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Odor-Active Headspace Components in Fermented Red Rice in the Presence of a *Monascus* Species

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Headspace components from rice and agar with (experimental) and without (control) inoculation with a *Monascus* spp. were investigated. Kinetics studies were carried out. Using rice as a substrate, 10 and 19 compounds were found for the control and the experimental groups, respectively, at day 14. Experimental group compounds were composed mainly of alcohols, ketones, and esters, whereas control group compounds were composed of aldehydes and ketones. With agar as a substrate, only five and three components were found in the control and experimental groups, respectively. Five alcohols, four esters, two ketones, and one furan with odor activity values (OAV) >1 dominated the overall flavor of the product. With liquid inoculation, the first six components with high OAVs were in the following order: 3-methyl-1-butanol (**17)** > ethanol (**14)** > ethyl acetate (**10**) > 2-methyl-1-propanol (**15**) > ethyl butanoate (**11**) > 3-methylbutyl acetate (**13)**. Kinetic studies showed that most compounds reached their maximum concentrations at 10−12 days. Many compounds identified in the model red rice were reported in commercial red sufus, and several appeared to contribute solely by red rice.

KEYWORDS: ***Monascus*; fermentation; aroma; fermented red rice**

INTRODUCTION

Fermented soybean curd, also known as sufu, is a popular condiment used in the preparation of southern Chinese dishes (*1*). On the basis of color, these curds are roughly divided into plain (or white) and red types of sufus in Hong Kong. The basic production steps involved in the two types of sufus are similar (*2*, *3*). For the red sufu, fermented red rice is introduced during the aging period to produce the reddish finished product (*1*, *4*).

During the preparation of fermented red rice, a *Monascus* spp. is introduced and red pigment is produced in ∼2 weeks (*4*). *Monascus* species comprise a group of filamentous fungi, which are widely used to provide colorants for various foods, such as in fish, meat, and some other Chinese food products such as rice wine and red sufu (*5*). The pigments can be yellow, orange, or red in color and are due to the presence of various compounds including monascin, ankaflavin, monascorubrin, rubropunctatin, monascorubramine, and rubropunctamine (*5*). Functionally, *Monascus-f*ermented rice was found to reduce the concentration of serum total cholesterol and triglyceride and to suppress atherosclerosis in animal models (*6*).

The major portions of the volatile flavor components in the red sufu were due to the presence of alcohols and esters in which ethyl 2-methyl propanoate, 2,3-butanedione, ethyl butanoate, ethyl 2-methylbutanoate, 3-(methylthio)propanal, benzeneacetaldehyde, and ethyl 3-phenylpropionate were predominant in quantity (*7*). Although the primary purpose of the addition of fermented red rice to red sufu is to provide red color to the product, it is likely that its alternate purpose may be to provide additional subtle flavor to the red sufu. So far there has not been any study examining the volatile components or their aroma contribution. To evaluate the contribution of the addition of the fermented red rice to the flavor quality in red sufu, the headspace composition of a model fermented red rice was studied.

MATERIALS AND METHODS

Samples. *Monascus serorubescens* sato was isolated from a commercial red rice product donated by a traditional condiment manufacturer in Hong Kong. The *Monascus* spp. was initially cultured in a complete medium agar plate at 37 °C for 2 weeks until mycelium and red pigment developed. The composition of the complete medium agar prepared in 1 L of water included the following compounds: $MgSO_4$ (0.5 g), KH_2PO_4 (0.46 g), K_2HPO_4 (1 g), peptone (2 g), dextrose (20 g), agar (15 g), yeast extract (2 g), and thiamin−HCl (0.5 mg). To produce a liquid fungal stock, two pieces of *Monascus*-infested agar (1 cm × 1 cm) were aseptically removed from the agar plate and transferred to a liquid complete medium. They were incubated at 37 °C for 2 weeks with continuous shaking.

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Rice Experiment. *M. serorubescens* sato was aseptically inoculated to both rice and agar. To prepare rice samples for the fungal inoculation, 300 g of rice (Golden Elephant Brand Premium Thai fragrant rice, 2 kg package, Golden Resources Development Ltd., Hong Kong SAR, China) was steamed with 800 mL of water and then cooled. The steamed rice (60 g) was transferred to three sets of three 150-mL conical flasks (i.e., a total of nine conical flasks), which were covered with aluminum foil. All rice-containing flasks were autoclaved at 121 °C for 15 min. Then, *Monascus* samples cultivated in both liquid (1 mL) and solid (1 cm × 1 cm agar) culture media were each aseptically transferred to one set of conical flasks and covered with the autoclaved aluminum foil. The remaining set of flasks was used as control without the inoculation of the *Monascus* species. Triplicate experiments were carried out.

Agar Experiment. For the agar experiment, 25 mL of the complete medium agar was prepared in two sets of three 150-mL conical flasks. The same liquid *Monascus* culture (1 mL) was aseptically inoculated to one set of conical flasks. The flasks without the inoculation of the fungal species were set as control. All flasks were aseptically covered with aluminum foil to provide an aerobic environment and incubated at 37 °C for 14 days. Headspace volatile components were collected after 14 days of incubation. Headspace gas (10 mL) was withdrawn using a 10-mL gastight syringe (Hamilton Co., Reno, NV) and injected into an analytical instrument. The agar experiment was repeated three times.

Kinetics Experiment. The setup of the inoculated rice and the control for the kinetic experiment was the same as described previously in the rice experiment. However, only the liquid *Monascus* stock and the control were used to study the temporal changes in the headspace volatile compounds. The prepared flasks were incubated at 37 °C for 14 days. Headspace volatile components were collected and analyzed at 2-day interval. Triplicate experiments were carried out.

Gas Chromatography−Mass Spectrometry (GC-MS). Headspace volatile components were manually collected by a 10-mL gastight syringe from each flask and were directly injected into a cooled-injection system (CIS; Gerstel, Baltimore, MD) set at −120 °C and at solvent vent mode installed in an HP 6890 gas chromatograph coupled with an HP 5973 mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). After injection, the CIS was immediately heated to 200 °C at a ramp rate of 10 °C/s and held at 200 °C for 10 min. A polar capillary column (Supelcowax 10, 60 m length × 0.25 mm i.d. × 0.25 μm d_f, Supelco, Inc., Bellefonte, PA) was used as the analytical column. GC oven conditions were initially at 35 °C for 5 min, programmed at 10 °C/min to 195 °C, and held for 13 min. Helium carrier gas flow rate was 30 cm/s. MS conditions were as follows: ion source temperature, 230 °C; MS quadrupole temperature, 106 °C; electron multiplier, 1200 V; and scan rate, 6.52 scans/s (*7*, *8*).

Compound Identification and Quantification. Volatile compounds were positively identified by comparing the retention time, linear retention index, and mass spectrum with those of an authentic compound (*7*−*9*). Initial identification of compounds was based on matching the mass spectrum with the Wiley library of mass spectral database (7th ed., Wiley, New York). Quantification of a compound was carried out by establishing a calibration curve with four different concentrations for each compound using authentic chemical (*10*). The response factor of each compound was derived from its standard curve for quantification.

Odor Activity Value (OAV). The OAV of a compound was calculated by dividing the concentration of a compound by its corresponding threshold value (*11*).

Statistical Analysis. Compounds from three different groups were analyzed by one-way analysis of variance (ANOVA) and compared by Tukey's studentized range test at a $p = 0.05$ level of significance. Compounds from two different groups were analyzed by independent Student's t test at a $p = 0.05$ level of significance (*12*).

RESULTS AND DISCUSSION

Rice Experiment. The headspace volatile components of the rice (control) and the fermented red rice (experimental) after 14 days of incubation are shown in **Table 1**. A total of 22 compounds were positively identified; 10 and 19 of them were found in the control and experimental samples, respectively. Seven compounds were common in both control and all experimental samples including benzaldehyde (**4**), 2-propanone (**5**), 2-heptanone (**6**), 1-phenylethanone (**7**), 3-methyl-1-butanol (**17**), 2-pentylfuran (**21**), and methylbenzene (**22**). In the control group, 2-propanone (**5**) was present in the highest concentration followed by *n*-hexanal (**3**). The former is described as having an apple, pear, grape, pineapple-like, ethereal, powerful flavor, whereas the latter was noted as having a fatty, green, and grassy flavor (*13*) and was identified in the red sufu (*7*). In each experimental group, ethanol (**14**) was the predominant compound in quantity in the headspace.

Although either liquid or solid inoculation of the *Monascus* spp. was used in the rice substrate, the fermentation produced the same headspace volatile composition. Quantitatively, solid inoculation contained more components with higher headspace concentrations than that of the liquid for most components except for acetic acid (**1**), benzaldehyde (**4**), 1-phenylethanone (**7**), ethyl butanoate (**11**), ethanol (**14**), 1-butanol (**16**), and 2-pentylfuran (**21**). However, from our observation, growth of the mycelia and distribution of the red pigments appeared to be more evenly distributed in the substrate inoculated with the liquid medium. Also, statistical analyses showed that there were no significant differences in the quantity in many components between the two inoculation methods except for those of benzaldehyde (**4**), 2-propanone (**5**), 1-butanol (**16**), and 2-pentylfuran (**21**) ($p > 0.05$). Besides, similar rankings from high to low concentrations among the components were observed in both solid and liquid inoculation methods. The order for the first nine compounds starting from the highest concentration is as follows: ethanol (**14**) > ethyl acetate (**10**) > 2-methyl-1-propanol (**15**) > 3-methyl-1-butanol (**17**) > 2-propanone (**5**) > ethyl butanoate (**11**) > 1-butanonl (**16**) > 2-heptanone (**6**) > 3-methylbutyl acetate (**13**). Overall, the qualities of the headspace volatile compositions are very similar when the rice is fermented by either inoculation method.

Comparison between the control and the two experimental groups also revealed that many components found in the experimental groups were not detected in the control group. Twelve compounds, mostly belonging to ester and alcohol classes, were not detected in the control. For the two chemical classes, esters generally have lower threshold values than alcohols, suggesting that the former class might be more likely to have much stronger aromas (**Tables 1** and **3**). Nevertheless, the odor impact of a compound also depends on its concentration (*11*). It appeared that the *Monascus* spp. might be able to utilize some of the compounds belonging to aldehydes, ketones, furans, and an aromatic compound in the cooked rice to biogenerate other products because the headspace concentration of many common components in the experimental groups was lower than that in the control group. Biogeneration of components from the existing substrate is not uncommon in fungus. For example, the fungus *Beauveria bassiana* synthezied raspberry ketone from *p*-hydroxybenzylidenacetone (*14*).

Ethanol (**14**) was clearly produced in much higher concentration compared to other headspace volatiles in the two inoculation methods (**Table 1**). Six alcohols were identified in the headspace of the current inoculated samples. All of them were reported in the commercial red sufu (*7*). However, neither 3-methyl-1-butanol (**17**) nor 2-heptanol (**18**) was found in Hwan and Chou's laboratory-type aging plain sufu in the absence and presence of ethanol (*15*). A major difference between the laboratory-type sufus and the commercial sufus was the addition of spices

Table 1. Headspace Volatile Components of Rice in the Absence (Control) and Presence of *Monascus* Species (Experimental) at Day 14 of the Rice Experiment

		compd present in sufus[b]							control		exptl (liquid)		exptl (solid)	
no.[a]	compd	R	Ao	Aw	descriptor[c]	RF[d]	*m/z*[e]	RI[f]	concn[g]	SD[h]	concn[g]	SD[h]	concn[g]	SD[h]
	acid (1)													
1	acetic acid	Y	Y	Y	strong, pungent sour odor (*13*)	7552	60	1487	- - -	- - -	0.14A	0.02	0.11A	0.03
	aldehydes (3)													
2	pentanal	N	N	N	woody, vanilla, fruity, nutty on dilution (*13*)	18454	44	968	1.1	0.3	- - -	- - -	- - -	- - -
3	n-hexanal	Y	Y	Y	fatty, green, grassy, powerful, penetrating (*13*)	6599	56	1072	7.7	0.7	- - -	- - -	- - -	- - -
4	benzaldehyde	Y	Y	Y	bitter almond, fragrant, aromatic, sweet (*13*)	23036	106	1520	0.19A	0.05	0.027B	0.005	0.016B	0.003
	ketones (5)													
5	2-propanone	N	N	N	apple, pear, grape, pineapple, ethereal, powerful (*13*)	4419	58	805	12A	1	9.4A	2.4	20B	2
6	2-heptanone	Y	N	N	fruity, green, fatty, sweet, ethereal (*13*)	27044	58	1167	0.61A	0.05	2.9B	0.3	3.0B	1.3
7	1-phenylethanone	N	N	N	sweet, hawthorn, floral, almond, fragrant, aromatic (*13*)	35516	105	1652	0.10	0.11	0.025A	0.000	0.023A	0.007
8	3-hydroxy-2-butanone	Y	N	N	buttery (*13*)	18076	45	1270	- - -	- - -	0.26A	0.06	0.34A	0.26
9	2-nonanone	Y	Y	Y	fruity, floral (*13*)	28559	58	1374	- - -	- - -	0.37A	0.11	0.58A	0.22
	esters (4)													
10	ethyl acetate	N	Y	Y	pineapple, ethereal (*13*)	4530	61	875	- - -	- - -	940A	260	1300A	110
11	ethyl butanoate	Y	Y	Y	fruity, fragrant, sweet, ethereal banana-pineapple undertones (*13*)	19163	71	1027	- - -	- - -	5.5A	1.1	5.2A	1.6
12	ethyl 2-methylbutanoate	Y	Y	Y	powerful, green, fruity, pungent (*13*)	16639	57	1043	- - -	- - -	0.23A	0.03	0.28A	0.03
13	3-methylbutyl acetate	Y	N	N	fruity, banana, sweet, fragrant (*13*)	23936	70	1108	- - -	- - -	1.1A	0.1	1.4A	0.5
	alcohols (6)													
14	ethanol	Y	Y	Y	pleasant odor (*22*)	8432	45	918	- - -	- - -	5400A	300	4000A	1000
15	2-methyl-1-propanol	Y	Y	Y	penetrating, wine-like (*13*)	10192	43	1070	- - -	- - -	140A	30	140A	40
16	1-butanol	Y	Y	Y	medicinal (*13*)	9352	56	1119	- - -	- - -	5.2A	1.1	3.0B	0.4
17	3-methyl-1-butanol	Y	N	N	fusel oil, whiskey (*13*)	13636	55	1178	0.22A	0.03	25B	8	32B	12
18	2-heptanol	Y	N	N	earthy, oily (*13*)	7303	55	1286	- - -	- - -	0.17A	0.03	0.29A	0.10
19	benzeneethanol	Y	Y	Y	rose, honey, fragrant, floral (*13*)	39037	91	1899	- - -	- - -	0.15A	0.02	0.17A	0.07
	furans (2)													
20	2-butylfuran	N	N	N	weak, noncharacteristic (*20*)	67969	81	1120	0.11	0.04	- - -	- - -	- - -	- - -
21	2-pentylfuran	Y	N	N	green bean, metallic, vegetable (*13*)	63003	81	1213	0.94A	0.40	0.41AB	0.06	0.16B	0.06
	aromatic compd (1)													
22	methylbenzene	N	Y	Y	benzene-like odor (*22*)	53377	91	1032	0.15A	0.01	0.13A	0.03	0.29A	0.23

[a] Compound number. [b] Y, compound was reported in commercial red sufu (R) (*7*), aging sufu without ethanol (Ao) (*15*), or aging sufu with ethanol (Aw) (*15*); N, compound was not reported. [c] Descriptors from the following references: Sigma-Aldrich 2003 (*13*), Fors, 1983 (*20*), and Budavari (*22*). [d] Response factor (digital counts/ng). [e] Mass/charge for quantification of compound. [f] Linear retention index. [g] Concentration of a compound in headspace (ng/mL of air); - - -, not detected; values in the same row with different letters are significantly different ($p < 0.05$). [h] Standard deviation of the concentration of a compound (ng/mL of air).

Table 2. Headspace Volatile Components of Agar in the Absence (Control) and Presence (Experimental) of *Monascus* Species Cultivated in Conical Flasks at Day 14 of the Agar Experiment

				control		exptl	
no.[a]	compd	*m/z*[b]	RI[c]	concn[d]	SD[e]	concn[d]	SD[e]
	aldehyde (1)						
4	benzaldehyde[f]	106	1520	0.50	0.07	0.046	0.007
	ketone (1)						
5	2-propanone[f]	58	805	6.1	1.2	1.6	0.5
	alcohols (2)						
16	1-butanol	56	1119	1.1	0.1	- - -[g]	- - -
17	3-methyl-1-butanol	55	1178	0.81	1.08	- - -	- - -
	aromatic compd (1)						
22	methylbenzene[f]	91	1032	0.14	0.01	0.77	0.32

[a] Compound numbers correspond to those in **Table 1**. [b] *m/z*, mass/charge for quantification of compound. [c] Linear retention index. [d] Concentration of compound in headspace (ng/mL of air). [e] Standard deviation the concentration of a compound (ng/mL of air). [f] Significance at 0.05 confidence level based on Student's *t* test. [g] Not detected/not calculated.

in the commercial ones during the aging period. The laboratory-type sufu represented a more realistic reference plain product in the absence of any added spices. During rice fermentation, acetic acid (**1**) was also produced. This compound was reported in the commercial red sufu as well as in the laboratory-type plain sufu, but was not identified in the commercial white ones (*8*). Acetic acid (**1**) found in the red sufus could be contributed by the fermented red rice when the rice was introduced to the red sufus during the aging period in addition to those already present in the aging plain sufu.

Four esters were produced throughout the rice fermentation, three of which have previously been reported in the red sufu, including ethyl butanoate (**11**), ethyl 2-methylbutanoate (**12**), and 3-methylbutyl acetate (**13**) (*7*). In our previous commercial red sufu investigation, both ethyl butanoate (**11**) and ethyl 2-methylbutanoate (**12**) were shown to be important flavor components in the red sufu, providing fruity and cantaloupe flavors (*7*). Comparison among the components identified in the commercial red sufu, the laboratory-type aging plain sufu from Hwan and Chou, and our current laboratory-scale model fermented red rice showed that 2-heptanone (**6**), 3-hydroxy-2-butanone (**8**), 3-methylbutyl acetate (**13**), 3-methyl-1-butanol (**17**), 2-heptanol (**18**), and 2-pentylfuran (**21**) were also aroma contributors to the red sufu because these compounds were found in only the red sufu, but not in the laboratory-type aging plain sufu (*7*, *8*, *15*) (**Table 1**).

Agar Experiment. Table 1 shows that more precise concentrations were found in the headspace components using the liquid cultivation method (i.e., with low standard deviations). The mean coefficients of variation (CV) from all components involving the liquid and solid inoculation methods were 18.3 and 33.5%, respectively. As a result of the lower mean CV, and a more evenly distributed growth and pigment development,

Table 3. Odor Activity Values (OAVs) of the Headspace Volatile Components at Day 14 of the Control Rice (without *Monascus* Species) and the Experimental Red Rice (with *Monascus* Species) of the Rice Experiment

			OAV[a]		
no.[b]	compd	threshold[c] (ng/mL)	control	exptl (liquid)	exptl (solid)
1	acetic acid	0.363/air[ii]	- - -	0.4	0.3
2	pentanal	0.0219/air[ii]	50.2	- - -	- - -
3	n-hexanal	0.05754/air[ii]	133.8	- - -	- - -
4	benzaldehyde	0.1862/air[ii]	1.0	0.1	0.1
5	2-propanone	34.67/air[ii]	0.3	0.3	0.6
6	2-heptanone	0.6761/air[ii]	0.9	4.3	4.4
7	1-phenylethanone	1.82/air[ii]	0.1	0.1>	0.1>
8	3-hydroxy-2-butanone	800/water[i]	- - -	0.1>	0.1>
9	2-nonanone	0.2291/air[ii]	- - -	1.6	2.5
10	ethyl acetate	9.772/air[i]	- - -	96.2	133.0
11	ethyl butanoate	0.1122/air[ii]	- - -	49.0	46.3
12	ethyl 2-methylbutanoate	0.1/water[iii]	- - -	2.3	2.8
13	3-methylbutyl acetate	0.123/air[i]	- - -	8.9	11.4
14	ethanol	54.95/air[ii]	- - -	98.3	72.8
15	2-methyl-1-propanol	2.57/air[ii]	- - -	54.5	54.5
16	1-butanol	1.5136/air[ii]	- - -	3.4	2.0
17	3-methyl-1-butanol	0.1622/air[ii]	1.4	154.1	197.3
18	2-heptanol	410000/water[v]	- - -	0.1>	0.1>
19	benzeneethanol	0.0871/air[ii]	- - -	1.7	2.0
20	2-butylfuran	10000/oil[iv]	0.1>	- - -	- - -
21	2-pentylfuran	0.0912/air[ii]	10.3	4.5	1.8
22	methylbenzene	5.888/air[ii]	0.1>	0.1>	0.1>

[a] Odor activity value (i.e., concn/threshold value); - - -, not calculated. [b] Compound number. [c] Threshold values from various sources: i, Buttery et al. (*17*); ii, Devos et al. (*18*); iii, Leffingwell and Associates (*23*); iv, Fors (*20*); v, Siek et al. (*19*).

the liquid inoculation method was used in subsequent investigations. In the agar experiment, the complete medium agar was used, on the assumption that it would support the normal growth of the *Monascus* species and produce headspace composition comparable to that using the rice as substrate. However, in reality, the number and quantity of the nutrients in the rice are in general much higher than that in the complete medium agar (*16*).

The headspace volatile components with and without the presence of *Monascus* in the agar experiment are shown in **Table 2**. A total of five compounds were detected in the headspace including benzaldehyde (**4**), 2-propanone (**5**), 1-butanol (**16**), 3-methyl-1-butanol (**17**), and methylbenzene (**22**). By comparison with the control, most compounds in the experimental group were fewer in number and were lower in concentration. One exception was the compound methylbenzene (**22**), which was more concentrated in the experimental group. Although this compound was not reported in either the commercial plain or red sufus (*7*, *8*), it was detected in the aging plain sufu in a laboratory-scale investigation carried out by Hwan and Chou (*15*). Benzaldehyde (**4**) was also identified in Hwan and Chou's samples and in the current two sets of agar samples, but not in the commercial sufu (*15*). By comparison of the rice and the agar experiments (**Tables 1** and **2**), many more compounds were found in both control and experimental groups with rice as the major substrate.

From these observations, different compositions of headspace volatile components would evolve if the *Monascus* spp. is cultivated in different media. Raw rice contains many nutrients including protein, fat, carbohydrate, water, minerals, and vitamins (*16*). Its presence during fermentation could provide some essential elements or conditions that the complete medium agar was unable to supply for the production of volatiles of similar composition. Further investigation in the rice composition may be necessary to pinpoint those essential components that are responsible.

Odor Activity Value. In **Table 3**, the OAVs of the headspace volatile components in the presence of rice were calculated on the basis of the threshold values found in the literatures (*17*−*20*). The majority of the chosen ones were from samples prepared and evaluated in air. However, exceptions were made when such conditions were not available. In the rice control, which did not contain the *Monascus* spp., pentanal (compound **2**, OAV = 50.2), *n*-hexanal (compound **3**, OAV = 133.8), benzaldehyde (compound **4**, OAV = 1.0), 3-methyl-1-butanol (compound **17**, OAV = 1.4), and 2-pentylfuran (compound **21**, OAV = 10.3) have OAVs ≥1. Many compounds in the rice control were reported in the cooked long-grain rice isolated by the Tenax trap method (*21*), in which *n*-hexanal had a much higher aroma activity than the rest of the compounds (*21*) and provided a fatty, green, grassy flavor. Pentanal (**2**) provides a woody, vanilla, fruity, nutty sensation; 2-petnylfuran (**21**) has a green bean, metallic vegetable-like aroma, whereas 3-methyl-1-butanol (**17**) has a fused oil, whiskey-like flavor (*13*).

In the presence of the *Monascus* spp., 3-methyl-1-butanol (compound **17**) (OAVs = 154.1 and 197.3) was identified as the most potent component in the two experimental groups. Ethanol (compound **14**, OAV = 98.3) and ethyl acetate (compound **10**, OAV = 133) came in second for the liquid and solid inoculation methods in the two groups, respectively. The former has a pleasant odor, whereas the latter has a pineapple, ethereal aroma. (*13*).

Overall, the dominating compounds based on the OAV in the control group generally belonged to aldehydes and furans, whereas in the two experimental groups, the dominating compounds were the alcohols, esters, and ketones. The rankings of OAV of the components from high to low are similar for both experimental groups (**Table 3**), particularly for the initial six compounds as follows: for liquid inoculation, **17** > **14** > **10** > **15** > **11** > **13**; for solid inoculation, **17** > **10** > **14** > **15** > **11** > **13**. The rest of the compounds, including **6**, **9**, **21**, **16**, **19**, and **12**, have much different orders. Nevertheless, within a chemical class, the rank of the compounds agrees well between the two inoculation methods. Except for *n*-hexanal (**3**), both experimental groups [i.e., rice + *Monascus* (liquid) and rice + *Monascus* (solid)] contained all of the compounds found in the commercial red sufus (**Table 1**) (*7*). As discussed in the previous section, compounds **6**, **13**, **17**, and **21** with OAVs >1 and compounds **8** and **18** with OAVs < 1 were not reported in Hwan and Chou's laboratory-type aging plain sufus (*15*), but were found in the commercial red ones (*7*). These observations implied that some of the odorous compounds in the commercial red sufus might be contributed by the fermented red rice, which was introduced at the start of the aging period. In fact, recalculations of the OAVs based on the concentrations of these six compounds from the previous commercial red sufus (*7*) and the current threshold values have shown that compounds **8** and **18** remained low in OAV (<1) among the samples. However, the rest of the compounds showed much higher odor contributions based on their OAVs. Compound **17** has the highest OAV range (9248−154131) followed by compound **21** (OAV = 899−4825), compound **6** (OAV = 222−1080), and compound **13** (OAV = 63−1707) in the original three samples (*7*). The magnitudes of these OAVs are either comparable or much higher than that of most compounds listed in the previous paper (*7*). Apparently, the presence of the *Monascus* is not only crucial for the production of natural pigments but also important for

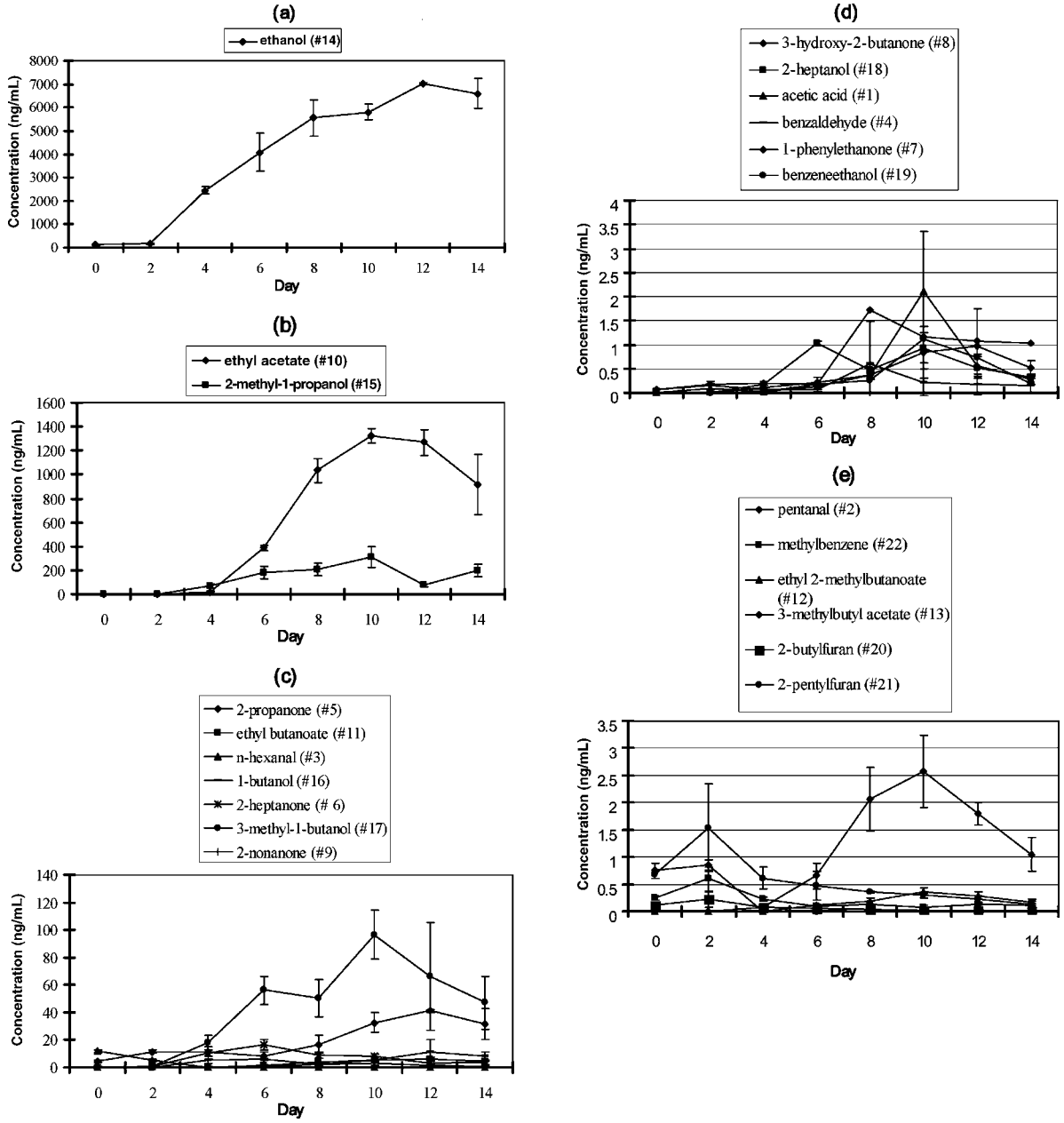

Figure 1. Time–concentration relationships among headspace volatile components in rice samples inoculated with *Monascus* spp. cultivated in liquid medium (experimental).

the synthesis of the characteristic flavor in the red rice, which provides additional odor to the red sufu (*5*).

Kinetics Experiment. Changes in the concentrations of the headspace components throughout the 14-day fermentation in the presence and absence of the *Monascus* were monitored and are shown in **Figures 1** and **2**, respectively. Most compounds reached their maximal concentrations between 10 and 12 days. For example, ethanol (**14**) had the highest concentration (>6000 ng/mL) at day 12, whereas both ethyl acetate (**10**) (>900 ng/mL) and 2-methyl-1-propanol (**15**) (>200 ng/mL) were maximum at day 10. For compounds with concentrations below 100 ng/mL, their maxima varied between days 2 and 12 (**Figure 1**).

Variations were observed in the mean concentrations of the common compounds between the present (kinetic) and the previous (rice) measurements at day 14. In the experimental samples with liquid *Monascus* inoculated, an independent Student's *t* test of the identified components found in the kinetic and the rice experiments showed that only 5 of 19 common components including 3-hydroxy-2-butanone (**8**), 2-nonanone (**9**), ethanol (**14**), 2-heptanol (**18**), and 2-pentylfuran (**21**) were significantly different ($p < 0.05$) in concentration. However, only 3 (**9**, **14**, and **21**) of the 12 odor-potent compounds (**6**, **9**−**17**, **19**, and **21**) discussed previously showed statistically significant difference in the kinetic experiment. This suggested that the majority of these aroma-contributing components remained dominant as the background flavor in the red rice even though the headspace volatiles were collected every 2 days to monitor their quantitative changes. It also implied that these compounds were continuously produced and replaced those that were removed from the fermentation process for analyses. The rest of the compounds were similar in concentration between the present (kinetics) and the previous measurements (rice) at day 14.

In the control samples, the concentrations of most compounds (**2**, **3**, **5**, **6**, and **20**−**22**) in the initial experiment were

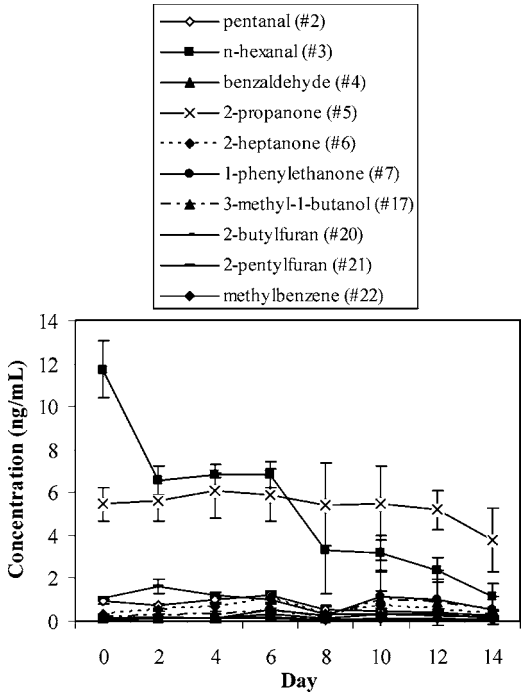

Figure 2. Time–concentration relationships among headspace volatile components in rice samples without *Monascus* spp. inoculation (control).

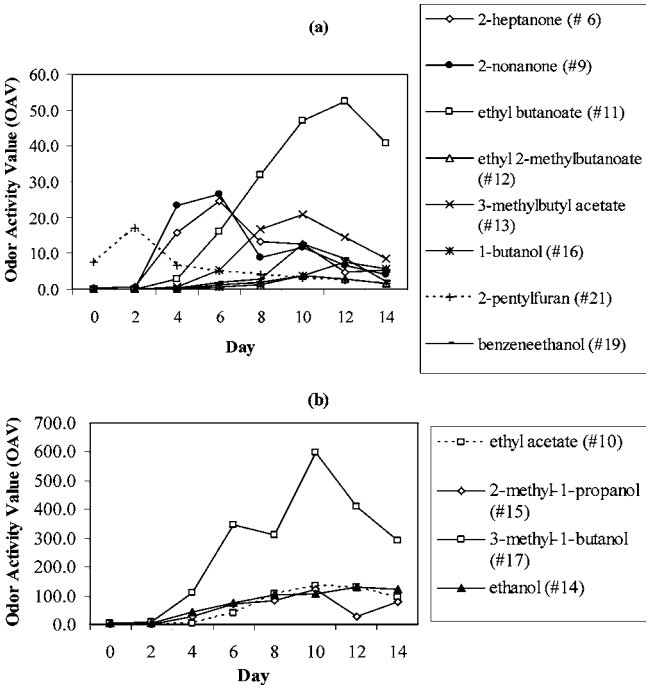

Figure 3. Time–odor activity value (OAV) relationships among headspace volatile components in rice samples inoculated with *Monascus* spp. grown in liquid medium (experimental).

significantly higher ($p < 0.05$) than those of the kinetics ones at day 14. The lower concentrations of volatile compounds in the kinetics experiment might be due to the continued samplings from the same sample. The removed portion of compounds was not replenished due to the absence of fermentation of the substrate in the absence of the *Monascus*. Nevertheless, qualitatively, there were no differences between the two sets of experiments at day 14.

In the absence of the *Monascus* (**Figure 2**), the concentrations of pentanal (**2**) and *n*-hexanal (**3**) decreased throughout the 14 days. Similarly, 2-propanone (**5**), 2-butylfuran (**20**), 2-pentylfuran (**21**), and methylbenzene (**22**) showed the same trend, but they all went through maxima before dropping below the initial concentrations. For compounds **4**, **6**, **7**, and **17**, there were initial increases in the concentration of the compounds before they finally fell at concentrations slightly higher than the initial concentrations at day 14. Generally, for most components in the control group, concentrations at day 14 were lower than those at day 0.

Changes in the OAV with time for both the experimental and control groups were plotted as shown in **Figures 3** and **4**, respectively. Among the alcohols in the experimental group, 3-methyl-1-butanol (**17**) and 1-butanol (**16**) had the highest and lowest OAVs at their maxima of 592.2 and 7.5, respectively. The highest OAVs were observed at day 10 for alcohols **14**, **15**, and **17** and at day 12 for **16** and **19**. Among the four potent esters, ethyl acetate (**10**) had the highest OAV followed by ethyl butanoate (**11**), 3-methylbutyl acetate (**13**), and ethyl 2-methylbutanoate (**12**). Except for ethyl butanoate (**11**), which had the maximum OAV at day 12, the rest of the compounds had their maximum OAV at day 10. Both ketones had the highest OAV at day 6, whereas one furan had its highest OAV at day 2. OAVs of many components started to fall between 10 and 12 days. Apparently, 12 days would be sufficient for many aroma-contributing components to reach their highest aroma impact in the headspace. Additional fermentation time at the same conditions probably would not bring any further advantage to the fermenting red rice for the production of aroma.

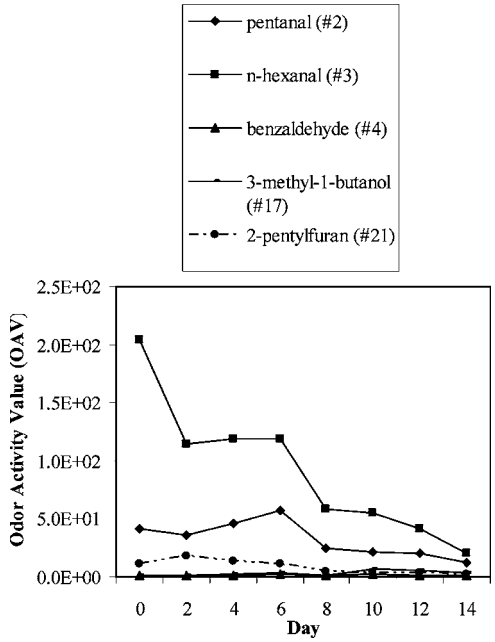

Figure 4. Time–odor activity value (OAV) relationships among headspace volatile components in rice samples without *Monascus* spp. inoculation grown in liquid medium (control).

In conclusion, fermented red rice contained several important aroma-contributing components including four esters, five alcohols, two ketones, and one furan based on their high OAVs (i.e., OAV ≥ 1). Among them, several have relatively high OAVs (i.e., OAV > 45) including ethyl acetate (**10**), ethyl butanoate (**11**), ethanol (**14**), 2-methyl-1-propanol (**15**), and 3-methyl-1-butanol (**17**). Most of them reached their maximum concentrations by day 12 under the current experimental conditions. Many of them were detected in the commercial red sufu, which was an indication of their likely contribution to the overall aroma of the product.

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