The Volatile Compounds and Aroma Profile of Some Pigmented Rice Brans After Fermentation

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Abstract
Pigmented rice is known to have nutritional and bioactive compounds which commonly concentrated in the bran layers. Solid-state fermentation is known to enhance the bioactive compounds of rice bran. The study aims to identify fermented rice bran’s volatile compounds and aroma attributes from some pigmented rice (Inpari 24, Saodah, Cempo Ireng and Jeliteng). The rice brans were sterilized at 121°C for 15 minutes and produced non-fermented rice bran and some of them were fermented for 72 hours at 30°C using *Rhizopus oligosporus*. Both non-fermented and fermented rice brans were analysed using solid-phase microextraction-gas chromatography/mass spectrometry (GC/MS) and qualitative descriptive analysis (QDA). The result showed that a total 114 of volatile compounds were identified from fermented and non-fermented rice bran. They consisted of 14 aldehydes, 12 ketones, 14 alcohols, 15 hydrocarbons, 8 acids, 23 esters, 9 benzenes, 5 phenols, 6 furans, 2 lactones, 1 monoterpene, 1 sesquiterpene, 1 thiazole, 1 pyrazine

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and 1 pyridine. The aroma attributes of fermented rice brans obtained by 10 trained panellists in QDA were sweet, caramel, vanilla, grass, milky, fatty, nutty, smoky, rancid, acid, cereal, pungent, earthy and fermented. The non-fermented rice bran has the same aroma as the corresponding fermented rice bran except fermented aroma. Furthermore, Pearson’s correlation test has resulted in several positive correlations between GC-MS results and QDA. These studies indicated that fermented rice bran might increase the volatile compound of rice bran; thus, it may provide opportunities to develop the production of fermented rice bran as a functional ingredient.

Introduction
Rice is one of major food in the world, and it has some varieties. Appearance-wise, there are pigmented and non-pigmented rice. The characteristics between pigmented and non-pigmented rice are differentiated by the bran. Pigmented rice contains anthocyanins in the aleurone (bran layers) as the pigment colorants, have functions as antioxidants and a higher nutritional content than non-pigmented rice. Indonesia has hundreds of rice varieties, both pigmented and non-pigmented. Inpari 24 (red rice) and Jeliteng (black rice) are improved pigmented rice varieties released by the Indonesian Agency for Agricultural Research and Development. Saodah (red rice) and Cempo Ireng (black rice) are local rice varieties in Yogyakarta Province. Pigmented rice contains bioactive compounds such as phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, c-oryzanol and phytic acid. The bioactive compound composition of pigmented depends on the cultivar (genetic), environment, cultivation practices, postharvest and processing.

Over the years, aroma has become one of consumer preference for rice. Volatile compounds play a key role in aroma formation in rice bran (RB) and are usually identified using gas chromatography-mass spectrometry (GC-MS). The volatile compound of RB consists of esters, alkanes, alcohol, ketones and aldehydes. Acid and aldehydes, especially hexanal and nonanal in high amount, are thought to form rancid aroma in RB because of lipid degradation reactions compounds. Our research have showed that the dominant volatile compounds of black RB were 2-furanmetanol, hexanal, naphthalene, 1R-α-pinene, and 4-ethyl-2-methoxyphenol; they produced burnt, nutty, fatty and pungent aromas.

Solid-state fermentation (SSF) is one of the fermentation techniques that can increase the content of bioactive components in foods and is thought to cause the reduction of lipid oxidation in RB. SSF using Rhizopus oryzae resulted in higher total phenolic content and antioxidant activity of Inpari 30 and Cempo Ireng RB; it also reduced the hexanal content due to hydrolases production during the fermentation process, which are responsible for the degradation of polysaccharides, oxidative and extracellular ligninolytic systems. SSF also increased the content of chlorogenic acid, p-hydroxybenzoic acid and vanillin, which gives the vanilla flavour in the bran.

Identification of volatile compounds in some varieties of fermented Indonesia RB has been examined using GC-MS. The developments of such studies are needed to expand our previous study and enhance the knowledge of volatile compounds as well as aroma profiles of Indonesian pigmented RB varieties. Thus, the objective of these studies was to identify the volatile compounds and aroma profiles that are responsible for the flavour attributes of fermented RB from pigmented rice varieties—Saodah, Inpari 24 (red rice), Jeliteng and Cempo Ireng (black rice)—using GC-MS and qualitative descriptive analysis (QDA) methods.

Materials and Methods
Rice Bran Preparation
The samples used in this study were red paddies (Saodah and Inpari 24 varieties) and black paddies (Cempo Ireng and Jeliteng varieties); they were obtained from farmers in Bantul and Sleman Regency, Yogyakarta, Indonesia. RB preparation
was done based on previous study with modification. Rice paddy was de-hulled using LM 24 to obtain brown rice. All samples were then polished using ICHI N50 resulting RB. RB samples were sterilized using an autoclave at 121°C for 15 minutes and stored at 5°C. The samples were divided into a non-fermented group: Inpari 24RB (Inp24NF), Saodah (SaodahNF), Cempo Ireng (CINF) and Jeliteng (Jeliteng NF), and a fermented group: Inpari 24 RB (Inp24F), Saodah (SaodahF), Cempo Ireng (CIF) and Jeliteng (JelitengF).

RB Fermentation
*Rhizopus oligosporus* with code 6010 was purchased from the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. Culture preparation and fermentation method refer to the previous study with a slight modification. *R. oligosporus* was inoculated with the pour plate method on potato dextrose agar (PDA). Fermented and non-fermented RB were dried using a freeze dryer (VirTis, SP SCIENTIFIC BenchTop Pro) for 2 days before further analysis.

Sample Extraction
HS-SPME method was used for sample extraction according to the prior study with modification. Briefly, 2cm of DVB/CAR/PDMS, 50/30 μm fibre (SUPELCO Bellefonte, PA USA) with 2,4,6-trimethyl pyridine as internal standard were used for the extraction. Three grams (±0.1 g) of the sample were put in a 22 mL headspace vial and sealed with Septa PTFE/Silicon septum. The sample was immersed in a water bath at 80°C and then extracted with DVB/CAR/PDMS, 50/30 μm fibre for 30 minutes. The fibres were removed from the vial and injected into the GC-MS injector at desorption for 10 minutes at 250°C in splitless mode.

Volatile Compound Identification
The identification of volatile compound in RB was done using GC-MS (GC Agilent Technologies 7890 A, MS Agilent 5975 C with triple exist detector XL EI/CI) that was equipped with a splitless mode injection port at 250°C. DBWax capillary column (30 m×0.25 mm×0.25 μm film thickness; Agilent Technologies) was used with a mass detector (TSQ Quantum XLS). The detector temperature was programmed at initial temperature of 40°C for 5 minutes, which was then increased to 110°C with 5°C/min speed and then increased again to 230°C at a speed of 8°C/min; finally, it was maintained for 5 min. Interface area temperature was set at 250°C. Helium is used as a carrier gas at a rate of 0.8 mL/min.

Evaluation of Aroma Attributes
QDA was used to evaluate the aroma attributes in RBs. The analysis was performed by 10 panellists (7 females and 3 males) who were trained based on ISO 8586-2012. Before evaluation, all panellists supplied an informed consent letter. The training was held 3 times (3 hours/time) with a final evaluation. Qualitative analysis was carried out by focus group discussions to obtain subjective data on the description of the aroma of fermented and non-fermented RB. Eight samples with trivial code consisting of fermented and non-fermented RB powder from four different varieties were presented individually to avoid bias during testing. Three-gram samples were served in odourless glasses at room temperature. The trained panellists provided an assessment of the aroma attributes present in the RB samples. Furthermore, the panellists were asked to inhale the aroma of the sample for 5 seconds and then neutralized it with the aroma of coffee and drink mineral water.

Data Analysis
The results obtained were processed by principal component analysis (PCA). The PCA results were visualized in the form of a biplot graphic using XLSTAT 2021 software. The correlation of RB volatile compound and aroma attributes from GC-MS and QDA were analysed using Pearson’s correlation with XLSTAT 2021.

Result and discussion
Volatile Compounds of Rice Bran
A total of 114 compounds were identified from GC–MS analysis in fermented and non-fermented RB; they consisted of 14 aldehydes, 12 ketones, 14 alcohols, 15 hydrocarbons, 8 acids, 23 esters, 9 benzenes, 5 phenols, 6 furans, 2 lactones, 1 monoterpen, 1 sesquiterpen, 1 thiazole, 1 pyrazine and 1 pyridine (Figure 1). The identified volatiles and their relative peak areas in the respective variety are summarized in Table 1.
### Table 1: Volatile Compounds in Various Varieties of Non-fermented RB

| No | LRI-Exp | LRI-Ref | Identification | Codes | Compounds | Description |
|----|---------|---------|----------------|-------|-----------|-------------|
|    |         |         |                |       | Fermented | Non-fermented | |
|    | Inp24   | Saodah  | CIF            | Jeliteng | Inp24 | Saodah | CINF | Jeliteng |
|    | F       | F       | NF             | NF       | F       | NF       | NF   | NF       |

#### Aldehydes

1. Hexanal
   - Relative peak area: 0.652 (F), 0.786 (Saodah), 0.528 (CIF), 0.01 (Jeliteng)
   - Description: Grass, tallow, fat
2. Heptanal
   - Relative peak area: nd (F), nd (Saodah), 0.601 (CIF), 0.184 (Jeliteng)
   - Description: Fat, citrus, rancid
3. Octanal
   - Relative peak area: 0.122 (F), nd (Saodah), 0.256 (CIF), 0.223 (Jeliteng)
   - Description: Lemon, green, fat, rancid
4. (2E)-hept-2-enal
   - Relative peak area: 0.42 (F), nd (Saodah), nd (CIF), nd (Jeliteng)
   - Description: Fatty
5. Nonanal
   - Relative peak area: 0.775 (F), 0.34 (Saodah), 0.411 (CIF), 0.7 (Jeliteng)
   - Description: Green, fat, citrus
6. Oct-2-enal
   - Relative peak area: 0.517 (F), 0.112 (Saodah), nd (CIF), nd (Jeliteng)
   - Description: Green, nut, fat
7. Furfural
   - Relative peak area: nd (F), nd (Saodah), 0.331 (CIF), 0.027 (Jeliteng)
   - Description: Sweet
8. Decanal
   - Relative peak area: 0.112 (F), 0.558 (Saodah), 0.049 (CIF), 0.226 (Jeliteng)
   - Description: Bitter, aldehyde, orange peel
9. Benzaldehyde
   - Relative peak area: 0.584 (F), 0.177 (Saodah), 0.297 (CIF), 0.866 (Jeliteng)
   - Description: Nutty, almond
10. Non-2-enal
    - Relative peak area: 0.098 (F), nd (Saodah), 0.066 (CIF), 0.025 (Jeliteng)
    - Description: Woody, fatty
11. Benzeneacetaldehyde
    - Relative peak area: nd (F), nd (Saodah), 0.431 (CIF), 0.104 (Jeliteng)
    - Description: Green, honey
12. 3-methylbenzeldehyde
    - Relative peak area: nd (F), nd (Saodah), 0.326 (CIF), 0.112 (Jeliteng)
    - Description: Cinnamon
13. Cinnamaldehyde
    - Relative peak area: nd (F), nd (Saodah), 0.081 (CIF), 0.028 (Jeliteng)
    - Description: Vaniilla
14. Vanillin
    - Relative peak area: 0.055 (F), 0.053 (Saodah), 0.116 (CIF), 0.035 (Jeliteng)
    - Description: Fruity
15. (3Z)-pent-3-en-2-one
    - Relative peak area: 1.175 (F), 0.632 (Saodah), nd (CIF), nd (Jeliteng)
    - Description: Butter, cream
16. 3-hydroxybutan-2-one
    - Relative peak area: nd (F), nd (Saodah), 0.133 (CIF), 0.068 (Jeliteng)
    - Description: Butter, cream

#### Ketones

1. 3-hydroxybutan-2-one
   - Relative peak area: nd (F), nd (Saodah), 0.133 (CIF), 0.068 (Jeliteng)
   - Description: Butter, cream

1. 3-methylbenzaldehyde
   - Relative peak area: nd (F), nd (Saodah), 0.326 (CIF), 0.112 (Jeliteng)
   - Description: Cinnamon
Table 1: Volatile Compounds in Various Varieties of Non-fermented RB

| No | LRI-Exp | LRI-Ref | Identifica- tion | Codes | Compounds | Relative peak area (µg/kg) | Description |
|----|---------|---------|-----------------|-------|-----------|--------------------------|-------------|
| 17 | 1408    | 1435    | MS+LRI          | Ke3   | (3E)-oct-3-en-2-one | 0.178 0.121 0.05 0.181 0.052 0.04 0.013 0.063 | Berry, nutty, fruity |
| 18 | 1582    | na      | MS              | Ke4   | 6-Methyl-3,5-heptadien-2-one | nd nd nd 0.215 nd nd nd nd | |
| 19 | 1648    | 1643    | MS+LRI          | Ke5   | 1-Phenylethanol | nd nd nd 0.236 0.024 0.042 0.072 0.042 | Must, flower, almond |
| 20 | 1667    | 1684    | MS+LRI          | Ke6   | 2(3H)-Furanone, 5-ethenylidihydromethyl-5-methyl-2,6,6-trimethyl-2-cyclohexene-1,4-dione | nd nd nd nd 0.084 nd nd nd | Musty, woody, tobacco, leafy |
| 21 | 1691    | 1677    | MS+LRI          | Ke7   | 2-Tridecanone | nd nd nd 0.145 nd 0.074 0.063 0.047 | Waxy, fatty, milky |
| 22 | 1803    | 1803    | MS+LRI          | Ke8   | (5Z)-6,10-dimethyl-4-hyldene-5,9-dien-2-one | nd nd nd 0.104 0.03 0.045 0.029 0.03 | |
| 23 | 1865    | 1840    | MS              | Ke9   | 1-(1H-pyrrol-2-yl)-2-one | nd nd nd 0.067 0.024 0.022 0.026 0.011 | Nut, walnut, bread |
| 24 | 1977    | 1967    | MS+LRI          | Ke10  | 1-(1H-pyrrol-2-yl)-2-one | nd nd nd 0.035 nd 0.012 0.071 0.009 | Floral |
| 25 | 1998    | 2006    | MS+LRI          | Ke11  | 6,10,14-Trime-thylpentadecan-2-one | 0.957 0.339 0.124 0.096 0.071 0.116 0.03 0.028 | |
| 26 | 2110    | 2131    | MS+LRI          | Ke12  | | | | |

F (Fermented) | NF (Non-fermented) | NF (Non-fermented) | N (Non-fermented)
### Table 1: Volatile Compounds in Various Varieties of Non-fermented RB

| No. | LRI-Exp | LRI-Ref | Identifier | Codes | Compounds                       | Fermented | Non-fermented | Description                      |
|-----|---------|---------|------------|--------|---------------------------------|-----------|---------------|----------------------------------|
|     |         |         |            |        |                                 | Inp24 F   | Saodah CIF | Jeliteng NF | Inp24 F | Saodah CINF | Jeliteng NF |            |
| 27  | 0       | 91335   | MS+LRI 01 | Ol1    | Ethanol                         | 8.763     | 2.689        | 1.217    | nd       | nd          | 2.828      | nd          | Sweet35  |
| 28  | 0       | 109317  | MS+LRI 02 | Ol2    | 2-Methylpropan-1-ol             | 3.504     | 1.041        | 1.686    | nd       | 0.563       | nd          | nd          | Wine35   |
| 29  | 1247    | 122038  | MS+LRI 03 | Ol3    | 3-Methylbutan-1-ol              | 4.026     | 1.544        | 3.173    | nd       | nd          | nd          | nd          | Fruity, whiskey, Fruity35 |
| 30  | 1441    | 144839  | MS+LRI 04 | Ol4    | Oct-1-en-3-ol                   | 0.38      | 0.244        | nd       | 0.143    | 0.178       | 0.019       | 0.122       | Raw mushroom34 |
| 31  | 1546    | 149436  | MS+LRI 05 | Ol5    | Butane-2,3-diol                 | 5.176     | 2.564        | 4.365    | nd       | nd          | 0.016       | nd          | fruity, creamy, Buttery34 |
| 32  | 1564    | 156639  | MS+LRI 06 | Ol6    | Octan-1-ol                      | nd        | nd           | nd       | 0.016    | nd          | nd          | nd          | Fatty, citrus34 |
| 33  | 1552    | 155037  | MS+LRI 07 | Ol7    | 3,7-Dimethylocta-1,6-dien-3-ol  | nd        | nd           | 0.204    | 0.078    | 0.043       | 0.047       | 0.082       | Floral, citrus34 |
| 34  | 1552    | 149436  | MS+LRI 08 | Ol8    | Butane-2,3-diol                 | 8.023     | 4.351        | 5.267    | nd       | nd          | nd          | nd          | Fruity, creamy, buttery34 |
| 35  | 1572    | na      | MS+LRI 09 | Ol9    | 1,3-Benzenediol, 4-ethyl-        | nd        | nd           | 0.205    | nd       | 0.062       | 0.044       | 0.073       | Slightly sweet35 |
| 36  | 1581    | 156839  | MS+LRI 10 | Ol10   | Butane-2,3-diol                 | 10.809    | 6.133        | 6.911    | nd       | nd          | 0.077       | nd          | Slightly sweet35 |
| 37  | 1879    | 187937  | MS+LRI 11 | Ol11   | Phenylmethanol                  | 0.311     | 0.302        | 0.515    | 0.027    | nd          | nd          | 0.035       | Mild rose35 |
| 38  | 1920    | 192039  | MS+LRI 12 | Ol12   | 2-Phenylethanol                 | 2.809     | 0.975        | 1.497    | 0.278    | 0.07        | 0.06        | 0.051       | Waxy34 |
| 39  | 2173    | 217135  | MS+LRI 13 | Ol13   | 4-Allyl-2-methoxy-              | nd        | nd           | nd       | nd       | 0.021       | nd          | nd          | Clove, honey34 |
| 40  | 2235    | na      | MS+LRI 14 | Ol14   | Pyridin-3-ylmethanol             | 0.268     | 0.193        | nd       | nd       | nd          | nd          | nd          | Waxy34 |
| 41  | 1131    | 113034  | MS+LRI 15 | Hc1    | 1,4-Dimethylbenzene             | nd        | nd           | nd       | nd       | 0.037       | 0.094       | 0.061       | Geranium35 |
| 42  | 1169    | 117438  | MS+LRI 16 | Hc2    | 1,2-Dimethylbenzene             | nd        | 0.187        | 0.208    | 0.208    | 0.072       | 0.076       | 0.125       | Geranium35 |
| No | LRI-Exp | LRI-Ref | Identification | Codes | Compounds | Fermented | Non-fermented | Description |
|----|---------|---------|----------------|-------|-----------|-----------|--------------|-------------|
|    |         |         |                |       |           | Inp24  | Saodah | CIF | Jeliteng | Inp24 | Saodah | CINF | Jeliteng |
| 43 | 1397    | na      | MS             | Hc3   | -benzene  | 0.476  | 0.242 | 0.386 | 0.504 | 0.176 | 0.219 | 0.115 | 0.178 | Alkane$^{35}$ |
| 44 | 1498    | na      | MS             | Hc4   | Tetradecane | 0.335  | 0.16  | 0.235 | nd    | 0.167 | 0.118 | 0.102 | nd    | Waxy$^{34}$ |
| 45 | 1738    | 1734$^{16}$ | MS+LRI       | Hc5   | Naphthalene | 1.273  | 0.74  | 1.534 | 0.962 | 0.264 | 0.386 | 0.313 | 0.399 | Camphor wood-like$^{40}$ |
| 46 | 1835    | 1802$^{18}$ | MS+LRI       | Hc6   | (1R,4R)-1,6-dimethyl-4-(propan-2-yl)-1,2,3,4-tetrahydronaphthalene | nd    | 0.068 | nd    | 0.18  | 0.046 | 0.076 | 0.065 | 0.056 | Herb, spice$^{35}$ |
| 47 | 1853    | 187$^{72}$ | MS+LRI       | Hc7   | 2-Methylnaphthalene | 0.231  | nd    | 0.255 | 0.207 | 0.045 | 0.058 | 0.05  | 0.075 | Sweet, floral, woody$^{44}$ |
| 48 | 1889    | na      | MS             | Hc8   | Naphthalene, 1-methyl-2-ethyl-naphthalene | nd    | 0.131 | 0.299 | 0.134 | 0.031 | nd    | 0.033 | 0.038 | Naphthyl$^{44}$ |
| 49 | 1950    | na      | MS             | Hc9   | 2,7-Dimethyl naphthalene | nd    | nd    | nd    | 0.066 | nd    | nd    | nd    | 0.017 |
| 50 | 1998    | na      | MS             | Hc10  | 2,6-Dimethyl naphthalene | 0.119  | nd    | 0.141 | 0.209 | 0.03  | 0.038 | 0.033 | 0.086 | Grass$^{35}$ |
| 51 | 2006    | 2012$^{22}$ | MS+LRI       | Hc11  | 2,3-Dimethyl naphthalene | nd    | nd    | nd    | 0.035 | nd    | nd    | nd    | 0.011 |
| 52 | 2073    | 2122$^{16}$ | MS+LRI       | Hc12  | 1,6,7-Trimethyl naphthalene | nd    | nd    | nd    | 0.033 | nd    | nd    | nd    | 0.012 |
| 53 | 2112    | 2122$^{16}$ | MS+LRI       | Hc13  | 2,3,6-Trimethyl naphthalene | nd    | nd    | 0.026 | 0.01  | 0.005 | nd    | nd    | 0.045 | Fruity$^{44}$ |
| 54 | 2120    | na      | MS             | Hc14  | 1H-indole | nd    | nd    | 0.105 | nd    | 0.011 | 0.017 | 0.037 | Mothball, burnt$^{35}$ |
| 55 | 2450    | 2376$^{16}$ | MS+LRI       | Hc15  | 1H-indole | nd    | nd    | 0.105 | nd    | 0.011 | 0.017 | 0.037 | Mothball, burnt$^{35}$ |
Table 1: Volatile Compounds in Various Varieties of Non-fermented RB

| No | LRI-Exp | LRI-Ref | Identification | Codes | Compounds | Relative peak area (µg/kg) | Description |
|----|---------|---------|---------------|-------|-----------|--------------------------|-------------|
|    |         |         |               | Fermented | Non-fermented |                       |             |
|    |         |         |               | Inp24 F | Saodah F | CIF Jeliteng F | Inp24 NF | Saodah NF | CINF Jeliteng NF | |
| 56 | 1450    | 1457²⁸  | MS+LRI Ac1    | Acetic acid | nd | nd | 0.301 | 0.192 | nd | 0.216 | 0.183 | Sharp, pungent, sour, vinegar³⁵ |
| 57 | 1628    | 1628²⁷  | MS+LRI Ac2    | Butanoic acid | nd | nd | 0.597 | 0.338 | 0.086 | 0.081 | 0.126 | Sharp acetic cheesy buttery fruity³⁹ |
| 58 | 1853    | 1846²⁷  | MS+LRI Ac3    | Hexanoic acid | 0.321 | 0.544 | 0.083 | 0.081 | 0.017 | nd | 0.0158 | Goaty, fatty acid, vegetable oil, Sweaty⁴⁶ |
| 59 | 1976    | 1971²³  | MS+LRI Ac4    | Heptanoic acid | nd | nd | 0.049 | 0.027 | nd | nd | 0.0158 | Rancid, sour, sweaty |
| 60 | 2065    | 2065²⁷  | MS+LRI Ac5    | Octanoic Acid | 0.344 | 0.238 | 0.02 | 0.018 | 0.034 | 0.029 | sweet, cheese, oily, Fatty⁴⁴ |
| 61 | 2165    | na      | MS Ac6        | Hexadecanoic acid | 3.818 | 1.623 | 0.238 | 0.089 | nd | 1.466 | Rancid, sour, waxy⁴⁵ |
| 62 | 2492    | 2502²⁷  | MS+LRI Ac7    | Dodecanoic acid | nd | nd | 0.056 | 0.0194 | 0.027 | 0.017 | 0.053 | Soapy, waxy⁴⁵ |
| 63 | 2706    | 2706²⁷  | MS+LRI Ac8    | Tetradecanoic acid | 0.491 | 0.271 | 0.095 | 0.06 | 0.082 | 0.158 | Waxy⁴⁴ |
| 64 | 1187    | 1177²⁶  | MS+LRI Es1    | Methyl hexanoate | nd | 0.756 | nd | nd | nd | nd | nd | Fruity⁴⁴ |
| 65 | 1288    | na      | MS Es2        | Methyl (E)-2-hexenoate | nd | 0.353 | nd | nd | nd | nd | nd | nd |
| 66 | 1373    | 1378²⁶  | MS+LRI Es3    | Methyl octanoate | 0.283 | 0.355 | 0.283 | nd | nd | nd | 0.077 | |
| 67 | 1592    | na      | MS Es         | 6-Methyl-3,5 | nd | nd | 0.215 | nd | nd | nd | 0.015 | |
Table 1: Volatile Compounds in Various Varieties of Non-fermented RB

| No | LRI-Exp | LRI-Ref | Identification | Codes | Compounds | Relative peak area (µg/kg) | Description |
|----|---------|---------|----------------|-------|-----------|--------------------------|-------------|
|    |         |         |                | Fermented | Non-fermented | | |
|    | Inp24 | Saodah | CIF | Jeliteng | Inp24 | Saodah | CIF | Jeliteng |
|    | F     | F      | NF   | NF     | F     | NF      | NF   | NF     |
| 68 | 1619   | 1601   | MS+LRI | Es5 | Methyl benzozate | 0.575 nd nd 0.135 0.047 0.068 nd nd | Pleasant smell |
| 69 | 1775   | 1755   | MS+LRI | Es6 | Methyl 2-hydroxybenzoate | nd nd nd 0.085 0.023 nd nd 0.028 | Peppermint |
| 70 | na      | MS     | Es7 | Methyl pyridine-3-carboxylate | nd nd nd nd 0.034 nd nd nd | |
| 71 | 1802   | 1795   | MS | Es8 | Methyl dodecanoate | 0.523 0.431 nd 0.119 0.015 nd nd nd | |
| 72 | 1845   | 1824   | MS+LRI | Es9 | Ethyl dodecanoate | 0.174 nd nd nd nd nd nd nd | Floral, honey |
| 73 | 2014   | 1994   | MS+LRI | Es10 | Methyl tetradecanoate | 2.212 1.144 0.42 0.107 0.053 0.033 0.044 0.023 | Orris |
| 74 | 2052   | 2044   | MS+LRI | Es11 | Ethyl tetradecanoate | 1.035 0.225 0.233 nd 0.017 nd nd 0.008 | Floral, honey |
| 75 | 2116   | 2108   | MS+LRI | Es12 | Methyl pentadecanoate | 0.185 0.126 0.039 nd nd nd nd nd | |
| 76 | 2224   | 2226   | MS+LRI | Es13 | Methyl palmitate | 28.735 14.219 3.831 0.562 0.438 0.198 0.245 0.138 | Waxy, fatty, oily, orris |
| 77 | na     | MS     | Es14 | Ethyl (9Z)-hexadec-9-enoate | 0.441 0.193 nd nd nd nd nd nd | |
| 78 | 2261   | 2259   | MS+LRI | Es15 | Ethyl hexadecanoate | 12.038 2.041 1.266 0.089 0.093 0.031 0.032 | Fatty acids, fruity, sweetish, rancid |
| No | LRI-Exp | LRI-Ref | Identification | Codes | Compounds | Fermented | Non-fermented | Description |
|----|---------|---------|----------------|-------|-----------|-----------|---------------|-------------|
|    |         |         |                |       |           | Inp24 F | Saodah F | CIF Jelteng | Inp24 F | Saodah F | CINF Jelteng | |
| 79 | 2279    | na      | MS             | Es16  | Ethyl (E)-hexadec-9-enoate | 0.494    | 0.158 | nd      | nd      | nd      | nd      | nd | |
| 80 | 2374    | na      | MS             | Es17  | Butyl hexadecanoate | 0.584 | 0.149 | 0.127 | nd      | nd      | nd      | nd | |
| 81 | 2430    | 2424    | MS+LRI         | Es18  | Methyl octadecanoate | 0.54 | 0.281 | 0.057 | nd | 0.003 | nd | nd | Oily, waxy |
| 82 | 2451    | na      | MS             | Es19  | Methyl (E)-octadec-9-enoate | 17.033 | 8.657 | 1.667 | 0.052 | 0.071 | 0.02 | nd | Waxy, fatty, oily |
| 83 | 2466    | 2450    | MS+LRI         | Es20  | Ethyl octadecanoate | 0.375 | nd | nd | nd | nd | nd | nd | Fatty acids |
| 84 | 2486    | na      | MS             | Es21  | Ethyl (9Z)-octadec-9-enoate | 9.541 | 2.027 | 0.725 | 0.456 | 0.05 | nd | 0.035 | Fatty acids, vegetable oil, rancid |
| 85 | 2502    | na      | MS             | Es22  | Methyl octadeca-9,12-dienoate | nd | 6.335 | 1.554 | 0.155 | 0.131 | 0.027 | 0.043 | 0.035 | Waxy, fatty, oily |
| 86 | 2535    | 2491    | MS+LRI         | Es23  | Ethyl (9Z,12Z)-octadeca-9,12-dienoate | 7.249 | 1.416 | 0.693 | nd | 0.026 | 0.014 | nd | nd | Fatty acids, vegetable oil, rancid |
| 87 | 2568    | na      | MS             | Es24  | Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate | nd | 0.218 | 0.046 | nd | nd | nd | nd | Oily fatty fruity |
| 88 | 1122    | 1115    | MS+LRI         | Bz1   | Ethylbenzene | nd | nd | nd | 0.24 | 0.066 | 0.128 | 0.051 | 0.093 | Gasoline |

### Table 1: Volatile Compounds in Various Varieties of Non-fermented RB

| No | LRI-Exp | LRI-Ref | Identification | Codes | Compounds | Fermented | Non-fermented | Description |
|----|---------|---------|----------------|-------|-----------|-----------|---------------|-------------|
|    |         |         |                |       |           | Inp24 F | Saodah F | CIF Jelteng | Inp24 F | Saodah F | CINF Jelteng | |
| 89 | 2535    | 2491    | MS+LRI         | Es23  | Ethyl (9Z,12Z)-octadeca-9,12-dienoate | 7.249 | 1.416 | 0.693 | nd | 0.026 | 0.014 | nd | nd | Fatty acids, vegetable oil, rancid |
| 90 | 2568    | na      | MS             | Es24  | Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate | nd | 0.218 | 0.046 | nd | nd | nd | nd | Oily fatty fruity |
| 91 | 1122    | 1115    | MS+LRI         | Bz1   | Ethylbenzene | nd | nd | nd | 0.24 | 0.066 | 0.128 | 0.051 | 0.093 | Gasoline |

**Benzenes**

| No | LRI-Exp | LRI-Ref | Identification | Codes | Compounds | Fermented | Non-fermented | Description |
|----|---------|---------|----------------|-------|-----------|-----------|---------------|-------------|
|    |         |         |                |       |           | Inp24 F | Saodah F | CIF Jelteng | Inp24 F | Saodah F | CINF Jelteng | |
| 92 | 1122    | 1115    | MS+LRI         | Bz1   | Ethylbenzene | nd | nd | nd | 0.24 | 0.066 | 0.128 | 0.051 | 0.093 | Gasoline |

**Relative peak area (µg/kg)**

- 79
- 80
- 81
- 82
- 83
- 84
- 85
- 86
- 87
- 88

**Description**

- Oily, waxy
- Fatty acids
- Fatty acids, vegetable oil, rancid
- Fatty acids, vegetable oil
- Fatty acids, vegetable oil, rancid
- Oily fatty fruity
- Gasoline
Table 1: Volatile Compounds in Various Varieties of Non-fermented RB

| No | LRI-Exp | LRI-Ref | Identification | Codes | Compounds | Relative peak area (µg/kg) | Description |
|---|---|---|---|---|---|---|---|
| | | | | Fermented | Non-fermented | | |
| | | | | Inp24 | Saodah | CIF | Jeliteng | Inp24 | Saodah | CINF | Jeliteng |
| 89 | 1250 | 1240 | MS+LRI | Bz2 | Styrene | 0.429 | 0.494 | 0.364 | 0.23 | nd | 0.208 | 0.143 | 0.219 | Balsamic, gasoline |
| 90 | 1266 | 1260 | MS+LRI | Bz3 | 1-Methyl-2-(propan-2-yl)benzene | nd | nd | 0.253 | 0.462 | 0.093 | 0.128 | 0.112 | 0.182 | |
| 91 | 1276 | 1269 | MS+LRI | Bz4 | 1,3,5-Trimethylbenzene | nd | 0.19 | nd | 0.302 | 0.078 | 0.1 | 0.078 | 0.128 | Sweet |
| 92 | 1419 | 1274 | MS+LRI | Bz5 | 1-Methyl-4-(propan-2-yl)benzene | nd | nd | nd | 0.056 | nd | nd | nd | 0.051 | Citrus |
| 93 | 1724 | 1721 | MS+LRI | Bz6 | 1,2-Dimethoxybenzene | nd | nd | 0.185 | nd | nd | nd | nd | 0.066 | Sweet, creamy, vanilla |
| 94 | 1828 | 1820 | MS+LRI | Bz7 | 1-methoxy-4-[(E)-prop-1-enyl]benzene | nd | nd | 0.302 | 0.171 | 0.052 | 0.1 | 0.075 | 0.063 | Sweet, licorice, medicinal |
| 95 | 1593 | 1593 | MS+LRI | Bz8 | (1R,4E,9S)-4,11,11-Trimethyl-8-methylidenecyclo[7.2.0]undec-4-ene | nd | nd | 0.337 | 0.22 | 0.275 | 0.094 | 0.127 | Clove, pepper, floral |
| 96 | 1140 | 1138 | MS+LRI | Bz9 | 3,7,7-trimethylbicyclo[4.1.0]hept-3-ene | 0.339 | 0.487 | 0.552 | 0.092 | 0.332 | 0.232 | 0.176 | Sweet, pungent |
| 97 | 1877 | 1872 | MS+LRI | Ph1 | 2-Methoxyphenol | 0.744 | 0.457 | 6.524 | 0.593 | 0.022 | 0.028 | 0.096 | 0.207 | Nutty |
| 98 | 1913 | 191224 | MS+LRI | Ph2 | 2,6-bis(1,1-dimethylmethyl)phenol | nd | nd | 0.09 | 0.09 | 0.025 | 0.028 | 0.025 | 0.017 | |
### Table 1: Volatile Compounds in Various Varieties of Non-fermented RB

| No | LRI-Exp | LRI-Ref | Identifi -cation | Codes | Compounds | Relative peak area (µg/kg) | Description |
|----|---------|---------|------------------|-------|-----------|----------------------------|-------------|
|    |         |         |                  |       | Fermented | Non-fermented              |             |
|    |         |         |                  |       | Inp24 F | Saodah F | CIF F | Jeliteng F | Inp24 NF | Saodah NF | CINF NF | Jeliteng NF |
| 99 | 2002    | 2000    | MS+LRI           | Ph3   | 1.021    | 0.383    | 0.664 | 0.051     | 0.014     | 0.073     | 0.019     | 0.02     | Sweet, medicinal |
| 100 | 2203 | 2200 | MS+LRI           | Ph4   | 0.536    | 0.147    | 0.258 | 0.507     | 0.074     | 0.231     | 0.093     | 0.179    | dry, woody, fresh, roasted |
| 101 | 2315 | 2317 | MS+LRI           | Ph5   | nd       | nd       | nd   | 0.077     | 0.03      | 0.055     | 0.034     | 0.031    |             |

**Furans**

| No | LRI-Exp | LRI-Ref | Identifi -cation | Codes | Compounds | Relative peak area (µg/kg) | Description |
|----|---------|---------|------------------|-------|-----------|----------------------------|-------------|
| 102 | 1230    | 1234    | MS+LRI           | Fu1   | 0.626    | 0.177    | 0.118 | 0.248     | 0.089     | 0.102     | 0.032     | 0.075    | Nutty, beany, buttery |
| 103 | 1426    | na      | MS               | Fu2   | nd       | nd       | nd   | 0.322     | nd        | 0.073     | 0.046     | 0.121    |             |
| 104 | 1667    | 1684    | MS+LRI           | Fu3   | nd       | nd       | nd   | nd        | 0.027     | nd        | nd        | nd       |             |
| 105 | 2037    | na      | MS               | Fu4   | nd       | nd       | 0.06 | nd        | nd        | nd        | nd        | nd       |             |
| 106 | 2391    | na      | MS               | Fu5   | 0.097    | 0.02     | 0.045 | 0.036     | 0.01      | 0.02      | 0.012     | 0.008    | Sweet |
| 107 | 2368    | na      | MS               | Fu6   | 0.201    | nd       | nd   | 0.112     | 0.045     | 0.052     | 0.023     | 0.03     |             |

**Lactone**

| No | LRI-Exp | LRI-Ref | Identifi -cation | Codes | Compounds | Relative peak area (µg/kg) | Description |
|----|---------|---------|------------------|-------|-----------|----------------------------|-------------|
| 108 | 1613    | na      | MS               | Fu7   | nd       | nd       | nd   | 0.123     | 0.039     | 0.045     | 0.05     | 0.042    | Creamy, fatty |
| 109 | 2037    | 2051    | MS+LRI           | Fu8   | 0.135    | 0.274    | nd   | 0.288     | 0.096     | 0.083     | 0.06     | 0.091    | Cotton candy |
| No. | LRI-Exp | LRI-Ref | Identification | Codes | Compounds                                      | Fermented Relative peak area (µg/kg) | Non-fermented Relative peak area (µg/kg) | Description              |
|-----|---------|---------|----------------|-------|------------------------------------------------|-------------------------------------|----------------------------------------|--------------------------|
|     |         |         |                 |       | Esehoxolan-2-one                                 | F 1.356 1.39 5.61 5.325 0.435 1.064 1.606 2.63 | F 1.39 5.61 5.325 0.435 1.064 1.606 2.63 | Lemon, orange            |
|     |         |         |                 |       | Monoterpenes (4R)-1-Methyl-4-prop-1-en-2-ylcyclohexene | F 0.151 nd nd 0.151 nd nd nd 0.054 | F nd nd nd nd nd nd nd |            |
|     |         |         |                 |       | Sesquiterpenoid (1R,8aS)-1,6-Dimethyl-4-(pr-3,7,8,8a-hexahydro-1,2,3,7,8,8a-hexahydra) | F 0.591 0.129 0.48 0.193 0.094 0.112 0.041 0.061 | F nd nd nd nd nd nd nd | Sulfurous, meaty        |
|     |         |         |                 |       | Thiazole 1,3-Benzothi-azole                       | F 0.384 nd nd nd nd nd nd nd | F nd nd nd nd nd nd nd | Coffee, caramellic      |
|     |         |         |                 |       | Pyridine 2,3-Dimethyl-pyridine                    | F nd nd nd nd nd nd nd nd | F nd nd nd nd nd nd nd | Roasted, green          |
|     |         |         |                 |       | Pyrazine 2-Methyl-5-[(E)-prop-1-enyl]pyrazine      | F nd nd nd 0.145 nd nd nd nd | F nd nd nd nd nd nd nd |            |

*MS, mass spectrum match to those NIST/EPA/NIH Mass spectral database; MS+LRI, mass spectrum match to those NIST/EPA/NIH Mass spectral databases and LRI match with literature value
nd: not detected
Volatile compounds in RB are probably composed of the original compounds, the compound resulting from the Maillard reaction due to the sterilization process and the compound resulting from the fermentation. The original compounds in RB were hexanal; heptanal; octanal; nonanal; benzaldehyde; (3E)-oct-3-en-2-one; 2,6,6-trimethyl-2-cyclohexene-1,4-dione; (5Z)-6,10-dimethylundeca-5,9-dien-2-one; 1-(1H-pyrrol-2-yl)ethan-1-one; oct-1-en-3-ol; octan-1-ol; 3,7-dimethylocta-1,6-dien-3-ol; phenylmethanol; 1,2-dimethylbenzene; tetradecane; pentadecane; acetic acid; butanoic acid; hexanoic acid; octanoic acid; phenol; 2-pentylfuran; 2,3-dihydro-1-benzofuran; (3R)-3,4,4-trimethyloxolan-2-one; (4R)-1-Methyl-4-prop-1-en-2-ylcyclohexene (d-limonene) and naphthalene.

Reaction between amino acids and carbohydrates in Maillard reaction were reported to form pyrazines such as 2-methyl-5-[(E)-prop-1-enyl]pyrazine, which contributed in roasted and green aroma. Heat treatment also allegedly formed vanillin and furfural, due to thermal degradation of ferulic acid and sugars, respectively. 4-ethenyl-2-methoxyphenol was also reported to be derived from thermal decarboxylation of ferulic acid in RB.

There were 20 volatile compounds that could have been formed during the fermentation process—as they were only found in fermented RB—as shown in Figure 2. They are oct-2-enal, (E); (3Z)-pent-3-en-2-one; 6-methyl-3, 5-heptadiene-2-one; 3-methylbutan-1-ol; butan-2,3-diol; pyridin-3-ylmethanol (nicotinyl alcohol); methyl hexanoate; methyl (E)-2-hexenoate; methyl octanoate; ethyl dodecanoate; methyl pentadecanoate; methyl (9Z)-hexadec-9-enoate; ethyl (E)-hexadec-9-enoate; butyl hexadecanoate; ethyl octadecanoate; methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate (methyl
linolenate); dihydro-3-hydroxy-4, 4-dimethyl-2(3H)-furanone; 2,3-dimethylpyridine and 2-methyl-5-[(E)-prop-1-enyl]pyrazine. This proved that fermentation might produce more volatile compounds in RB.

Esters were reported as the major volatiles constituent in fermented RB. The major esters compounds identified in the fermented RB were methyl palmitate, 9-octadecenoic acid, methyl ester, (E)- and ethyl hexadecanoate. Those compounds are responsible for waxy, fatty and oily odor and were increasing due to the fermentation process. Esters mostly are formed by esterification between acids and alcohols during fermentation. The second most common substances in fermented RB were alcohols. Among them were the contents of butane-2,3-diol, 3-methylbutan-1-ol and phenylmethanol. Butane-2,3-diol is thought to provide a characteristic of buttery and creamy aroma, and is formed from glucose catabolism via the glycolysis pathway. Phenylmethanol is known to be formed from the reduction of benzoic acid assisted by microorganisms. Similar to 3-methyl-3-butanal, phenylmethanol tends to increase in RB treated with fermentation due to the metabolic activity of microbes that form in RB through the glycolysis pathway.

Amyl alcohols (3-methylbutan-1-ol and 2-methylbutan-1-ol) have fermented and malt-like odour notes. These are also detected in other fermented rice such as makegolli (Korean rice wine). Another alcohol compound that was identified in fermented RB is 2-phenylethanol; it is thought to have a slightly rose floral scent. The formation of 2-phenylethanol might be from hydrolysis of phenylethyl ester and phenylethyl acetate.

In the group of aldehydes, hexanal, nonanal and benzaldehyde were the compounds with the highest relative peak areas found in fermented RB. These three compounds tend to increase compared to non-fermented RB. Saturated aldehydes such as hexanal and nonanal might be formed by linoleic acid (C18:2) oxidation as one of the main fatty acids in RB. Linoleic acid might be oxidized to form 9-OOOH and 13-OOOH hydrogen peroxides, which are further degraded to form saturated aldehydes such as oct-2enal and hexanal. Hexanal allegedly contributed to grass, tallow and fat aroma; meanwhile, nonanal might have contributed to green, fat and citrus aroma. Benzaldehyde may be the odour-active compounds in RB. The content of amino acids in RB such as valine, isoleucine, leucine and phenylalanine can be transformed into Strecker aldehydes, leading to 2-methylpropanal, 2-methylbutanal, 3-methylbutanal and phenylacetaldehyde, which is also the most effective precursor for the production of benzaldehyde. Benzaldehyde is responsible for giving the nutty and almond aroma.
In addition, other aldehydes found in fermented rice bran in relatively low peak areas include heptanal, octanal, (2E)-hept-2-enal, oct-2-enal, furfural, decanal, non-2-enal, benzeneacetaldehyde, 3-methylbenzaldehyde and vanillin. Furfural, which provides bread, almond and sweet aroma, was derived from the thermal degradation of sugars such as fructose and glucose. Unsaturated aldehydes such as (2E)-hept-2-enal, oct-2-enal and non-2-enal are the products of linoleic acid oxidation that provides fat and green, nut and fat aroma, respectively. Non-2-enal and hexanal were also reported to cause rancid defects in virgin olive oil. Benzeneacetaldehyde was found in all varieties of non-fermented RB, while in fermented RB, it was only identified in Jeliteng, and the content tends to increase due to the fermentation process. Benzeneacetaldehyde may be formed from phenylalanine precursor. Vanillin, which gives vanilla aroma, originates from lignin degradation in aerobic conditions and might be formed by the thermal degradation of ferulic acid.

Naphthalene was the highest content of hydrocarbon that was found in RB. The relative peak areas of naphthalene which contributed to camphor wood-like aroma tends to increase due to fermentation. In other prior research, naphthalene was also found to be an odour-active compound in red and black rice.

Principle Component Analysis (PCA) of Volatile Compounds in RB

The PCA biplot of volatile compounds in fermented and non-fermented RB is shown in Figure 3.
Fig. 3: Principal Component Analysis (PCA) biplot of: a) non-fermented RB, b) fermented RB, c) fermented and non-fermented RB. The variable descriptions were referred to their corresponding compound in Table 1.

![Biplot](image)

Fig. 4: Summary of major volatile compounds (ppb) of Inpari 24 non-fermented (Inp24NF); Inpari 24 fermented (Inp24F); Saodah non-fermented (SaodahNF); Saodah fermented (SaodahF); Cempo Ireng non-fermented (CINF); Cempo Ireng fermented (CIF); Jeliteng non-fermented (JelitengNF); and Jeliteng fermented (JelitengF).
PCA was used to analyse the grouping of fermented and non-fermented RB in all varieties and to determine the volatiles characteristic in each group. The data variation (F1 and F2) of non-fermented, fermented and RB groups were 82.15%, 87.08% and 72.11%, respectively (Figure 3). Figure 3a showed that non-fermented RB variety Cempo Ireng, Inpari 24 and Saodah were grouped together with hexadecanoic acid (Ac6), methyl palmitate (Es13), (4R)-1-methyl-4-prop-1-en-2-ylcyclohexene (d-limonene) (Mt1), benzaldehyde (Al9), nonanal (Al5) and octanal (Al3) as the dominant compounds, while Saodah was dominated with ethanol (Ol1). The result of fermented RB is shown in Figure 3b. Saodah and Inpari 24 RB were dominated by methyl palmitate (Es13), 9-octadecenoic acid, methyl ester, (E)- (Es19), butane-2,3-diol (Ol10), ethyl hexadecanoate (Es15) and ethanol (Ol1), while Jeliteng and Cempo Ireng had the higher amount of (4R)-1-methyl-4-prop-1-en-2-ylcyclohexene (d-limonene) (Mt1), hexadecanoic acid (Ac6), 2-methoxyphenol (Ph1) and naphthalene (Hc5).

Cempo Ireng and Jeliteng might be in a group since they have the same pigment: black rice. Figure 3c showed the PCA of fermented and non-fermented RB. Fermented RB of Inpari 24, Saodah and Cempo Ireng were grouped together and their dominant compounds were methyl palmitate (Es13), 9-octadecenoic acid, methyl ester, (E)- (Es19), butane-2,3-diol (Ol10) and ethanol (Ol1) which contribute to oily, waxy, fatty, orris, fruity, creamy, buttery and sweet odours. Meanwhile, fermented Jeliteng was in the same group with all non-fermented RB. This group was characterized by higher amount of (4R)-1-methyl-4-prop-1-en-2-ylcyclohexene (d-limonene) (Mt1), hexadecanoic acid (Ac6), nonanal (Al5), naphthalene (Hc5) and benzaldehyde (Al9), which provided lemon, orange, green, fat, champor wood-like and almond aroma.

The dominant aroma of fermented RB were esters, especially methyl palmitate; ethyl hexadecanoate; 9-octadecenoic acid, methyl ester; ethyl (9Z)-octadec-9-enoate and methyl octadeca-9,12-dienoate (Figure 4). Esters were formed by the esterification of acids and alcohol, and provide fruity and floral notes. Therefore, JelitengF had the lowest relative peak areas of esters due to a few numbers of alcohols. The relative peak areas of esters might be affected by sugar content of RB, yeast strains, the temperature of fermentation and aeration. Alcohols that dominated the fermented RB were ethanol; 2-methylpropan-1-ol; 3-methylbutan-1-ol; butane-2,3-diol and 2-phenylethanol. Alcohol in fermented RB might be derived by sugar fermentation or amino acids catabolism, so the content of alcohol in fermented RB might be differed by sugar and amino acid availability. Other compounds that dominated fermented RBs were hydrocarbons. Tetradecane, pentadecane and naphthalene were the most abundant hydrocarbons contained in fermented RBs. Even so, it might have a little contribution on RB aroma because generally hydrocarbons have high threshold values. Acids also became one of main compounds in fermented RB. Prior study stated that acid was the most abundant volatile oil in red and black rice and tends to provide an unpleasant aroma. In this study, the relative peak areas of acids were relatively lower than other compounds such as esters and phenols. The differences might be affected by the degree of oxidation. RBs used in this study were fresh so the level of oxidation could be minimized and result in a lower level of acids.

QDA of Aroma Attributes
Aroma attributes of fermented and non-fermented RBs from each variety are shown in Figure 5. The QDA result showed that both the fermented and non-fermented RBs had a similar aroma attribute (sweet, caramel, rancid, acid, pungent, fatty, milky, woody, sour, cereal, vanilla, nutty, smokey) except “fermented” aroma, which was only identified in fermented RBs. PCA analysis was used group varieties that had some similarities and matching aromas, based on panellists’ identification. Both fermented and non-fermented RBs tended to have sweet, acid, vanilla, cereal and caramel aromas as their major attributes. The differences between these RBs were in the number of panellists that recognized the aroma. Aroma attributes of fermented RBs were noticed more by panellists; this might conclude that the intensities of aromas in fermented RBs were higher than non-fermented RBs. The aroma attribute similarities between fermented and non-fermented RBs were allegedly because the RBs used in this study were fresh; thus, they were not dominated by unwanted aroma like pungent and rancid. Sweet, caramel and vanilla aroma might have been derived from the Maillard reaction.
Fig. 5: Aroma attributes of a) non-fermented RB and b) fermented RB by QDA
Fig. 6: Principal Component Analysis (PCA) biplot of: a) non-fermented RB, b) fermented RB, c) fermented and non-fermented RB by QDA.
Non-fermented RBs in varieties Jeliteng and Inpari 24 were in the same group with cereal and acid as the dominant aromas, while Saodah and Cempo Ireng were in the same group with the dominant aromas of sweet, vanilla and caramel (Figure 6a). Biplot aroma of fermented RB is shown in Figure 6b. Inpari 24 and Cempo Ireng were in the same group with cereal, acid and sweet as the dominant aromas, while Saodah and Jeliteng were in the same group that were characterized by vanilla and caramel aromas. Figure 6c shows the PCA of fermented and non-fermented RB aromas. Fermented RB varieties Inpari 24, Jeliteng, Cempo Ireng are in the group with non-fermented Inpari 24. These RBs are characterized by acid and cereal aromas. Non-fermented RB varieties such as Jeliteng, Cempo Ireng and Saodah are in the same group with Saodah F; they were characterized by sweet, vanilla and caramel aromas. Figure 6a shows that non-fermented RB in Jeliteng and Inpari 24 varieties were in the same group with cereal and acid as the dominant aromas, while Saodah and Cempo Ireng were in the same group with the dominant aromas of sweet, vanilla and caramel. Biplot aroma of fermented RBs is shown in Figure 6b. Inpari 24 and Cempo Ireng were in the same group with cereal, acid and sweet as the dominant aromas, while Saodah and Jeliteng were in the same group that were characterized by vanilla and caramel aromas. Figure 6c shows the PCA of fermented and non-fermented RB aromas. Fermented RBs in Inpari 24, Jeliteng, and Cempo Ireng varieties were the group with non-fermented Inpari 24. These RBs are characterized by acid and cereal aromas. Non-fermented RB varieties such as Jeliteng, Cempo Ireng and Saodah were in the same group with the fermented Saodah; they were characterized by sweet, vanilla and caramel aromas.

**Pearson’s Correlation of Volatile Compounds and Aroma Profile**

The correlation between volatile compounds of RB are identified by GC-MS, and the aroma attributes are identified by QDA (Table 2). It shows that some volatile compounds have positive correlation with aroma description obtained by QDA. Hexanal has positive correlation with grass aroma. This is in accordance with the study that stated grass, tallow and fat as the aromas of hexanal. Oct-2-enal has positive correlation with fatty aroma, similar to a previous study’s description. The 2-methylpropan-1-ol and 3-methylbutan-1-ol were positively correlated with fermented aroma. It is also in accordance with the description by another previous study. Acetic acid was described to have sharp, pungent, sour and vinegar aroma and the Pearson’s correlation showed that acetic acid correlated with pungent aroma. Ethyl hexadecanoate and ethyl octadecanoate have positive correlation with fatty aroma, while ethyl (9Z)-octadec-9-enoate positively correlated with acid aroma. 2-methyl-5-[(E)-prop-1-enyl] pyrazine correlated to smokey aroma. These correlations match with the aroma description by previous studies.

**Table 2: Pearson’s Correlation between RB Volatile Compounds by GC-MS and Aroma Description by Panelists**

| Variables | Hexanal | 2-Octenal, (E) | 1-Propanol, 2-methyl | 1-Butanol, 3-methyl | Acetic acid | Ethyl hexadecanoate | Ethyl stearate | Ethyl oleate | Pyrazine, 2-methyl-5-(1-propenyl), (E) |
|-----------|---------|----------------|----------------------|-------------------|------------|--------------------|----------------|-------------|-------------------------------------|
| Sweet     | -0.035  | 0.232          | 0.003                | -0.160            | -0.177     | 0.165              | 0.189          | 0.163       | 0.567                               |
| Caramel   | 0.307   | -0.418         | -0.267               | -0.195            | -0.294     | -0.251             | -0.574         | -0.421      | 0.082                               |
| Vanilla   | -0.274  | -0.549         | -0.353               | -0.221            | 0.002      | -0.526             | -0.570         | -0.541      | -0.114                              |
| Grass     | 0.895   | 0.519          | 0.278                | 0.135             | -0.003     | 0.452              | 0.424          | 0.503       | 0.424                               |
| Milky     | -0.384  | -0.257         | -0.152               | -0.156            | 0.142      | -0.194             | -0.087         | -0.244      | -0.087                              |
| Fatty     | -0.086  | 0.517          | 0.311                | 0.085             | 0.001      | 0.502              | 0.607          | 0.494       | 0.087                               |
| Nutty     | 0.312   | 0.264          | 0.339                | 0.236             | -0.603     | 0.257              | 0.215          | 0.248       | 0.277                               |
| Smokey    | 0.259   | 0.422          | 0.376                | 0.284             | 0.179      | 0.458              | 0.535          | 0.459       | 0.555                               |
| Rancid    | -0.836  | -0.359         | -0.192               | -0.018            | 0.163      | -0.303             | -0.293         | -0.337      | -0.293                              |
| Acid      | 0.356   | 0.473          | 0.375                | 0.447             | 0.266      | 0.482              | 0.459          | 0.510       | 0.459                               |
| Cereal    | 0.197   | 0.497          | 0.627                | 0.760             | -0.114     | 0.560              | 0.509          | 0.557       | 0.218                               |
| Pungent   | -0.197  | -0.292         | -0.462               | -0.446            | 0.543      | -0.313             | -0.238         | -0.305      | 0.143                               |
| Earthly   | 0.094   | -0.598         | -0.633               | -0.648            | 0.661      | -0.588             | -0.488         | -0.582      | 0.683                               |
| Fermented | 0.183   | 0.294          | 0.586                | 0.735             | -0.259     | 0.382              | 0.314          | 0.371       | 0.314                               |
Conclusion
Volatiles compounds found in RB consist of ester, hydrocarbon, aldehyde, ketone, acid, phenol, furan, lactone, monoterpenes, thiazole, sesquiterpenes, pyridine and pyrazine. A total of 114 volatile compounds were found, out of which 106 were contained in fermentation RB and 94 in non-fermentation RB. Fermentation on RB formed some new volatile compounds such as oct-2-enal; (3Z)-pent-3-en-2-one; 6-methyl-3,5-heptadiene-2-one; 3-methylbutan-1-ol; butane-2,3-diol; 3-pyridinemethanol (nicotinyl alcohol); methyl hexanoate; methyl (E)-2-hexenoate; methyl octanoate; ethyl dodecanoate; methyl pentadecanoate; methyl (9Z)-hexadec-9-enoate; ethyl (E)-hexadec-9-enoate; butyl hexadecanoate; ethyl octadecanoate; methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate (methyl linolenate); dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone; 2,3-dimethylpyridine and 2-methyl-5-[(E)-prop-1-enyl]pyrazine. The result between GC-MS identification has some positive correlation with QDA; hexanal was correlated with grass; oct-2-enal, ethyl octadecanoate and ethyl hexadecanoate were correlated with fatty; 2-methylpropan-1-ol and 3-methylbutan-1-ol were correlated with fermented aroma; acetic acid was correlated with pungent; ethyl (9Z)-octadec-9-enolate was correlated with acid and 2-methyl-5-[(E)-prop-1-enyl] pyrazine was correlated with smoky.

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Conflict of interest
All authors declare no conflict of interest.

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