)** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Glutamic acid** | 0.0019 | 0.0031 | 0.0030 | 0.0000 | 0.0036 | 0.0000 | 0.0040 | 0.0002 |
| **Lysine** | 0.0005 | 0.0003 | 0.0002 | 0.0002 | 0.0002 | 0.0002 | 0.0000 | 0.0003 |
| **Tyrosine** | 0.0003 | 0.0005 | 0.0003 | 0.0040 | 0.0002 | 0.0003 | 0.0002 | 0.0004 |
| **Arginine** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Histidine (MA)** | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **Cystine** | 0.0009 | 0.0023 | 0.0011 | 0.0138 | 0.0007 | 0.0143 | 0.0003 | 0.0022 |

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