Impact of Different Raw Materials on Changes of Volatile Compounds during Moromi Fermentation

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Abstract: The composition and ratio of volatile compounds in soy sauce have a major impact on its organoleptic properties.Considering the important influence of long-term (3 months) moromi fermentation on the aroma formation of the soy sauces from different materials (soybean, Rice, Black Bean, Wheat, Wheat flour, and mungbean), the volatile compounds of 24 samples in total, taken from three different stages of moromi fermentation, were analyzed by solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC-MS). The results showed a total of 77 volatile compounds, including acids (4), alcohols (14), phenols (6), aldehydes (12), esters (26), ketones (5), furan(one)s (5), and pyrazine (5), and the majority of the compounds were common. Among all samples, The highest amount of volatile compounds (5528.58 ± 1308 μg/L) was detected in the moromi made from the combination of soybean, black bean, and wheat flour on the first month of fermentation, and the sample that had the lowest amount of volatile compounds (63.25 ± 1.70 μg/L) was detected in the moromi sample from the combination of soybean and wheat flour on day 0. During the three months of moromi fermentation, the relative contents of acids, alcohols, phenols, aldehydes, esters, ketones, furan(one)s, and pyrazines changed gradually. Finally, the total presence of volatile compounds identified in the 24 samples increased from 0 days to one month and from month to month perfectly.

Keywords: Volatile compounds; Raw materials; moromi fermentation; Soy sauce; GC-MS; HS-SPME

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1. INTRODUCTION

Soy sauce is a dark brown liquid from a fermented soybean and wheat blend or wheat flour, which originated in China and was brought to Cambodia by Chinese people who immigrated there long ago. Chinese Cambodians mainly produce soy sauce in Cambodia, and over 90% of Cambodians consume soy sauce (Theary et al., 2013; Williams, 1967). It is now Asia and Western countries' most widely recognized fermented soyfood (Liu, 2008). Soy sauce is consumed as a culinary ingredient rather than a preservative. Its peculiar flavor has a strong umami, salty, and caramel-like character that complements various foods' savory taste and scent. On the other hand, the processes of soy sauce manufacture are related to the country of origin. For example, the Chinese style employs 80:20 and 70:30 soybeans to wheat or wheat flour ratios, respectively, whereas the Japanese style uses equal proportions of each (ratio 50:50) (Diez-Simon et al., 2020a). Koji is made with Aspergillus oryzae and then fermented at a greater salt concentration (160–180 g/l NaCl) at a controlled or uncontrolled temperature. Fermentation of moromi is a complicated process. For 3-6 months of fermentation, flavor, and microbes, such as lactic acid bacteria and yeasts, are added to the fermentation process (Jiang et al., 2021). Although physicochemical properties, particularly formaldehyde nitrogen, were crucial in determining the quality of soy sauce by the China National Standard (GB18186-2000,

fermented soy sauce), volatile characteristics and distinct flavors related to the fermentation process were also important (Zheng et al., 2013). The composition and ratio of volatile chemicals in soy sauce significantly impact its organoleptic properties (Fukushima, 1979). During the moromi fermentation process in traditional Chinese soy sauce, significant changes in acids, alcohols, esters, aldehydes, ketones, and furans were examined (X. L. Gao et al., 2010). The most common and robust technique to analyze (and identify) volatile aroma compounds is Gas Chromatography-Mass Spectrometry (GC-MS) (Diez-Simon et al., 2021). GC separates the volatile in a sample, whereas the analyte is fragmented by GC-MS and identified by its mass (Lee & Khor, 2015). Not only are soybeans and wheat used as materials to make soy sauce, but other kinds of beans, such as black beans, peas, and rice, have also been used to make soy sauce (Yamana et al., 2020). The volatile compounds in moromi from different materials have yet to be widely studied.

The primary purpose of this study was to evaluate and differentiate the number and concentration of the volatile compounds extracted and detected from soy sauces during moromi fermentation of different materials of its respective day zero, one month, two months, and three months old.

2. METHODOLOGY

2.1. Materials

Soy sauces were made using different raw materials (SBW: Soybeans 50%+black beans 30%+wheat flour 20%; SWFB: Soybeans 50%+wheat flour 30%+mung beans 20%; SR: Soybeans 50%+rice 50%; SW: Soybeans 50%+wheat 50%; SWF: Soybean 50%+wheat flour 50% and SRW: Soybeans 50%+rice 30%+wheat 20%). The ingredients (Soybeans, black beans, mung beans, wheat, wheat flour, and salt) for making soy sauce were purchased from supermarkets in Phnom Penh, Cambodia. Samples were coded with 0 to 3, meaning from day 0 to 3 months (e.g., SBW0=SBW on day 0).

2.2. Sample preparation

Samples were directly prepared for Koji fermentation (3 days) and then soaked into a brine solution to continue as moromi fermentation when the first stage of the sample was taken as day zero (Only when Koji soaked into brine). Samples were aged at room temperature for a month, two months, and three months, which was when the sample's second, third, and fourth stages were taken, respectively. Therefore, samples had to be filtered by cheesecloth and placed into a falcon. After that, samples were centrifuged at 5000rpm, one accel, 4 ℃, for 20min (Luo et al., 2009) and kept at a temperature of -20 ℃ for further experiments.

2.3. Equipment

The key for this experiment is the gas Chromatography-Mass Spectrometry (GC-MS) apparatus, Shimadzu GCMS-QP2010 Ultra with AOC-5000 (Japan). On the other hand, an SPME fiber 50/30um DVB/CAR/PDMS, Stableflex (2cm) 24Ga including its holder (bought from Sigma-Aldrich and Supelco, from Merck KGaA, Darmstadt, Germany), 20mL gas-tight glass vial with PTFE septum in an aluminum cap, a magnetic heating stirrer including a stirring bar; micropipettes, a thermometer and a salt meter were used for the SPME extraction of the volatile compounds through a headspace. The column used in this study was SH-Rxi-5SilMS with 30 m of length, 0.32 mm of diameter, and 0.1 μm of thickness.

2.4. SPME extraction of volatile compounds

In the first stage, after thawing, each sample had to be checked for salt content by the salt meter (a few drops of soy sauce were put on the salt meter in the sensor section to measure its salt). Then, salt (sodium chloride: NaCl) was gradually weighted and adjusted to 25% of the 5ml sample. Salt and 5ml of soy sample were then placed into a 20ml vial, and the vial was covered with an aluminum cap (once a reagent was put into the vial, they were immediately capped to avoid some contaminations) (Yan et al., 2008). In the next step, 10 μL of the internal standards, 2-methyl-3-heptanone (it was first diluted with

methanol before being added and had a final concentration obtained of 20mg/L) (Feng et al., 2013) and 10μL of 4-nonanol (with the concentration of 0.082g/L) were added (Kesen et al., 2018). In addition, a magnetic stirring bar was placed in the vial to balance the mixture with a magnetic stirrer in the following stage. The vial was then carefully and tightly closed. It was then submerged in water in a beaker set over a heating stirrer until the mixture level sank; as a result, the mixture was homogenized for 20 minutes at each absorption time and temperature condition. The calibrated fiber was injected into the headspace and inside the vial after being equilibrated (the fiber needle was thought not to have been in direct contact with the liquid phase of the mixture), and the extraction of volatile compounds was carried out at a specific temperature for a specific amount of time (40°C, 40min; in this study).

2.5. GC-MS analysis of volatile compounds

Some equipment had to be calibrated before the extraction of volatile compounds by the SPME technique to prevent errors. The SPME fiber needed to be injected into the GC-MS injector port at 250 °C at least 30 minutes before use to ensure that there were no remaining compounds in the fiber, the GC-MS apparatus used in this work was made up of an SH-Rxi-5SilMS column with the following specifications: 30m in length, 0.32 mm in diameter, and 1 m thick film (J&W Scientific, Folsom, CA). A 26.6 mL/min flow of 99.999% pure helium gas was used, and the injection mode was split. The GC oven's temperature was maintained at 40°C for 2min before being raised to 250°C at a rate of 5°C/min and maintained for 5min. The ion source's mode was electron ionization, and its temperature was 230°C with an electron voltage of 70eV. The ions produced by ionization were scanned between 34 and 348m/z during the study of volatiles.

Following the SPME extraction of the volatile compounds from the soy sauce, the extracted analytes were desorbed by injecting the SPME fiber onto the GC injector port at 250°C for 10 minutes. Once injected, a computer system attached to the GC-MS equipment was used to control the detection procedure. The GC-MS Real-Time Analysis application ran the analysis of the volatile compounds for 59 minutes for each sample. Each sample was analyzed in triplicate.

2.6. Identification and semi-quantification of volatile compounds

2.6.1. Identification

The identification of the volatile compounds in this study was evaluated by the mass spectra depending highly on the measure of similarity score was at least 85% that was matched to the NIST20 library. Then, each unknown compound was confirmed by calculating the retention indices (RI) using series n-alkane C7-C40.

2.6.2. Semi-quantification

The quantification of volatile compounds was calculated by the peak area ratio to the internal standard and multiplied by the concentration of the internal standard. Semi-qualification of aldehydes, esters, ketone, phenols, and Furan(one)s was calculated by using 2-methyl-3- heptanone as internal standard, while acid, alcohols, and pyrazines were calculated by using 4-nonanol as internal standard (Kilic-Buyukkurt, 2021). Semi-quantification was calculated by the following formula:

$$\text{UC conc.} = \frac{\text{UC peak area}}{\text{IS peak area}} \times \text{IS conc.}$$

where:

UC conc. = Unknown compound concentration (μg/L)

IS conc. = Internal standard concentration (μg/L)

UC peak area = Unknown compound peak area

IS peak area = Internal standard peak area

3. RESULTS AND DISCUSSION

3.1. Volatile compounds identification of 24 soy sauces

The identification of volatile components in the extracts was done using GC-MS. While the RI (retention index) of unknown compounds was calculated using GC retention index standards (hydrocarbons from straight chain C7-C40) and compared to the RI of the standards or those reported in the published work, the majority of compounds were identified by comparison of their spectra (Gao et al., 2009). Six soy sauces were produced from different raw materials, and each sample was taken four times from different stages of moromi fermentation: day zero, one month, two months, and three months. Thus, there would be 24 samples in total that were examined. In this study, around 300 volatile compounds were observed in the 24 soy sauce samples using SPME-GC-MS methods. But not all those substances were retrieved to be examined or discussed. Most compounds were assumed to be rejected from semi-quantification since they had similarity scores below 85 and could not be confirmed with retention index. As shown in Table 1, 77 of 300 volatile compounds were quantified using internal standards (2-Methyl-3-Heptanone and 4-Nonanol) and considered into eight compound groups. The eight groups were acid (4), alcohol (14), phenols (6), aldehydes (12), esters (26), ketones (5), furan(one)s (5), and pyrazine (5).

3.2.1. Alcohols

Alcohols can be formed through many pathways, like aldehydes (Diez-Simon et al., 2019). Most of the alcohols in soy sauce are produced during the fermentation process under aerobic conditions from sugars and amino acids (Sun et al., 2010a). Alcohols may specifically arise during the fermentation process of the fermented soy sauce type at the moromi stage of soy sauce production. Alcohols could occur during the moromi stage either through spontaneous fermentation or through the addition of LAB and certain yeast species, as well as through the reduction of aldehydes molecules (Luh, 1995). Moreover, the amino-acid catabolic and biosynthetic pathways can decarboxylate and then decrease the keto acids, equivalent to the alcohols, to make higher alcohols (Van Der Sluis et al., 2001). A total of 15 alcohols were identified in the 24 soy sauces. Among these, 2-methyl-1-butanol and 1-hexanol were presented in almost all samples tested; however, phenyl ethyl alcohol seemed to be the predominant alcohol. 2-methy-1-propanol and 2-methyl-1-butanol are compounds that are mainly produced through the Ehrilch pathway during fermentation (Feng et al., 2013); however, the breakdown of 2-methyl-1-butanal also results in the production of 2-methyl-1-butanol, which add to the malty aroma. Moreover, 2-methyl-1-propanol, 2-methyl-1-butanol were also found in Chinese soy sauces which were made by a high-salt-diluted state fermentation in a previous study (Sun et al., 2010a)Therefore, 2-ethyl-1-hexanol was found in each of the samples but was able to identify only in 12 samples; SBW0, SWF0, SWFB0, SR2, SR0, SRW0, SW2, SW0, SRW1, SWFB2, SW1, and SWFB1; and provided the highest concentration 123.16±22.51 μg/L in SBW0, followed by SWF0. Some alcohols are also produced by the oxidation of fat during koji incubation; *Aspergillus oryzae* produced lipase to break fat long chain molecules, which is why it is prone to fat oxidation and produces alcohol such as 1-hexanol, 3-octanol, 2-octen-1-ol, 1-octen-3-ol. The highest amount of 1-hexanol, 3-octanol, and 1-octen-3-ol was 90.07-112.44 μg/L, 3.92-4.67 μg/L, and 158.58-177.30 μg/L, respectively; thus, during the moromi fermentation the mixtures of the sample did not break down sufficiently to form a higher concentration of alcohol, but according to Gao *et al.* (2010), alcohols started respectively picking up their concentration from day 0 to first and second month while alcohols peaking up at third month which is similar to this study as presented in Fig 1

Table 1 Volatile compounds in SRW from day 0 to week 3.

RT[(a)]	Compounds[(b)]	RIE[(c)]	RIL[(d)]	Odour descriptors	Mean concentration ± SD (μg/L)			
					SRW0	SRW1	SRW2	SRW3
Acid								
1.2867	Acetic acid	<700	610	Acidic	n.d.	96.29 ± 4.89	38.46 ± 0.05	84.41 ± 7.44
5.0229	Butanoic acid, 3-methyl-	844	850	Rancid	n.d.	22.19 ± 2.35	4.96 ± 0.2	3.82 ± 0.17
5.439	Butanoic acid, 2-methyl-	856	861	Cheesy	n.d.	12.19 ± 0.75	n.d.	n.d.
	Octanoic acid		1180	Cheesy	n.d.	n.d.	n.d.	n.d.
Alcohol								

1.485	1-Propanol, 2-methyl-	<700	624	Wine	0.51 ± 0.5	n.d.	9.62 ± 0.37	22.96 ± 0.74
	1-Butanol		659	Fruity	n.d.	n.d.	n.d.	n.d.
	1-Butanol, 2-methyl-		739	Malty	n.d.	n.d.	n.d.	n.d.
5.3255	2-Furanmethanol	852	860	Baked	n.d.	n.d.	1.29 ± 0.09	2.52 ± 0.09
5.6829	1-Hexanol	866	868	Floral, green	2.14 ± 0.1	6.68 ± 0.33	3.63 ± 0.09	20.55 ± 1.9
8.654	1-Heptanol	971	970	Fruity	n.d.	n.d.	n.d.	1.32 ± 0.09
8.9007	1-Octen-3-ol	979	980	Mushroom	73.12 ± 0.64	36.04 ± 1.05	5.78 ± 1.06	n.d.
9.3984	3-Octanol	996	993	Mushroom	0.83 ± 0.05	n.d.	n.d.	n.d.
10.4324	1-Hexanol, 2-ethyl-	1031	1030	Floral	2.76 ± 0.05	1.79 ± 0.07	n.d.	n.d.
10.5742	Benzyl alcohol	1036	1036	Floral	n.d.	n.d.	3.58 ± 0.7	20.65 ± 1.23
	2-Octen-1-ol, (E)-		1067	Baked	n.d.	n.d.	n.d.	n.d.
	2-Octen-1-ol, (Z)-		1068	Floral	n.d.	n.d.	n.d.	n.d.
	1-Octanol		1070	Fruity	n.d.	n.d.	n.d.	n.d.
	Phenylethyl Alcohol		1116	Rosy, honey	n.d.	13.12 ± 1.01	30.44 ± 2.7	103.07 ± 7.1
Aldehydes								
1.6868	Butanal, 3-methyl-	<700	652	Malty	n.d.	9.15 ± 0.92	7.77 ± 1.62	7.85 ± 0.46
1.7881	Butanal, 2-methyl-	<700	662	Malty	n.d.	6.04 ± 0.32	5.39 ± 1.35	n.d.
3.8675	Hexanal	789	801	herbaceous	n.d.	n.d.	n.d.	19.21 ± 1.53
4.7206	3-Furaldehyde	827	831	Almond-like	n.d.	n.d.	n.d.	n.d.
7.7210	Furfural	828	829	Sweet	n.d.	0.69 ± 0.08	n.d.	n.d.
	Heptanal		901	Rancid	n.d.	n.d.	n.d.	n.d.
	Methional		907	Mashed potato	n.d.	n.d.	n.d.	n.d.
8.2653	Benzaldehyde	959	962	Fruity	2.42 ± 0.23	56.35 ± 1.26	114.53 ± 30.18	43.38 ± 6.4
10.8437	Benzeneacetaldehyde	1044	1045	Honey-like	0.15 ± 0.01	9.26 ± 0.53	11.55 ± 2.09	15.17 ± 1.11
11.2966	2-Octenal, (E)-	1059	1060	Fatty	0.77 ± 0.07	0.72 ± 0.02	n.d.	5.87 ± 0.28
12.6861	Nonanal	1105	1104	Fatty	n.d.	n.d.	1.87 ± 0.18	6.9 ± 0.24
15.9031	2,4-Nonadienal, (E,E)-	1215	1216	Floral, fatty	n.d.	n.d.	n.d.	3.21 ± 0.13
Esters								
		<700						220.13 ±
1.3992	Ethyl Acetate		612	Fruity	n.d.	45.54 ± 3.17	97.79 ± 22.71	13.74
2.4975	Propanoic acid, ethyl ester	705	705	Fruity	n.d.	0.2 ± 0.02	n.d.	1.07 ± 0.06
	Butanoic acid, ethyl ester		802	Fruity	n.d.	n.d.	n.d.	n.d.
	Acetic acid, butyl ester		812	Fruity	n.d.	n.d.	n.d.	n.d.
5.1531	Butanoic acid, 2-methyl-, ethyl ester	845	849	Fruity	n.d.	1.3 ± 0.79	2.06 ± 0.49	6.85 ± 0.68
	Butanoic acid, 3-methyl-, ethyl ester		853	Fruity	n.d.	n.d.	n.d.	n.d.
5.8818	1-Butanol, 3-methyl-, acetate	873	876	Banana-like	n.d.	n.d.	n.d.	1.19 ± 0.18
	1-Butanol, 2-methyl-, acetate		879	Fruity	n.d.	n.d.	n.d.	n.d.
	Hexanoic acid, methyl ester		925	Fruity	n.d.	n.d.	n.d.	n.d.
9.5227	Hexanoic acid, ethyl ester	999	999	Fruity	n.d.	n.d.	n.d.	34.9 ± 5.91
	Heptanoic acid, methyl ester		1023	Fruity	n.d.	n.d.	n.d.	n.d.
12.401	Benzoic acid, methyl ester	1095	1094	Floral, honey	n.d.	2.13 ± 0.15	3.13 ± 0.76	n.d.
12.5231	Heptanoic acid, ethyl ester	1099	1098	Fruity	n.d.	n.d.	n.d.	7.1 ± 0.95
	Octanoic acid, methyl ester		1126	Fruity	n.d.	n.d.	n.d.	n.d.
14.6641	Benzoic acid, ethyl ester	1172	1172	Fruity, floral	n.d.	1.76 ± 0.21	12.64 ± 2.98	31.8 ± 3.23
	Benzeneacetic acid, methyl ester		1178	Honey-like	n.d.	n.d.	n.d.	n.d.
15.0129	Butanedioic acid, diethyl ester	1184	1181	Fruity	n.d.	n.d.	n.d.	3.87 ± 0.17
15.442	Octanoic acid, ethyl ester	1198	1196	Fruity	n.d.	n.d.	2.32 ± 0.65	22.29 ± 3.44
16.8066	Benzeneacetic acid, ethyl ester	1247	1247	Floral	n.d.	0.7 ± 0.02	5.5 ± 1.21	12.06 ± 0.7
17.1384	Acetic acid, 2-phenylethyl ester	1259	1258	Honey-like	n.d.	n.d.	n.d.	1.86 ± 0.09
	Decanoic acid, ethyl ester		1396	Wax-like	n.d.	n.d.	n.d.	n.d.
	Dodecanoic acid, methyl ester		1526	Floral	n.d.	n.d.	n.d.	n.d.
	Dodecanoic acid, ethyl ester		1594	Wax-like	n.d.	n.d.	n.d.	n.d.
32.7043	Hexadecanoic acid, methyl ester	1928	1926	Wax-like	0.45 ± 0.08	n.d.	n.d.	n.d.

Table 1 continued

RT[a]	Compounds[b]	RIE[c]	RIL[d]	Odour descriptors	Mean concentration ± SD (µg/L)			
					SRW0	SRW1	SRW2	SRW3
34.0109	Hexadecanoic acid, ethyl ester	1996	1993	Wax-like	n.d.	n.d.	4.7 ± 1.06	41.46 ± 6.26
37.1808	9-Octadecenoic acid, ethyl ester	2169	2141	Floral	n.d.	n.d.	n.d.	21.16 ± 4.33
Furan(one)s								
	2(3H)-Furanone, dihydro-3-methyl-		953	Creamy	n.d.	n.d.	n.d.	n.d.
	3(2H)-Furanone, 4-hydroxy-5-methyl-		955	Caramel-like	n.d.	n.d.	n.d.	n.d.
9.2361	Furan, 2-pentyl-	990	993	Green bean	n.d.	n.d.	n.d.	11.72 ± 1.74
	2(3H)-Furanone, 5-ethyldihydro-		1056	Caramel-like	n.d.	n.d.	n.d.	n.d.
	2(3H)-Furanone, dihydro-5-pentyl-		1365	Coconut-like	n.d.	n.d.	n.d.	n.d.
Ketone								
2.3098	Acetoin	713	713	Butter-like	0.23 ± 0.09	n.d.	1.71 ± 0.42	2.67 ± 1.16
6.2811	2-Heptanone	888	891	Fruity	n.d.	0.27 ± 0.01	1.02 ± 0.27	1.26 ± 0.07
	Butyrolactone		916	Creamy	n.d.	n.d.	n.d.	n.d.
9.1202	3-Octanone	987	986	Pungent	11.82 ± 0.71	6.23 ± 0.12	10.45 ± 1.64	n.d.
26.5063	Benzophenone	1634	1635	Rose-like	n.d.	n.d.	0.6 ± 0.19	1.27 ± 0.01
Phenol								
	Phenol, 2-methoxy-		1090	Smoky, burnt	n.d.	n.d.	n.d.	n.d.
14.5765	Phenol, 4-ethyl-	1170	1168	Spicy	n.d.	3.63 ± 0.01	17.74 ± 3.81	12.87 ± 0.51
16.0725	4-Vinylphenol	1222	1223	Spicy	3.46 ± 0.08	2.97 ± 0.28	n.d.	n.d.
17.7484	Phenol, 4-ethyl-2-methoxy-	1282	1282	Spicy	n.d.	28.24 ± 0.15	219.16 ± 49.03	202.59 ± 8.39
18.7017	2-Methoxy-4-vinylphenol	1317	1316	Spicy	8.2 ± 0.06	n.d.	n.d.	n.d.
23.7694	2,4-Di-tert-butylphenol	1516	1514	Phenol	1.24 ± 0.12	1.73 ± 0.33	4.35 ± 2.4	n.d.
Pyrazine								
4.5171	Pyrazine, methyl-	820	829	Nutty	0.58 ± 0.22	15.65 ± 0.89	4.74 ± 0.38	4.63 ± 0.37
6.9912	Pyrazine, 2,5-dimethyl-	915	917	Roasted nut	n.d.	n.d.	n.d.	0.91 ± 0.13
	Pyrazine, 2,6-dimethyl-		917	Roasted cocoa	n.d.	n.d.	n.d.	n.d.
7.1351	Pyrazine, 2,3-dimethyl-	921	920	Roasted nut	n.d.	2.91 ± 0.29	1.36 ± 0.16	n.d.
	Pyrazine, 2-ethyl-3-methyl-		1004	Caramel-like	n.d.	n.d.	n.d.	n.d.

[a] Retention time of each compound after integration and identification.
[b] Compounds were selected by searching mass spectra with the library NIST 20 and at least 85% similarity, then confirmed with retention indices in the NIST 20 library.
[c] Calculated retention indices from the experiment using series n-alkane C7-C40 standards.
[d] Retention indices literature from NIST 20 library on semi-standard non-polar GC-MS column.
n.d., not detected by GC-MS

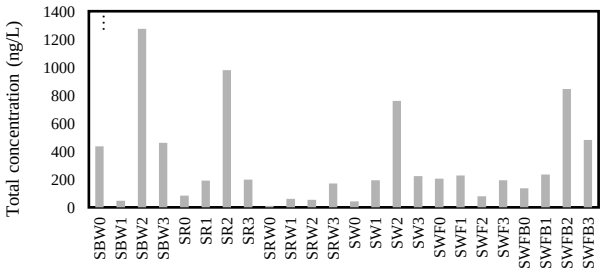

Figure 1. Total alcohol semi-quantification of each sample tested.

3.2.2. Phenols

Five phenols were identified in this work. Within 24 samples, there are only SBW0 that didn't detect in the sample. and 455.037±17.576 μg/L in SW3, except for 4-Ethylphenol, which was not present in SWF2. However, 4-ethylphenol is also a significant phenol compound in soy sauces. They have been previously reported in Japanese, Korean, and Thai soy sauce(Wanakhachornkrai and Lertsiri, 2003). These compounds are generated from the degradation of lignin glycoside in cereal bran during fermentation (Van Der Sluis et al., 2001).

The enzyme peroxide can also produce 4-ethyl guaiacol, which causes aromatic amino acids to break down. When wheat bran is used for Moromi fermentation, a microbe called Torulopsis transforms ferulic acid into 4-ethyl guaiacol, which can be thought of as one of the desirable volatile components for soy sauces (Devanthi & Gkatzionis, 2019). Soy sauces have a smoky aroma from 4-ethyl guaiacol, but these sauces also have spicy and sweet vanilla scents. 4-ethyl-2-methoxyphenol was also reported in Japanese raw and thermally treated soy sauces (Meng et al., 2017), according to Sun et al. (2010). There are 16 samples detected 2,4-Di-tert-butylphenol in the samples; it is a bioactive, antifungal, and also found in rice and some plants (Zhao et al., 2020). From Fig 2., phenols in SW2 contained the high total concentration (1114.75 μg/L), followed by SW2 (485.71 μg/L); however, SWFB0 had the lowest among all presented phenols in the soy sauces.

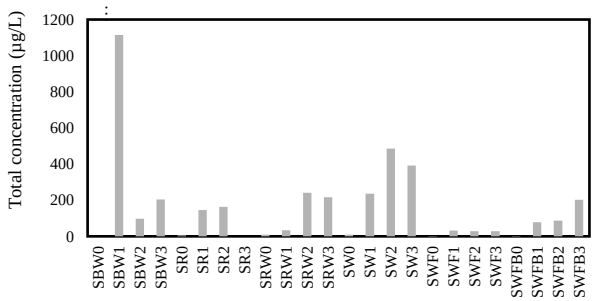

Figure 2. Total phenol semi-quantification of each sample tested.

3.2.3. Acids

Microbial mechanisms in fermented soy sauces produce acids during the fermentation process, but in acid-hydrolyzed soy sauces, acids are byproducts of lipid destruction aided by heat (S. M. Lee et al., 2006). Among the 24 soy sauces, four acids were identified: (2-methyl-butanoic acid, 3-methyl-butanoic, acetic acid, and octanoic acid. Acids start detected from the first month of fermentation, that were found ranged from 2.46±0.1 μg/L to 148.78±31.47 μg/L. The lowest concentration is 2.46±0.1 μg/L. Acetic acid produced by lactic acid bacteria during fermentation gives a sour odor to soy sauce and contributes substantially to its aromatic profile. Acetic acid can react with alcohols to generate the corresponding acetate esters, which impart various fruity aromas (Harada et al., 2018). Acetic acid is one of the most vital acids in soy sauces (Diez-Simon et al., 2020b)

From a previous study, the total semi-quantification of acids increased gradually from day 0 to the third month and slightly decreased after that (X. L. Gao et al., 2010). Therefore, the semi-quantification of acids in this study seemed to be similar to the above-referenced research except for the SBW sample where SBW2 (1-month age of soy sauce) picked right up to 148.78±31.47 μg/L as the highest among all samples tested. This might be caused by the formation of esters related to the metabolism of lipids by yeast, which provides many acids and alcohols that may undergo esterification to yield a variety of esters (S. M. Lee et al., 2006).

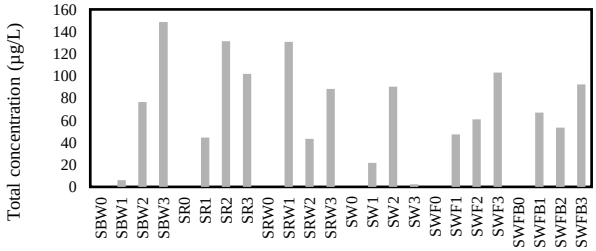

Figure 3. Total acids semi-quantification of each sample tested.

3.2.4. Esters, furan(one)s and pyrazine

Esters also played essential roles as volatile compounds in soy sauce. Esters are mainly formed from the esterification of alcohols with fatty acids during fermentation(Van Der Sluis et al., 2001). Furthermore, because most of the microorganisms found in moromi fermentation have active lipase systems that can break down triglycerides into free fatty acids and glycerol, monoglycerides, and diglycerides, fatty acids are considered to be degradation products of soybean fat and significantly contribute to the flavor of soy sauce. However, only a small number of fatty acids were found in the initial research and the presence of other fatty acids in the matching esters. In contrast, other fatty acids were absent, suggesting that most acids generated esters through an esterification reaction during moromi fermentation (X. L. Gao et al., 2010). Many eaters were present in this study; 26 esters were detected. Ethyl acetate was the compound that had much, especially in SBW1, which has between 443.08 μg/L to 738.21 μg/L.

Esters formed from the esterification of alcohols with fatty acid during moromi fermentation. Following Fig 4., SBW1 contained the highest total semi-quantification (775.84 μg/L) of esters identified. However, esters found in each sample's 1-month and 3-month age were agreeably increased so that esterification would be worked extensively and form higher esters.

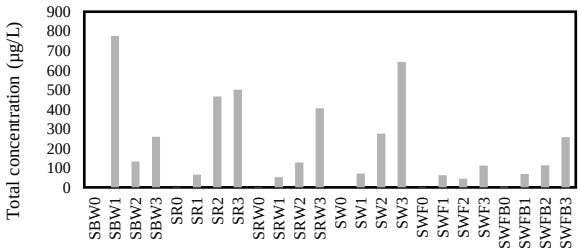

Figure 4. Total ester semi-quantification of each sample tested.

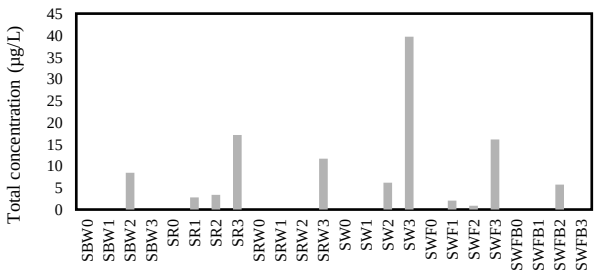

Figure 5. Total furan(one)s semi-quantification of each sample tested.

Five furans were identified in this study; 2-pentylfuran-, 2(3H)-Furanone, dihydro-3-methyl-, 3(2H)-Furanone, 4-hydroxy-5-methyl-, 2(3H)-Furanone, 5-ethyldihydro-,2(3H)-Furanone, dihydro-5-pentyl-; that found their highest concentration in SW3 (39.71±5.04 μg/L). Furans can be formed through a Maillard reaction of pentose during

heating or by a biosynthesis pathway involving yeasts (Dahlen et al., 2001). In Fig 5., the furan group was mostly found in the 3-month age of soy sauces and provided the highest total concentration in SW3; however, furans were also found during day 0; this might happen from the roasted raw materials for the Koji stage (Sun et al., 2010). In addition, furans were produced during moromi fermentation and slightly increased the following date (X. L. Gao et al., 2010); that's why in the first month of fermentation, furans were also able to observe but could not be identified as they had low similarity and peak area.

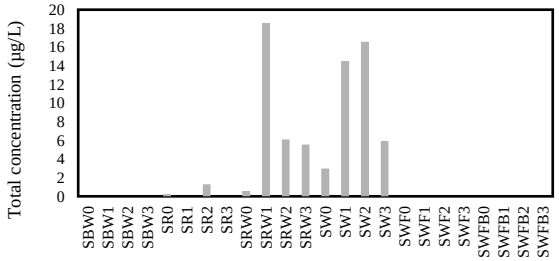

Figure 6. Total furan(one) semi-quantification of each sample tested.

The pyrazines have essential characteristics resulting from the presence of two nitrogen atoms. The Maillard reaction between saccharide and amino residues and the ambient temperature reaction of microbial metabolites can produce pyrazines (Fan et al., 2007). Many pyrazines were detected in the samples, such as methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-3-methylpyrazine,2,6-dimethylpyrazine.In these samples, only nine samples found pyrazines, such as ten samples in code SR, SRW, SRW0, and SW.

3.2.5. Aldehydes and ketones

Twelve aldehydes and five ketones were identified in the 24 soy sauces, including five and 12 aldehydes. These arise mainly from the raw materials and the fermentation procedure. The most critical aldehydes were 2-methylbutanal and 3-methylbutanal, which had the highest concentration in SBW1 (71.9±31.177 μg/L and 16.8±53.535 μg/L, respectively). The 2-methylbutanal, which gives a malty aroma, is a crucial aroma compound in Japanese and Korean soy sauces (Lee et al., 2006). Two aromatic aldehydes, benzaldehyde and benzeneacetaldehyde, were both detected. Benzaldehydes were detected and identified in almost all samples excepted, whereas benzeneacetaldehydes, were not found on day 0 of SW. Benzaldehyde was seen in SBW1 with 3197 μg/L; this amount was higher than benzaldehyde from other samples because these compounds came from black beans (Han et al., 2022). Ketone is one of the essential smells in soy sauce. During fermentation, some amino acids break down and produce ketones, and some ketones form from the oxidation of alcohol (Waterhouse et al., 2016).

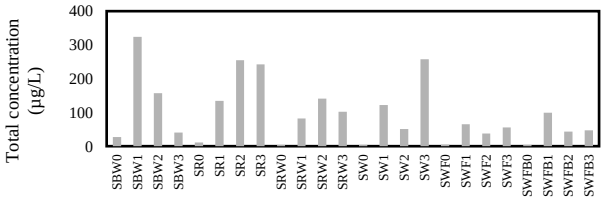

Figure 7. Total aldehydes semi-quantification of each sample test.

Five ketones are detected in samples: acetoin, 2-Heptanone, 3-Octanone, butyrolactone, and benzophenone. ketones found in SBW1 and SRW0 has the total amount 63.29±21 μg/L.1 and 51.9 ±0.71 μg/L, respectively.

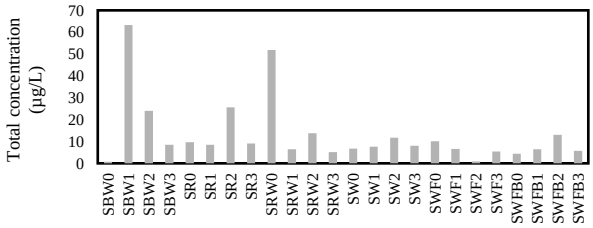

Figure 8. Ketone semi-quantification of each sample tested.

3.2.6. All volatile compounds inclusion

Volatile compounds found in the study were reported in many previous works of traditional fermented soy sauce(Gao et al., 2009). However, the amount of the identified volatile compounds in this work gradually increased from month to month as shown in Fig 7. Moreover, during the moromi fermentation of the 6 types of samples, volatile compounds were found between 11 to 17 volatile compounds during 0 days of fermentation and about 30 to around 40 volatile compounds were discovered during the 3-month age of samples.

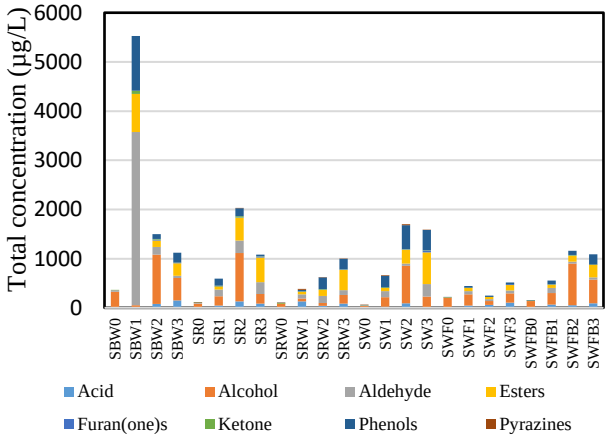

Figure 9. Total presence of volatile compounds in the 24 samples.

Furthermore, during the liquid fermentation, the mixture of soy sauces started breaking down from day to day, which is why many more compounds were presented on the following date, even though some compounds that were present during the 0-day age were degraded but produced newer compounds (which caused aroma of soy sauce more interesting). The more unique volatile compounds, such as acids and aldehydes produced by microbial mechanisms during fermentation, rose interestingly(X. L. Gao et al., 2010).

4. CONCLUSION

In conclusion, this study has demonstrated the changes in volatile compounds during moromi fermentation. Long-term moromi fermentations are necessary for aroma formation. Based on the results, it was determined that the number of volatile compounds mostly steadily rose from 0-day to month and month to month. Alcohols were increased from 0 days to 2 months, and most ester compounds were increased from 2 months to 3 months. Additionally, given that most volatile compounds were produced during the early moromi fermentation stage, further research on optimizing the Koji-culturing process is crucial and is now being done to improve the flavor of soy sauce. There were 77 volatile compounds identified from 24 soy sauces that were detected by SPME-GC-MS and classified into eight groups of compounds, including acids (4), alcohols (15), phenols (5), aldehydes (12), esters (26), ketones (5), furan(one)s (5), and pyrazine (5). Of these 8 groups, they had respectively highest concentration__ 5528.58±1308.05 µg/L, 2024.7±209.74 µg/L, 1697.49±59.63 µg/L, and 1588.4±149.49 µg/L, respectively; in SBW1,

SR2, SW2, and SW3, whereas the lowest concentration were in 0-day of SW0, SRW0, SR0, and SWFB0 with the concentration of 63.25±1.7 μg/L, 311.87±1.36 μg/L, 112.88±5.77 μg/L, 148.19±3.72 μg/L, respectively. Using different materials to make soy sauce, the volatile compound in soy sauce is also changed based on the materials it made. After three months of fermentation, SW3 was high in esters, furan(one)s, phenols, and pyrazines. SBW3 is high in acids, SR3 is high in aldehydes, and SWFB3 is high in alcohol.

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