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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 30ºC 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, smokey, 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.[1] 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.[2] Saodah (red rice) and Cempo Ireng (black rice) are local rice varieties in Yogyakarta Province.[3,4] Pigmented rice contains bioactive compounds such as phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, c-oryzanol and phytic acid.[1] The bioactive compound composition of pigmented depends on the cultivar (genetic), environment, cultivation practices, postharvest and processing.[1]

Over the years, aroma has become one of consumer preference for rice.[5] 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.[6] Acid and aldehydes, especially hexanal and nonanal in high amount, are thought to form rancid aroma in RB because of lipid degradation reactions compounds.[6,7] Our research have showed that the dominant volatile compounds of black RB were 2-furanmetanol, hexanal, naphthalene, 1R-α -pinene, and 4-ethenyl-2-methoxyphenol, while for non-pigmented RB, they were 2-furanmetanol, nonanal, methyl tetradecanoate, phenol and 4-ethenyl-2-methoxyphenol; they produced burnt, nutty, fatty and pungent aromas.[8]

Solid-state fermentation (SSF) is one of the fermentation techniques that can increase the content of bioactive components in foods[9-11] 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.[8,12] SSF also increased the content of chlorogenic acid, p-hydroxybenzoic acid and vanillin, which gives the vanilla flavour in the bran.[13]

Identification of volatile compounds in some varieties of fermented Indonesia RB has been examined using GC-MS.[8] The developments of such studies are needed to expand our previous study[8] 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[3] 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[8] 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[14] 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 40ºC for 5 minutes, which was then increased to 110ºC with 5ºC/min speed and then increased again to 230ºC at a speed of 8ºC/min; finally, it was maintained for 5 min. Interface area temperature was set at 250ºC. 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.[15]

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 monoterpene, 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	Relative peak area (µg/kg)								Description
						Fermented				Non-fermented				
						Inp24 F	Saodah F	CIF	Jeliteng F	Inp24 NF	Saodah NF	CINF	Jeliteng NF	
					Aldehydes									
1	0	1065[16]	MS+LRI	AI1	Hexanal	0.652	0.786	nd	0.528	0.01	0.38	0.023	0.386	*Grass, tallow, fat*[44]
2	1182	1186[17]	MS+LRI	AI2	Heptanal	nd	nd	nd	0.601	0.184	0.204	nd	0.263	*Fat, citruss, rancid*[44]
3	1284	1286[18]	MS+LRI	AI3	Octanal	0.122	nd	nd	0.256	0.223	0.193	0.116	0.24	*Lemon, green, fat*[44]
4	1318	1320[19]	MS+LRI	AI4	(2E)-hept-2-enal	0.42	nd	nd	nd	nd	nd	nd	0.174	*fatty*[35]
5	1390	1392[16]	MS+LRI	AI5	Nonanal	0.775	0.34	0.411	0.7	0.529	0.45	0.167	0.412	*Green, fat, citruss*[35]
6	1418	1424[19]	MS+LRI	AI6	Oct-2-enal	0.517	0.112	nd	nd	nd	nd	nd	nd	*Green, nut, fat*
7	1485	1462[18]	MS+LRI	AI7	Furfural	nd	nd	nd	0.331	0.027	nd	0.006	0.115	*Bread, almond, sweet*[44]
8	1496	1513[16]	MS+LRI	AI8	Decanal	nd	nd	nd	0.558	0.049	0.226	nd	0.204	*Bitter, aldehyde, orange peel*[45]
9	1516	1525[17]	MS+LRI	AI9	Benzaldehyde	0.584	0.177	0.297	0.866	0.562	0.391	0.039	0.297	*Nutty, almond*[44]
10	1519	1530[19]	MS+LRI	AI10	Non-2-enal	0.098	nd	nd	0.066	0.025	nd	0.013	0.009	*Woody, fattty*[35]
11	1643	1643[17]	MS+LRI	AI11	Benzeneacetaldehyde	nd	nd	nd	0.431	0.104	0.12	0.065	0.148	*Green, honey*[35]
12	1644	na	MS	AI12	3-methylbenzaldehyde	nd	nd	nd	0.326	0.112	nd	nd	0.122	
13	2047	2045[20]	MS+LRI	AI13	Cinnamaldehyde	nd	nd	nd	0.081	nd	0.028	0.019	0.014	*Cinnamon*[35]
14	2578	2578[21]	MS+LRI	AI14	Vanillin	0.055	nd	0.053	0.116	0.035	0.023	0.024	0.035	*Vanilla*[35]
					Ketones									
15	1125	na	MS	Ke1	(3Z)-pent-3-en-2-one	1.175	0.632	nd	nd	nd	nd	nd	nd	*Fruity*[44]
16	1283	1273[18]	MS+LRI	Ke2	3-hydroxybutan-2-one	nd	nd	0.133	nd	0.068	nd	nd	nd	*Butter, cream*[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 N	
17	1408	1435[22]	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*[44]
18	1582	na	MS	Ke4	6-Methyl-3,5-heptadien-2-one	nd	nd	nd	0.215	nd	nd	nd	nd	
19	1648	1643[18]	MS+LRI	Ke5	1-Phenylethan-1-one	nd	nd	nd	0.236	0.024	0.042	0.072	0.042	*Must, flower, almond*[35]
20	1667	1684[23]	MS+LRI	Ke6	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	nd	nd	nd	nd	nd	nd	nd	0.038	
21	1691	1677[20]	MS+LRI	Ke7	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	nd	nd	nd	nd	0.084	nd	nd	nd	*Musty, woody, tobacco, leafy*[44]
22	1803	1803[15]	MS+LRI	Ke8	2-Tridecanone	nd	nd	nd	0.145	nd	0.074	0.063	0.047	*Waxy, fatty, milky*[44]
23	1865	1840[17]	MS	Ke9	(5Z)-6,10-dimet-hylundeca-5,9-dien-2-one	nd	nd	nd	0.104	0.03	0.045	0.029	0.03	
24	1977	1967[24]	MS+LRI	Ke10	1-(1H-pyrrol-2-yl)-2-one	nd	nd	nd	0.067	0.024	0.022	0.026	0.011	*Nut, walnut, bread*[35]
25	1998	2006[16]	MS+LRI	Ke11	pentadecan-2-one	nd	nd	nd	0.035	nd	0.012	0.071	0.009	*Floral*[44]
26	2110	2131[25]	MS+LRI	Ke12	6,10,14-Trime-thylpentadec-an-2-one	0.957	0.339	0.124	0.096	0.071	0.116	0.03	0.028	*Fat*[44]

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	
					Alcohols									
27	0	913[26]	MS+LRI	OI1	Ethanol	8.763	2.689	1.217	nd	nd	2.828	nd	nd	*Sweet*[35]
28	0	1093[27]	MS+LRI	OI2	2-Methylp`-ropan-1-ol	3.504	1.041	1.686	nd	nd	0.563	nd	nd	*Wine*[35]
29	1247	1220[26]	MS+LRI	OI3	3-Methyl-butan-1-ol	4.026	1.544	3.173	nd	nd	nd	nd	nd	*Fermented, whiskey, Fruity*[35]
30	1441	1448[28]	MS+LRI	OI4	Oct-1-en-3-ol	0.38	0.244	nd	nd	0.143	0.178	0.019	0.122	*Raw mushroom*[44]
31	1546	1494[16]	MS+LRI	OI5	Butane-2,3-diol	5.176	2.564	4.365	nd	nd	nd	nd	nd	*fruity, creamy, Buttery*[44]
32	1564	1566[19]	MS+LRI	OI6	Octan-1-ol	nd	nd	nd	nd	0.016	nd	nd	nd	*Fatty, citruss*[44]
33	1552	1550[67]	MS+LRI	OI7	3,7-Dimethylocta-1,6-dien-3-ol	nd	nd	nd	0.204	0.078	0.043	0.047	0.082	*Floral, citrus*[44]
34	1552	1494[16]	MS+LRI	OI8	Butane-2,3-diol	8.023	4.351	5.267	nd	nd	nd	nd	nd	*Fruity, creamy, buttery*[44]
35	1572	na	MS	OI9	1,3-Benzenediol, 4-ethyl-	nd	nd	nd	0.205	nd	0.062	0.044	0.073	
36	1581	1568[29]	MS+LRI	OI10	Butane-2,3-diol	10.809	6.133	6.911	nd	nd	nd	0.077	nd	*Fruity, creamy, buttery*[44]
37	1879	1879[17]	MS+LRI	OI11	Phenylmethanol	0.311	0.302	0.515	0.027	nd	nd	nd	0.035	*Slightly sweet*[35]
38	1920	1920[30]	MS+LRI	OI12	2-Phenylethanol	2.809	0.975	1.497	0.278	0.07	0.06	0.051	0.062	*Mild rose*[35]
39	2173	2171[31]	MS+LRI	OI13	4-Allyl-2-metho-xyphenol	nd	nd	nd	nd	nd	nd	0.021	nd	*Clove, honey*[44]
40	2235	na	MS	OI14	Pyridin-3-ylme-thanol	0.268	nd	0.193	nd	nd	nd	nd	nd	*Waxy*[44]
					Hydrocarbons									
41	1131	1130[14]	MS+LRI	Hc1	1,4-Dimethyl-benzene	nd	nd	nd	nd	0.037	0.094	0.061	nd	
42	1169	1174[18]	MS+LRI	Hc2	1,2-Dimethyl	nd	0.187	nd	0.208	nd	0.072	0.076	0.125	*Geranium*[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	
					-benzene									
43	1397	na	MS	Hc3	Tetradecane	0.476	0.242	0.386	0.504	0.176	0.219	0.115	0.178	*Alkane*[35]
44	1498	na	MS	Hc4	Pentadecane	0.335	0.16	0.235	nd	0.167	0.118	0.102	nd	*Waxy*[44]
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*[46]
46	1835	1802[18]	MS+LRI	Hc6	(1R,4R)-1,6-dimethyl-4-(propan-2-yl)-1,2,3,4-tetrahydronaph-thalene	nd	0.068	nd	0.18	0.046	0.076	0.065	0.056	*Herb, spice*[35]
47	1853	1877[32]	MS+LRI	Hc7	2-Methylnap-hthalene	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-	nd	0.131	0.299	0.134	0.031	nd	0.033	0.038	*Naphthyl*[44]
49	1950	na	MS	Hc9	2-Ethylnaphthalene	nd	nd	nd	0.066	nd	nd	nd	0.017	
50	1998	na	MS	Hc10	2,7-Dimethyl naphthalene	nd	nd	nd	0.078	0.02	0.014	0.02	0.045	
51	2006	2012[32]	MS+LRI	Hc11	2,6-Dimethyl naphthalene	0.119	nd	0.141	0.209	0.03	0.038	0.033	0.086	*Grass*[35]
52	2073	2122[16]	MS+LRI	Hc12	2,3-Dimethyl naphthalene	nd	nd	nd	0.035	0.012	nd	nd	0.011	
53	2112	2122[16]	MS+LRI	Hc13	1,6,7-Trimethylnaphthalene	nd	nd	nd	0.033	0.006	nd	nd	0.012	
54	2120	na	MS	Hc14	2,3,6-Trimethylnaphthalene	nd	nd	nd	0.026	0.01	0.005	nd	nd	*Fruity*[44]
55	2450	2376[16]	MS+LRI	Hc15	1H-indole	nd	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	
					Acids									
56	1450	1457[68]	MS+LRI	Ac1	Acetic acid	nd	nd	nd	0.301	0.192	nd	0.216	0.183	*Sharp, pungent, sour, vinegar*[35]
57	1628	1628[17]	MS+LRI	Ac2	Butanoic acid	nd	nd	nd	nd	nd	nd	0.017	nd	*Sharp acetic cheesy buttery fruity*[19]
58	1853	1846[27]	MS+LRI	Ac3	Hexanoic acid	0.321	0.597	0.544	0.338	0.083	0.086	0.081	0.126	*Goaty, fatty acid, vegetable oil, Sweaty*[45]
59	1976	1971[33]	MS+LRI	Ac4	Heptanoic acid	nd	nd	nd	0.049	0.027	nd	nd	0.0158	*Rancid, sour, sweaty*
60	2065	2065[17]	MS+LRI	Ac5	Octanoic Acid	0.344	0.252	0.238	0.103	0.02	0.018	0.034	0.029	*sweet, cheese, oily, Fatty*[44]
61	2165	na	MS	Ac6	Hexadecanoic acid	3.818	1.532	1.623	4.096	0.238	0.089	nd	1.466	
62	2492	2502[27]	MS+LRI	Ac7	Dodecanoic acid	nd	nd	0.056	0.194	0.041	0.027	0.017	0.053	*Soapy, waxy*[45]
63	2706	2706[27]	MS+LRI	Ac8	Tetradecanoic acid	0.491	0.225	0.271	0.4	0.095	0.06	0.082	0.158	*Waxy*[44]
					Esters									
64	1187	1177[16]	MS+LRI	Es1	Methyl hex anoate	nd	0.756	nd	nd	nd	nd	nd	nd	*Fruity*[44]
65	1288	na	MS	Es2	Methyl (E)-2-hexenoate	nd	0.353	nd	nd	nd	nd	nd	nd	
66	1373	1378[16]	MS+LRI	Es3	Methyl octa noate	0.283	0.355	nd	0.283	nd	nd	nd	nd	
67	1592	na	MS	Es	6-Methyl-3,5	nd	nd	nd	0.215	nd	nd	nd	0.077	

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	
					-heptadien-2-one									
68	1619	1601[16]	MS+LRI	Es5	Methyl benzoate	0.575	nd	nd	0.135	0.047	0.068	nd	nd	*Pleasant smell*[47]
69	1775	1755[34]	MS+LRI	Es6	Methyl 2-hydroxybenzoate	nd	nd	nd	0.085	0.023	nd	nd	0.028	*Peppermint*[35]
70	1780	na	MS	Es7	Methyl pyridine-3-carbo-xylate	nd	nd	nd	nd	0.034	nd	nd	nd	
71	1802	1795[35]	MS	Es8	Methyl dodecanoate	0.523	0.431	nd	0.119	0.015	nd	nd	nd	
72	1845	1824[18]	MS+LRI	Es9	Ethyl dodecanoate	0.174	nd	nd	nd	nd	nd	nd	nd	*Floral, honey*[48]
73	2014	1994[18]	MS+LRI	Es10	Methyl tetradecanoate	2.212	1.144	0.42	0.107	0.053	0.033	0.044	0.023	*Orris*[35]
74	2052	2044[36]	MS+LRI	Es11	Ethyl tetradecanoate	1.035	0.225	0.233	nd	0.017	nd	nd	0.008	*Floral, honey*[48]
75	2116	2108[28]	MS+LRI	Es12	Methyl pentadecanoate	0.185	0.126	0.039	nd	nd	nd	nd	nd	
76	2224	2226[25]	MS+LRI	Es 13	Methyl palmitate	28.735	14.219	3.831	0.562	0.438	0.198	0.245	0.138	*Waxy, fatty, oily, orris*[44]
77	2244	na	MS	Es14	Methyl (9Z)-hex-adec-9-enoate	0.441	0.193	nd	nd	nd	nd	nd	nd	
78	2261	2259[25]	MS+LRI	Es15	Ethyl hexade-canoate	12.038	2.041	1.266	0.089	nd	0.093	0.031	0.032	*Fatty acids, fruity, sweetish, rancid*[45]

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	
79	2279	na	MS	Es16	Ethyl (E)-hexadec-9-enoate	0.494	0.158	nd	nd	nd	nd	nd	nd	
80	2374	na	MS	Es17	Butyl hexadecanoate	0.584	0.149	0.127	nd	nd	nd	nd	nd	
81	2430	2424[16]	MS+LRI	Es18	Methyl octadecanoate	0.54	0.281	0.057	nd	0.003	nd	nd	nd	*Oily, waxy*[44]
82	2451	na	MS	Es19	Methyl (E)-octadec-9-enoate	17.033	8.657	1.667	0.052	0.071	0.02	nd	0.035	*Waxy, fatty, oily*[44]
83	2466	2450[37]	MS+LRI	Es20	Ethyl octadecanoate	0.375	nd	nd	nd	nd	nd	nd	nd	*Fatty acids*[45]
84	2486	na	MS	Es21	Ethyl (9Z)-octadec-9-enoate	9.541	2.027	0.725	0.456	0.05	nd	0.035	nd	*Fatty acids, vegetable oil, rancid*[45]
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*[44]
86	2535	2491[18]	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*[45]
87	2568	na	MS	Es24	Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	0.415	0.218	0.046	nd	nd	nd	nd	nd	*Oily fatty fruity*[44]
					Benzenes									
88	1122	1115[16]	MS+LRI	Bz1	Ethylbenzene	nd	nd	nd	0.24	0.066	0.128	0.051	0.093	*Gasoline44*

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	
89	1250	1240[16]	MS+LRI	Bz2	Styrene	0.429	0.494	0.364	0.23	nd	0.208	0.143	0.219	*Balsamic, gasoline*[44]
90	1266	1260[16]	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[38]	MS+LRI	Bz4	1,3,5-Trimethylbenzene	nd	0.19	nd	0.302	0.078	0.1	0.078	0.128	*Sweet*[44]
92	1419	1274[17]	MS+LRI	Bz5	1-Methyl-4-(propan-2-yl)benzene	nd	nd	nd	0.056	nd	nd	nd	0.051	*Citrus*[35]
93	1724	1721[27]	MS+LRI	Bz6	1,2-Dimethoxybenzene	nd	nd	nd	0.185	nd	nd	nd	0.066	*Sweet, creamy, vanilla*[44]
94	1828	1820[27]	MS+LRI	Bz7	1-methoxy-4-[(E)-prop-1-enyl]be-nzene	nd	nd	0.302	0.171	0.052	0.1	0.075	0.063	*Sweet, licorice, medicinal*[44]
95	1593	1593[39]	MS+LRI	Bz8	(1R,4E,9S)-4,11,11-Trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	nd	nd	nd	0.337	0.22	0.275	0.094	0.127	*Clove, pepper, floral*[44]
96	1140	1138[18]	MS+LRI	Bz9	3,7,7-trimethylbicyclo[4.1.0]hept-3-ene	0.501	0.339	0.487	0.552	0.092	0.332	0.232	0.176	*Sweet, pungent*[44]
					Phenols									
97	1877	1872[17]	MS+LRI	Ph1	2-Methoxyphenol	0.744	0.457	6.524	0.593	0.022	0.028	0.096	0.207	*Nutty*[44]
98	1913	191224	MS+LRI	Ph2	2,6-bis(1,1-dimethylethyl)-4-methylphenol	nd	nd	nd	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	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	
99	2002	2000[40]	MS+LRI	Ph3	Phenol	1.021	0.383	0.664	0.051	0.014	0.073	0.019	0.02	*Sweet, medicinal*
100	2203	2200[41]	MS+LRI	Ph4	4-Ethenyl-2-methoxyphenol	0.536	0.147	0.258	0.507	0.074	0.231	0.093	0.179	*dry, woody, fresh, -roasted*[35]
101	2315	2317[29]	MS+LRI	Ph5	2,4-Ditert-buty -lphenol	nd	nd	nd	0.077	0.03	0.055	0.034	0.031	
					Furans									
102	1230	1234[42]	MS+LRI	Fu1	2-Pentylfuran	0.626	0.177	0.118	0.248	0.089	0.102	0.032	0.075	*Nutty, beany, buttery*[44]
103	1426	na	MS	Fu2	Furan-3-car -baldehyde	nd	nd	nd	0.322	nd	0.073	0.046	0.121	
104	1667	1684[23]	MS+LRI	Fu3	5-Ethenyl-5 -methyloxol -an-2-one	nd	nd	nd	nd	0.027	nd	nd	nd	
105	2037	na	MS	Fu4	3-Hydroxy-4, -4-dimethylox -olan-2-one	nd	nd	0.06	nd	nd	nd	nd	nd	
106	2391	na	MS	Fu5	2,3-dihydro-1-benzofuran	0.097	0.02	0.045	0.036	0.01	0.02	0.012	0.008	*Sweet*[44]
107	2368	na	MS	Fu6	4,4,7a-Trime -thyl-5,6,7,7a -tetrahydro-1 -benzofuran-2(4H)-one	0.201	nd	nd	0.112	0.045	0.052	0.023	0.03	
					Lactone									
108	1613	na	MS	Fu7	Dihydrofuran -2(3H)-one	nd	nd	nd	0.123	0.039	0.045	0.05	0.042	*Creamy, fatty*[44]
109	2037	2051[43]	MS+LRI	Fu8	(3R)-3,4,4-trim	0.135	0.274	nd	0.288	0.096	0.083	0.06	0.091	*Cotton candy*[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	
					ethyloxolan-2-one									
					Monoterpene									
110	1190	1299[24]	MS+LRI	Mt1	(4R)-1-Methyl-4-prop-1-en-2-ylcyclohexene	1.356	1.39	5.61	5.325	0.435	1.064	1.606	2.63	*Lemon, orange*[35]
					Sesquiterpenoid									
111	1757	na	MS	St1	(1R,8aS)-1,6-Dimethyl-4-(pr-opan-2-yl)-1,2,-3,7,8,8a-hexahy-dronaphthalene	nd	nd	nd	0.151	nd	nd	nd	0.054	
					Thiazole									
112	1948	1950[30]	MS+LRI	Tz1	1,3-Benzothi-azole	0.591	0.129	0.46	0.193	0.094	0.112	0.041	0.061	*Sulfurous, meaty*[35]
					Pyridine									
113	1255	1358[24]	MS+LRI	Pd1	2,3-Dimethyl-pyridine	nd	nd	nd	0.384	nd	nd	nd	nd	*Coffee, caramellic*[44]
					Pyrazine									
114	1820	na	MS	Pz1	2-Methyl-5-[(E)-prop-1-enyl]pyrazine	nd	nd	nd	0.145	nd	nd	nd	nd	*Roasted, green*[44]

*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 witch literature value
nd: not detected

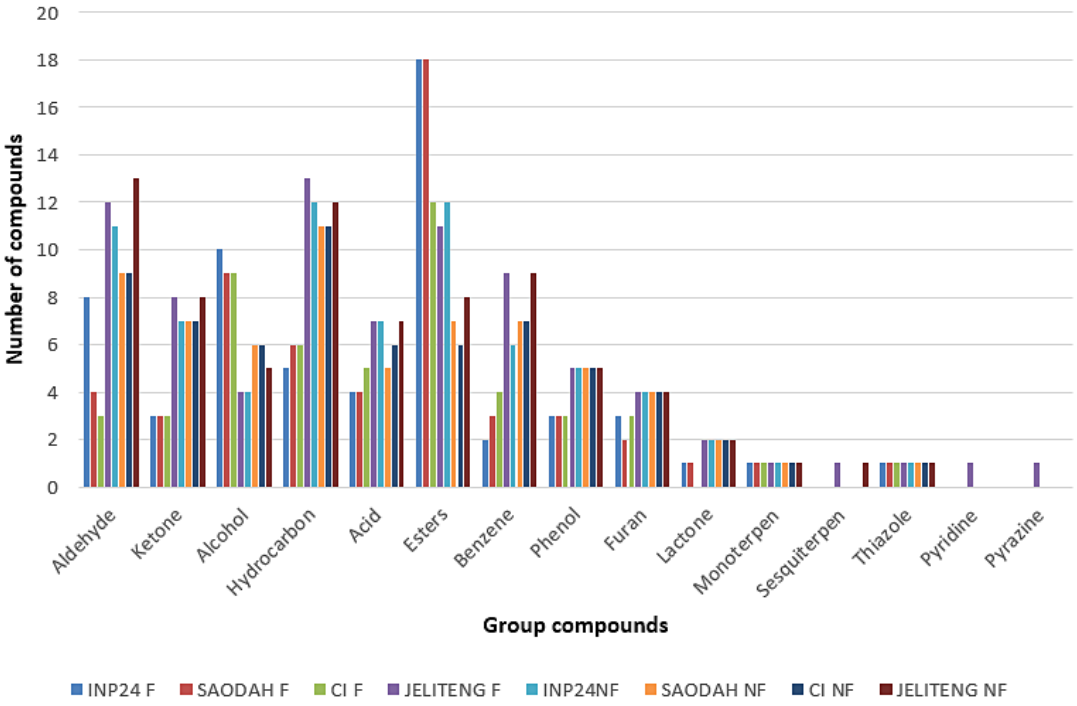


Fig. 1: Volatile compounds of fermented RB: Inpari 24 RB (Inp24F), Saodah (SaodahF), Cempo Ireng (CIF), and Jeliteng (JelitengF), and non-fermented RB: Inpari 24 (Inp24NF), Saodah (SaodahNF), Cempo Ireng (CINF), Jeliteng (Jeliteng NF)

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.[5,19,49,50]

The RBs might be subjected to the Maillard reaction and lipid oxidation might due to heat treatment, which is the sterilization process in this study.[19] In the Maillard reaction, the sugar's reactive carbonyl group combined with the nucleophilic amino group of acid and formed new volatile compounds.[51] 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.[52,53] Heat treatment also allegedly formed vanillin and furfural, due to thermal degradation of ferulic acid and sugars, respectively.[19] 4-ethenyl-2-methoxyphenol was also reported to be derived from thermal decarboxylation of ferulic acid in RB.[54]

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;butane-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.

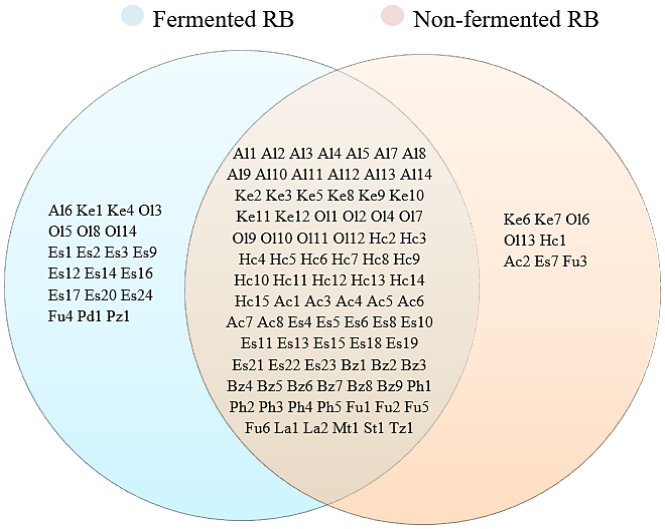


Fig. 2: Venn diagram of fermented and non-fermented RB volatile compounds. Code of compounds were shown in Table 1

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[44] and were increasing due to the fermentation process. Esters mostly are formed by esterification between acids and alcohols during fermentation.[55] 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.[56,57] Phenylmethanol is known to be formed from the reduction of benzoic acid assisted by microorganisms.[58] 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-oland 2-methylbutan-1-ol) have fermented and malt-like odour notes.[48] These are also detected in other fermented rice such as makgeolli (Korean rice wine).[48] Another alcohol compound that was identified in fermented RB is 2-phenylethanol; it is thought to have a slightly rose floral scent.[59] The formation of 2-phenylethanolmight be from hydrolysis of phenylethyl ester and phenylethyl acetal.[60]

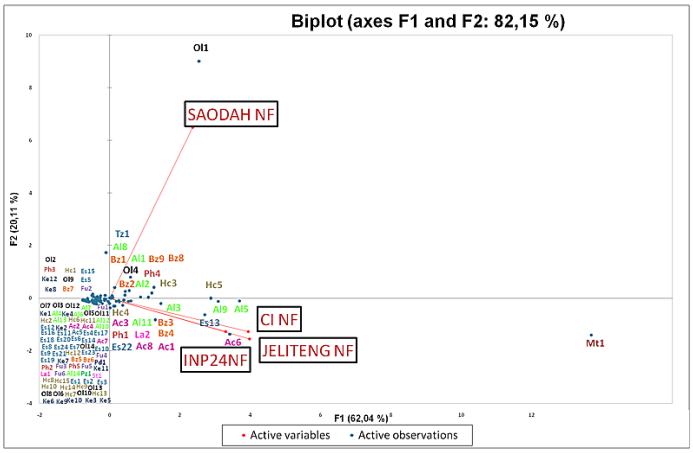
In the group of aldehydes, hexanal, nonanal and benzaldehyde were the compounds with the highest relative peak areas found in fermented RB. These three compounds tend to increase compared to non-fermented RB. Saturated aldehydes such as hexanal and nonanal might be formed by linoleic acid (C18:2) oxidation 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-2-enal and hexanal.[61] 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.6 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.[7,62] Benzaldehyde is responsible for giving the nutty and almond aroma.[44]

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,[44] was derived from the thermal degradation of sugars such as fructose and glucose.[63] 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.[44,61] Non-2-enal and hexanal were also reported to cause rancid defects in virgin olive oil.[64] 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.[7] Vanillin, which gives vanilla aroma, originates from lignin degradation in aerobic conditions and might be formed by the thermal degradation of ferulic acid.[19]

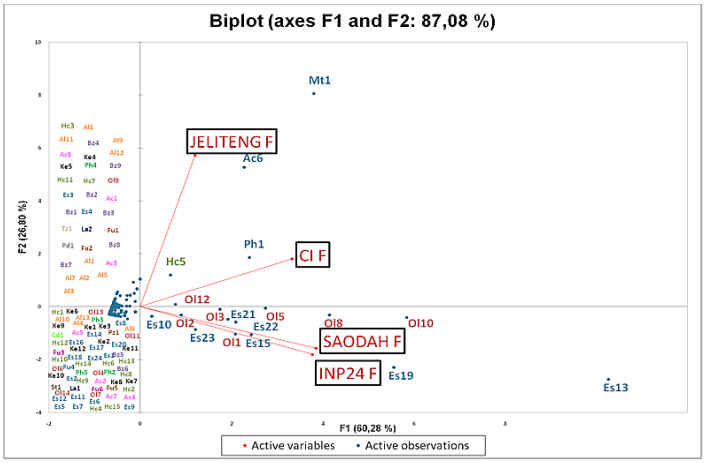
Naphthalene was the highest content of hydrocarbon that was found in RB. The relative peak areas of naphthalene which contributed to camphor wood-like aroma tends to increase due to fermentation. In other prior research, naphthalene was also found to be an odour-active compound in red and black rice.[6]

Principle Component Analysis (PCA) of Volatile Compounds in RB

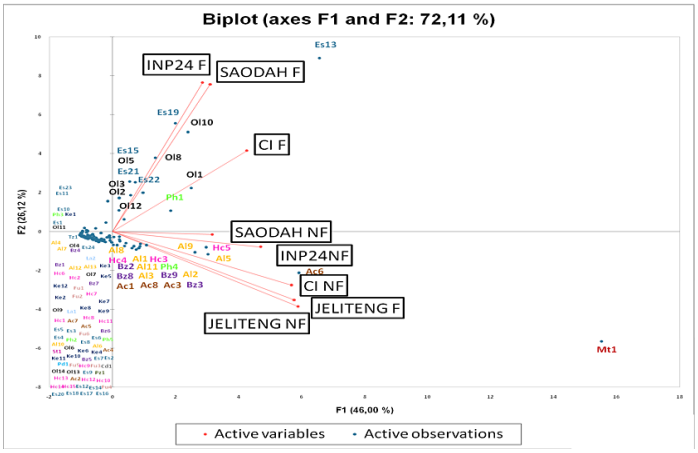
The PCA biplot of volatile compounds in fermented and non-fermented RB is shown in Figure 3.



(a)



(b)



(c)

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

Codes	Volatile compounds	Inp24 NF	Inp24 F	Saodah NF	Saodah F	CI NF	CI F	Jeliteng NF	Jeliteng F
	Alcohols								
Ol1	Ethanol								
Ol2	2-methylpropan-1-ol								
Ol3	3-methylbutan-1-ol								
Ol5	Butane-2,3-diol								
Ol8	2,3-Butanediol								
Ol10	2,3-Butanediol								
Ol12	2-Phenylethanol								
	Monoterpene								
Mt1	(4*R*)-1-Methyl-4-prop-1-en-2-ylcyclohexene (D-Limonene)								
	Aldehydes								
Al1	Hexanal								
Al2	Heptanal								
Al3	Octanal								
Al5	Nonanal								
Al8	Decanal								
Al9	Benzaldehyde								
Al11	Benzeneacetaldehyde								
	Esters								
Es13	Methyl palmitate								
Es15	Ethyl hexadecanoate								
Es19	9-Octadecenoic acid, methyl ester								
Es21	Ethyl (9Z)-octadec-9-enoate								
Es22	Methyl octadeca-9,12-dienoate								
	Phenols								
Ph1	2-Methoxyphenol								
Ph4	4-Ethenyl-2-methoxyphenol								
	Hydrocarbons								
Hc3	Tetradecane								
Hc4	Pentadecane								
Hc5	Naphthalene								
	Benzenes								
Bz2	Styrene								
Bz3	1-Methyl-2-(propan-2-yl)benzene								
Bz8	Caryophyllene								
Bz9	3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene (3-carene)								
	Acids								
Ac1	Acetic acid								
Ac3	Hexanoic acid								
Ac6	Hexadecanoic acid								
Ac8	Tetradecanoic acid								

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).

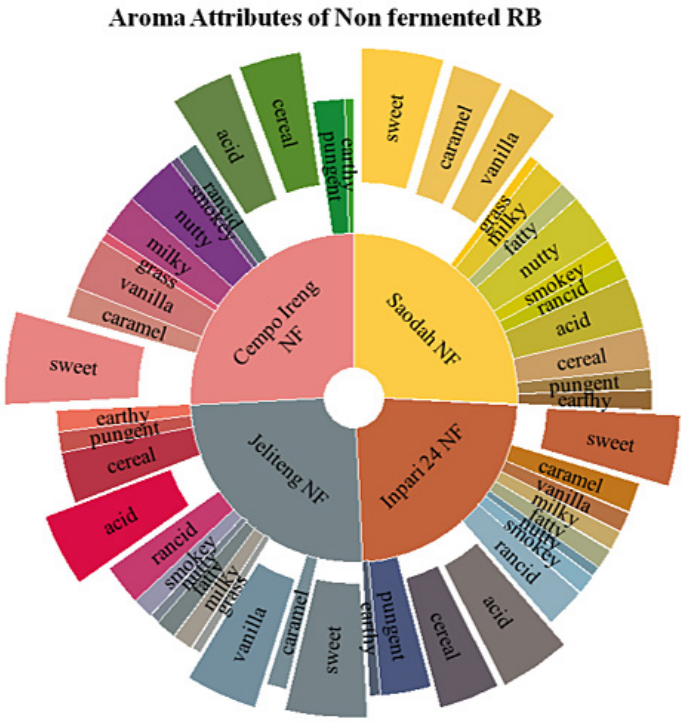
Cempo Ireng and Jeliteng might be in a group since they have the same pigment: black rice. Figure 3c showed the PCA of fermented and non-fermented RB. Fermented RB of Inpari 24, Saodah and Cempo Ireng were grouped together and their dominant compounds were methyl palmitate (Es13), methyl (E)-octadec-9-enoate (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.[55] 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.[48] 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.[65] 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.[66] 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.50 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.[6] 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.

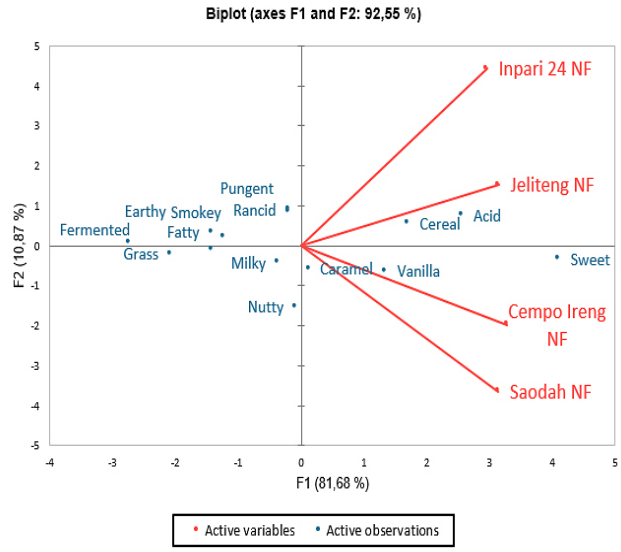
Both fermented and non-fermented RBs tended to have sweet, acid, vanilla, cereal and caramel aromas as their major attributes. The differences between these RBs were in the number of panellists that recognized the aroma. Aroma attributes of fermented RBs were noticed more by panellists; this might conclude 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.[52,53]



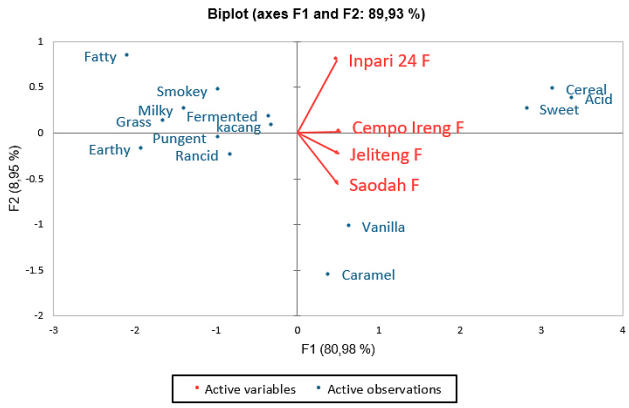
a

b

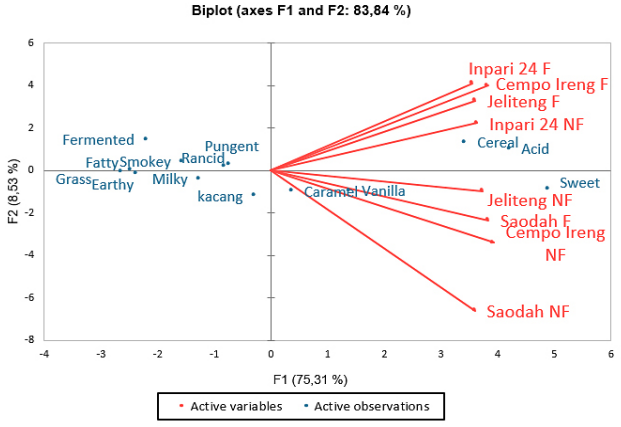
Fig. 5: Aroma attributes of a) non-fermented RB and b) fermented RB by QDA



a



b



c

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 RBs varieties such as Jeliteng, Cempo Ireng and Saodah are in the same group with SaodahF; 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 6.c 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 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[44] 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.[44] 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.[35] Acetic acid was described to have sharp, pungent, sour and vinegar aroma[35] 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-enoatepositively 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.[44,45]

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	-0.451	-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	0.535
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
Earthy	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

Volatile compounds found in RB consist of ester, hydrocarbon, aldehyde, benzene, alcohol, ketone, acid, phenol, furan, lactone, monoterpene, thiazole, sesquiterpene, pyridine and pyrazine. A total 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-enoate was correlated with acid and 2-methyl-5-[(E)-prop-1-enyl] pyrazine was correlated with smokey.

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Conflict of interest

All authors declare no conflict of interest.

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