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Comparison of Aroma Compounds in Traditional Fermented and Inoculated Douchies, A Chinese Traditional Fermented Soybean Food

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Douchi is a Chinese traditional fermented food of soybean. In this study, the cooked soybean fermented by Multi-strains (*Aspergillus oryzae* and *Aspergillus niger*), traditional method and pure *Aspergillus oryzae* were analyzed and compared for volatile compound by GC-MS, using the simultaneous distillation and solvent extraction (SDE) combined technique, a total of 152 volatile compounds including 30 hydrocarbons, 21 alcohols, 8 aldehydes, 33 esters, 7 ketones, 6 phenols, 18 acids, 20 heterocyclic compounds, 3 sulfo compounds and 6 other compounds were identified. Results showed that the Multi-strains fermented samples had higher contents of volatile compounds than the pure *Aspergillus oryzae* inoculated samples. The predominant volatile compounds in *Aspergillus oryzae* and *Aspergillus niger* fermented soybeans included alcohols, acids, esters and pyrazines, and the major volatile compounds in the pure *Aspergillus oryzae* fermented soybean included hydrocarbons, aldehydes, acids and alcohols. Multi-strains fermented douchi has the most volatile compounds of the naturally fermented douchi.

Key words: Douchi; fermentation; volatile compounds.

Douchi is a Chinese traditional soybean product, particularly in the southern part of China. It has been generally used as seasoning for food. Like other soybean products such as natto, tempe and chungkuk-jang, douchi has been appreciated by consumers as healthy food due to its nutritional attributes and unique flavor and taste[1,2]. The traditional preparation method of douchi is based on the multi-strains fermentation using natural micro-ûora, including *Saccharomyces*, *Aspergillus oryzae* and so on. Recently, increasing attention has been drawn to the pure strain fermentation method, which requires the inoculation of different microbes on the soybeans. Generally, there are four types of douchi that are produced by pure strain fermentation using *Mucor, Rhizopus*, *Bacteria* and *Aspergillus*. Among them, *Aspergillus*-fermented douchi is the most popular one, the production of which can be traced back at least 2000 years ago.

Douchi produced by natural fermentation has the characteristic flavor, palatable soft texture, and a bright black color. It usually takes as long as one year for the fermentation process to produce douchi using traditional method. Compared with naturally fermented douchi, douchi produced from pure strain inoculation method requires a much shorter production cycle, which is only about 30 days. However; the quality may not as good as naturally fermented douchi. Usually, naturally fermented douchi has particular aroma, due to the

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presence of many volatile compounds generated during the long-term fermentation process. In this process, numerous enzymatic and nonenzymatic reactions occur, such as protein degradation, Strecker degration and Maillard reactions. These reactions generated various volatile compounds, such as aldehydes, acids, alcohols, ketones, esters, as well as sulphur and many other compounds.

The volatile compounds in various fermented soybean products have been reported previously. For instance, it was found that the predominant volatile compounds in pure *Bacillus*-fermented included 2,5-dimethylpyrazine, 2-methylbutanoic acid, 2,3,5-trimethylpyrazine, 2-methylpropanoic acid and acetic acid, while the major volatile compounds in the naturally fermented soybean included 2,5-dimethylpyrazine, benzaldehyde, 5-methyl-3-hexanone, 2-butanone and 3-methyl-2-pentanone[3]. Zhao[4] identiûed several aroma active compounds for naturally fermented Chinese soybean paste, including 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone, ethyl linoleate, 2,3-butanediol, acetic acid, fufural, benzene acetaldehyde and pyrazine 2,6-dimethyl. It was detected different major compounds in pure-starter culture natto, which were 2,5-dimethylpyrazine, 2-methylbutanoic acid, acetone, 2,3,5-trimethylpyrazine and 2,3-butanedione[5,6]. Studies indicate that the highest contents of pyrazines in African soumbala, fermented by pure-starter *B. subtilis*, gave signicantly preferable scores to naturally-fermented products, having greater aldehyde contents[7]. The correlation between volatile compounds and sensory attributes in Korean doenjang has also been evaluated by Lee and Ahn[8] who found that high furfuryl alcohol and maltol were associated with a sweet-grain attribute. The major classes of volatile compound in three commercial douchies have been reported as esters (29), acids (18), alcohols (16), pyrazines (14), ketones (13), aldehydes (12), phenols (6), hydrocarbons (5), furans(5), sulphur-containing compound (5), pyridines (4), pyrimidines (2), and miscellaneous compound (2)[9].

Although numerous reports have suggested the antioxidant and anti-hypertensive activities of douchi, the reports about profiles of volatile compounds of douchi is scarce. For the sake of improving the aroma of douchi, the aroma active compounds in traditionally-fermented douchi need to be identiûed the modiûcation of production process. The objective of this study was to compare the volatile compounds in traditionally-fermented douchi and the one fermented by Multi-strains (*Aspergillus oryzae* and *Aspergillus niger*). The volatile compounds of douchies were collect by using a simultaneous steam distillation and extraction apparatus (SDE), and the extracts were then analyzed by gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Strains and culture condition

Aspergillus oryzae 3.042 and *Aspergillus niger* 3.350 were bought from China Center of Industrial Culture Collection, This strains were routinely cultured on nutrient agar and maintained at “20°C, isolated in 20% glycerol. For preparation of the inoculum, the strains cultivate in czapek’s medium at 37°C for 72 h. The cells were subsequently harvested, suspended with sterile distilled water and carefully adjusted to achieve a concentration of 10^6 CFU/mL. The suspension was used as the inocula for soybean fermentation.

Preparation of douchi

Protein-rich black soybeans, supplied by local manufacturing company (Nanchang, Jiangxi, China), were used as raw material of Douchi Soybeans were washed and soaked in tap water for 1.5h at 35°C. Boiling for 2h, cooled to 30-35°C, and inoculated immediately with 10^6 CFU (per gram of sterilized soybeans) of *Aspergillus oryzae* 3.042 starter culture. Subsequently, the inoculated soybeans were incubated at 30°C for 72h under 80% relative humidity in an incubator. In contrast, for Multi-strains fermentation, cooked and cooled soybeans were inoculated with 10^6 CFU(per gram of sterilized soybeans) of Multi-strains (*Aspergillus oryzae* 3.042: *Aspergillus niger* 3.350 of 1:1), incubated at 30°C for 48h, under 80% relative humidity in an incubator. Semi-ûnished products were called the douchi qu (koji). The douchi qu were salted as far as the content of NaCl reached about 8% (w/w). The sample was ripened for four weeks at 45-50°C in the same incubator. The sufficient fermented soybeans were pulverized and frozen at “20°C for further research.

Collection of volatile component

A Likens–Nickerson type SDE apparatus (model 523010-000, Anhui, China) was used to collect the volatile compounds. For the analysis, sample (100 g each) was dissolve by 200 mL of distilled water and the aliquot was loaded in a 500 mL flask. Five millilitres of internal standard (IS) [2-methyl-1-pentanol (10 µg/mL in methanol)] was added to the sample before extraction. Each sample was extracted with 100 mL of redistilled dichloromethane and carried out for 4 h till the distilled water started to boil. The extraction was dried over Na_2SO_4 overnight and concentrated to 2 mL using a rota vapor (Büchi, Switzerland) equipped with a Büchi 461 water bath. The temperature of the water bath was 35–36°C and the reducing pressure was 100"150 mm Hg. The solvent was further removed under a purified nitrogen stream to 1 mL ultimately. The concentrated extraction was stored at "20°C for further analysis.

Gas chromatography–mass spectrometry (GC–MS) analyses

GC–MS analyses were performed on an Agilent Technologies (Paolo Alto, CA, USA) gas chromatograph model 6890A with a mass selective detector model 7673. GC operating conditions were: column HP-5MS (Agilent J & WGC column), 30 m × 0.25 mm i.d., ûlm thickness 0.25 µm, the column temperature programmed from 70°C isothermal for 2 min, then increased to 200°C at a rate of 3°C min^{-1} and held isothermal for 15 min. Carrier gas was heliumat ûow rate 1 mL min^{-1}. Injector temperature was 250°C. Volume injected was 1 µL and split ratio was 1:50. Mass spectrometry conditions were: ionisation voltage 70eV, ion source temperature 280°C, mass scan range 30-450 mass units.

Identification and quantification of volatile compounds

The GC-MS was calibrated daily by running 0.1 µL of a 100 ppm standard mixture of C_5–C_{25} n-alkanes. Qualities of volatile compounds were identiûed by comparing linear retention indices (LRI) with those standard compounds and mass spectra of compounds by comparison with the bibliographic data of known compounds from the mass spectral database. The quantities of each compound were determined by comparison of the area to the integrated of peaks of the total ion chromatogram and count by comparing peak area with that of the 1,2-dichlorobenzene internal standard.

Statistical analysis

All data were subjected to analysis of variance (ANOVA), and Duncan's multiple range test (DMRT) was used to compare significant difference of means at $P \leq 0.05$. Most experiments were performed in triplicate.

RESULTS AND DISCUSSIONS

Profiles of the volatile compounds in three different douchies (soybean fermented by pure *Aspergillus oryzae*, soybeans fermented by traditional method and soybean fermented by Multi-strains) are shown in Fig. 1 and Table 1.

Similar volatile compounds were identified in the chromatograms of the traditional fermented douchi and Multi-strains fermented douchi and traditional fermented Chinese soybean pastes[10,11]. However, the difference in the peak intensity of the individual volatiles was found in present study (Fig. 1),.The number of peaks of the pure *Aspergillus oryzae* fermented douchi was less than the other two. The identified volatile compounds were shown in Table 1. Variation in the volatile compounds was not only appeared in the inoculated and traditional fermented samples, but also within the natural fermented samples. The douchi inoculated pure *Aspergillus oryzae* had the least amounts of volatile compounds, whereas the traditionally-fermented samples had the most abundant volatile. A total of 152 compound were identified from the three samples, including 30 hydrocarbons, 8 aldehydes, 21 alcohols, 33 esters, 7 ketones, 18 acids, 6 phenols, 20 heterocyclic compound, 3 sulfo compound and 6 other compound. Esters, alcohols and acids were the major, which accounted for approximately 50% of the total volatile compounds. However, only 22 compounds out of 152 were identified in all the samples, which may reveal the better process and quality control of the douchi were needed.

Hydrocarbons

As shown in table 1, a total of 30 hydrocarbons were identified from three different samples. The pure *Aspergillus oryzae* fermented and Multi-strains fermented douchi contained 13 hydrocarbons while traditionally-fermented douchi contained 25 hydrocarbons, including a large

Table 1. GC-MS analysis of volatile compound of three different type douchies

Components	RT[a]	CASRegistry number[b]	MW[c]	Content (μg/g)[d]		
				A[e]	B[f]	C[g]
Hydrocarbons (30)				8.47	3.66	4.21
Benzene,1,2,3-trimethyl-	4.41	000526-73-8	120	1.51	0.58	0.37
Benzene,1-ethyl-3-methyl-	4.65	000620-14-4	120	nd[h]	0.2	nd
Allylidenecyclohexane	9.04	005664-10-8	122	nd	0.05	nd
Tetradecane	9.90	000629-59-4	198	0.11	nd	0.49
Heptadecane	10.12	000629-78-7	240	0.23	0.1	nd
Dodecane, 2,6,11-trimethyl-	10.69	031295-56-4	212	nd	0.07	0.72
Hexadecane	10.78	000544-76-3	226	nd	0.06	
1-Methyl-4-isopropylbenzene	11.79	000099-87-6	134	nd	nd	0.07
Dodecane	11.81	000112-40-3	170	nd	0.03	nd
Cyclohexane, 2-propenyl-	11.86	002114-42-3	124	nd	0.02	0.29
Tetratetracontane	12.20	007098-22-8	619	2.06	nd	nd
Pentadecane	12.84	000629-62-9	212	nd	0.27	
Pentadecane, 2,6,10-trimethyl-	12.94	003892-00-0	254	nd	0.12	0.23
Hexatriacontane	12.99	000630-06-8	507	0.1	0.04	nd
Eicosane, 9-cyclohexyl-	13.09	004443-61-2	364	0.14	0.07	nd
Tridecane, 7-hexyl-	13.58	007225-66-3	280	nd	0.16	0.23
Octadecane, 2-methyl-	14.09	001560-88-9	268	nd	nd	0.06
Cyclotetradecane	14.21	000295-17-0	196	1.36	0.08	nd
2-Tetradecene, (E)-	14,29	035953-53-8	196	nd	0.05	nd
7-Hexadecene, (Z)-	14.36	035507-09-6	224	1.14	0.04	nd
3-Hexadecene, (Z)-	14.40	034303-81-6	224	1.5	0.04	nd
Octadecane	14.61	000593-45-3	254	0.08	0.40	nd
Hexadecane, 2,6,10,14-tetramethyl-	14.70	000638-36-8	282	nd	0.17	0.83
1-Heptadecene	15.06	006765-39-5	238	nd	0.05	nd
Tricosane	19.39	000638-67-5	324	0.16	0.31	nd
Cycloeicosane	20.37	000296-56-0	280	nd	0.23	0.25
Eicosane	23.02	000112-95-8	282	nd	nd	0.63
Hexacosane	24.68	000630-01-3	366	0.08	0.05	nd
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all- E)-	28.07	000111-02-4	410	nd	0.11	0.06
Squalene	28.40	007683-64-9	410	nd	0.13	0.08
Alcohols(21)				10.17	19.97	19.82
3-Hexanol, 2,3-dimethyl-	3.74	004166-46-5	130	nd	3.12	4.56
3-Pentanol, 3-ethyl-	3.75	000597-49-9	116	0.64	3.9	3.21
9-Oxabicyclo[3.3.1]nonan-2-ol	9.04	133521-31-0	142	nd	0.05	nd
2-Heptanol, 6-methyl-	9.75	004730-22-7	130	0.21	nd	1.13
1-Eicosanol	10.57	000629-96-9	298	nd	0.02	1.5
1-Nonadecanol	10.63	001454-84-8	284	nd	0.03	nd
2-Benzylidenecyclohexanol	10.89	034492-42-7	188	nd	nd	0.14
Benzeneethanol	10.91	60-12-8	122	0.12	0.09	nd
Cyclopropanol,2,2-dimethyl-3-(2-phenylethynyl)-	11.22	1000271-82-1	186	nd	nd	1.84
tert-Hexadecanethiol	11.74	025360-09-2	258	nd	0.05	2.37
1-Decanol, 2-hexyl-	11.78	002425-77-6	242	nd	0.53	0.18
1-Tetracosanol	11.86	000506-51-4	354	nd	0.67	nd
1-Hexadecanol, 2-methyl-	13.09	002490-48-4	256	0.87	nd	0.84
Cyclododecanol, 1-ethenyl-	14.15	006244-49-1	210	nd	4.56	0.68
E-2-Hexadecacen-1-ol	14.18	1000131-10-1	240	nd	0.02	0.21
Ethanol, 2-(tetradecyloxy)-	14.21	002136-70-1	258	0.16	0.18	nd

2-Ethyl-1-dodecanol	15.73	019780-33-7	214	nd	4.39	2.26
Phytol	16.17	000150-86-7	296	0.5	0.96	0.23
Ethanol, 2-(octadecyloxy)-	16.64	002136-72-3	314	0.15	0.82	0.39
1-Hexadecanol, 3,7,11,15-tetramethyl-	18.18	000645-72-7	298	7.52	nd	0.28
(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	18.92	030689-78-2	254	nd	0.58	nd
Aldehydes (8)			23.71	10.7	13.59	
Benzaldehyde	3.65	000100-52-7	106	nd	2.65	1.59
Benzeneacetaldehyde	4.98	000122-78-1	120	21.3	3.50	12.8
Benzeneacetaldehyde, .alpha.-ethylidene-	8.44	004411-89-6	141	0.9	nd	nd
1H-Indene-4-carboxaldehyde, 2, 3-dihydro-	8.44	051932-70-8	146	nd	0.2	nd
Benzeneacetaldehyde, .alpha.-(2-methylpropylidene)-	9.73	026643-91-4	174	1.02	0.07	0.13
2-Furanacetaldehyde, .alpha.-isopropylidene-	9.80	031681-28-4	150	nd	0.58	nd
5-Methyl-2-phenyl-2-hexenal	11.23	021834-92-4	188	0.43	1.16	0.85
Tetradecanal	20.20	000124-25-4	212	0.06	1.54	0.22
Ketones (7)				1.92	2.52	2.97
7-Oxabicyclo[4.1.0]heptan-2-one, 4,4,6-trimethyl-	6.94	010276-21-8	154	nd	nd	1.35
Cyclohexanone, 3,3,5,5-tetramethyl	7.12	014376-79-5	154	0.3	nd	1.2
Ethanone, 1-(2-hydroxy-5-methylphenyl)-	9.34	001450-72-2	150	0.19	0.85	nd
4-Hydroxy-3-methylacetophenone	9.52	000876-02-8	150	0.81	0.64	nd
2-Benzylcyclohexanone	10.91	000946-33-8	188	0.62	nd	0.36
2-Cyclopenten-1-one,4-hydroxy-2-m ethyl-3-phenyl-	11.23	069745-73-9		nd	0.86	nd
2-Piperidinone,1-(4-bromobutyl)-	13.19	195194-80-0	234	nd	0.17	0.06
30.05	27.8					
Pentanoic acid, 3-methyl-	3.17	000105-43-1	116	5.74	2.34	6.36
3-Methyl-2-furoic acid	3.71	004412-96-8	126	nd	nd	0.2
2,3,4-Trimethylpentanoic acid	3.74	090435-18-0	144	4.63	3.62	2.24
2-Butenoic acid, 3-methyl-	3.90	000541-47-9	100	1.57	0.03	nd
5-Benzoylpentanoic acid	4.14	097547-93-8	206	nd	1.2	nd
Hexanoic acid	4.79	000142-62-1	116	nd	0.59	2.33
Butanoic acid, 3-methyl-	4.96	000503-74-2	102	nd	0.27	1.25
Pentadecanoic acid	15.36	001002-84-2	242	1.58	1.04	nd
Hexadecanoic acid, 2-methyl-	15.93	027147-71-3	270	0.32	nd	0.22
n-Hexadecanoic acid	16.76	000057-10-3	256	14.58	4.68	7.84
Tetradecanoic acid	16.82	000544-63-8	228	3.54	2.51	0.46
Tridecanoic acid	16.88	000638-53-9	214	nd	4.89	2.13
n-Decanoic acid	16.96	000334-48-5	172	1.14	1.97	nd
9,12-Octadecadienoic acid (Z,Z)-	18.20	000060-33-3	280	6.44	5.44	3.3
1-Heneicosyl formate	18.22	077899-03-7	340	2.65	nd	0.32
9-Undecenoic acid, 2,6,10-trimethyl-	18.57	1000131-86-2	226	nd	nd	0.18
Nonahexacontanoic acid	19.73	040710-32-5	999	nd	nd	0.29
22-Tricosenoic acid	19.78	065119-95-1	352	nd	1.47	nd
Ester(33)			6.42	12.07	17.71	
o-Acetyl-L-serine	3.17	5147-00-2	147	nd	nd	0.19
Pentanoic acid,2-hydroxy-4-methyl-, methyl ester	3.21	40348-72-9	146	nd	0.64	nd
Heptanoic acid, methyl ester	3.86	000106-73-0	144	0.63	nd	0.96
Butanoic acid, 3-methyl-, 1-methyl	3.90	032665-23-9	144	nd	nd	0.11
Acetic acid, 2-phenylethyl ester	8.13	000103-45-7	164	0.38	0.93	0.24
Bromoacetic acid, 2-phenylethyl						

ester	8.21	003785-33-9	243	nd	nd	0.32
.beta.-Phenylethyl butyrate	8.28	000103-52-6	192	nd	1.68	0.36
Methoxyacetic acid, 2-tetradecyl ester	10.69	1000282-04-8	286	nd	0.46	0.18
Hexanoic acid, 2-ethylhexyl ester	11.44	016397-75-4	228	nd	0.59	0.31
Hexanoic acid, 3-tetradecyl ester	11.58	1000279-29-7	312	nd	nd	0.02
Pentadecyl heptafluorobutanoate	11.86	1000216-79-3	424	nd	nd	0.03
2-Tridecanyl methoxyacetate	14.70	1000282-04-5	272	nd	nd	0.14
Didodecyl phthalate	15.43	002432-90-8	278	nd	0.06	0.79
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	15.43	000084-69-5	502	nd	0.77	0.61
1,2-Benzenedicarboxylic acid, dipropyl ester	15.50	000131-16-8	250	nd	0.26	0.80
Hexadecanoic acid, methyl ester	15.93	000112-39-0	270	0.28	0.31	1.8
Pentadecanoic acid, 14-methyl-, methyl ester	16.13	005129-60-2	270	nd	0.5	0.30
1,2-Benzenedicarboxylicacid, 1-butyl 2-(8-methylnonyl) ester	16.39	000089-18-9	362	nd	0.53	Nd
Dibutyl phthalate	16.40	000084-74-2	278	nd	0.2	1.72
1,2-Benzenedicarboxylic acid, bis(1-methylethyl) ester	16.58	000605-45-8	284	0.56	0.96	1.28
9,12-Octadecadienoicacid, methyl ester	17.60	002462-85-3	294	nd	nd	0.43
9,12-Octadecadienoicacid, methyl ester, (9E,12E)-	17.60	002566-97-4	294	nd	nd	0.5
10,13-Octadecadienoic acid methyl ester	17.60	056554-62-2	294	nd	nd	0.43
Linoleic acid ethyl ester	18.20	000544-35-4	308	1.62	0.84	1.83
2- Chloropropionic acid, octadecyl ester	18.24	088104-31-8	361	nd	nd	0.36
Ethyl Oleate	18.24	000111-62-6	310	2.6	0.63	1.36
Heptadecanoic acid, heptadecyl ester	18.92	036617-50-2	508	nd	0.3	0.58
2-Propenoic acid, 3-(4-methoxy phenyl)-, 2-ethylhexyl ester	19.78	005466-77-3	290	0.08	0.28	0.8
1-Cyclopentene-1-decanoic acid, .et a .-hydroxy-3-oxo-2-pentyl-, methyl ester	19.88	055521-10-3	352	nd	0.19	nd
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	22.32	004376-20-9	278	0.15	0.45	nd
1,2-Benzenedicarboxylic acid, ditridecyl ester	22.32	000119-06-2	530	nd	0.46	0.65
Di-n-octyl phthalate	22.35	000117-84-0	390	0.12	0.45	0.29
1,2-Benzenedicarboxylic acid, diisooctyl ester	22.35	027554-26-3	390	nd	0.58	0.32
heterocyclic compounds(20)			6.57	14.05	10.35	
2-Methylpyrazine	4.68	109-08-0	94	2.08	1.62	nd
2,5-Dimethyl pyrazine	5.41	123-32-0	108	nd	1.18	3.54
3a,6-Methano-3aH-indene, 2,3,6, 7-tetrahydro	6.49	98640-29-0	132	nd	0.07	nd
1H-Indene, 2,3-dihydro-5-methyl-	7.05	000874-35-1	132	nd	0.07	nd
Azulene	7.18	000275-51-4	128	nd	0.27	0.25
Naphthalene	7.23	000091-20-3	128	nd	0.28	nd
1H-Indene, 1-methylene-	7.30	002471-84-3	128	nd	0.09	nd

number of alkanes and alkenes. This may be explained by the fact that during traditional fermentation process, some spices were usually added, such as ginger, pepper, garlic, which presumably consisted of many hydrocarbon compounds. Squalene was detected in traditionally-fermented and Multi-strains fermented douchies. Squalene, known as the active ingredients of plant oil, has a unique aroma, good oxygen ability, thus anti-hypoxia and anti-fatigue, and enhance human immunity and enhance the function of the gastrointestinal tract[12].

Alcohols

Alcohol is the third largest class containing 21 different compounds. The 3-pentanol, 3-ethyl-, phytol and ethanol, 2-(octadecyloxy)- were detected in all three douchies. Alcohols provide pleasant aromas and sweet flavors[13,14]. Previous research has shown that the quality of miso due to the alcohol contents[15,16]. The pure *Aspergillus oryzae* fermented douchi contained much less alcohols than the tranditionally-fermented and Multi-strains fermented douche. This was possibly because during the fermentation of the pure *Aspergillus oryzae* douchi, the temperature was high, the salt tolerant yeast could not grow resulting in the production of less alcohols. By contrast, traditionally-fermented douchi due to long period of fermentation, temperature was low, the salt tolerant yeast had sufficient time to complete alcohols and ester reaction. TheMulti-strains fermented douchi exhibited similar profile of alcohols to the traditionally-fermented douche, probably due to the addition of yeast during post-fermentation as well as the lower fermentation temperature.

Aldehydes

A total of 8 aldehydes were identified from three different samples. Benzeneacetaldehyde was the most abundant compound in the pure *Aspergillus oryzae* fermented douchi, Some aldehydes found in douchi extraction have been reported in other soy products. Benzaldehyde identified in this work was reported in sufu[17,18], vmiso[19,20], natto[21], and soy sauce[22]. Phenylacetaldehyde found in douchi extracts was previously reported in sufu, miso, soy sauce, and the unflavoured textures soy protein[23]. 5-Methyl-2-phenyl-2-hexenal was reported in sufu, the favorable odor of aldehyde compound such as benzaldehyde (cherry or almond-like odor), benzeneacetaldehyde (rosy-like odor) and 2-phenyl-2-butenal (floral, prune-like odor) can be considered to enhance the flavor of douchi. These aldehydes can be produced by lipid oxidation and degradation during fermenting.

Ketones

In the present study, a total of 7 ketones were detected from three different douchies, i.e. we identified 7-oxabicyclo[4.1.0], heptan-2-one and 4,4,6-trimethyl only in multi-strains fermented douchi, 2-Cyclopenten-1-one,4-hydroxy-2-methyl-3-phenyl- was only identified in traditional fermented douchi, besides we identified ethanone, 1-(2-hydroxy-5-methylphenyl)- and 4-hydroxy-3-methylacetophenone both in pure *Aspergillus oryzae* fermented and traditional fermented douchi, 3,3,5,5-tetramethyl cyclohexanone and 2-benzylcyclohexanone were both found in pure *Aspergillus oryzae* fermented and multi-strains fermented douchi, 2-Piperidinone,1-(4-bromobutyl)- was found in traditional fermented and multi-strains fermented douchi. Ketones can be formed by fungal enzymatic actions on lipids and/or amino acids, or by the Maillard reaction[24]. Some ketones such as 4-hydroxy-3-methylacetophenone 2-benzylcyclohexanone were found in douchi have also been reported in soy sauces[25].

Acids

Acids containing 18 compounds were the most abundant class in three douchies. 3-methyl-pentanoic acid, 2,3,4-trimethylpentanoic acid, n-Hexadecanoic acid, tetradecanoic acid and 9,12-octadecadienoic acid (Z,Z)- were detected in all three samples. 3-methyl-2-furoic acid, 2,6,10-trimethyl-9-undecenoic acid and nonahexacontanoic acid were only found in Multi-strains fermented samples, 5-benzoylpentanoic acid and 22-tricosenoic acid were only found in traditional fermented samples, 3-methyl-2-butenoic acid, pentadecanoic acid and n-decanoic acid were detected in pure *Aspergillus oryzae* and traditionally-fermented samples. Hexanoic acidÿ3-methyl-butanoic acid and tridecanoic acid were detected in traditional and multi-strains fermented samples. Hexanoic acid was derived from the oxidation of hexanal, has been described as having a “sweat-like” odor[26] as well as possessing a cheesy, fatty, sweaty, sour, rancid and pungent-like odor(11). In general, these acids are described as having cheesy odours, including butanoic acid (cheesy, sharp, rancid, sweaty, sour), 2/3-methylbutanoic acid (cheese, rancid, sweaty) and hexanoic acid (cheesy, fatty, sweaty, sour, rancid, pungent)[27], and pentanoic acid (sweaty, rancid) mainly contribute oily odours. 2-methylpropanoic acid and 3-methylbutanoic acid were derived from valine and leucine degradation[28].

Esters

In the present study, a total of 33 esters were detected from three different douchies. Most esters have pleasant aromas and enhance douchi flavour quality[29]. Acetic acid 2-phenylethyl ester, hexadecanoic acid methyl ester, 1,2-Benzenedicarboxylic acid bis(1-methylethyl) ester, linoleic acid ethyl ester, ethyl oleate, 3-(4-methoxyphenyl)-2-propenoic acid 2-ethylhexyl ester and di-n-octyl phthalate were detected in all three samples. Hexadecanoic acid methyl ester, 1,2-benzenedicarboxylic acid bis(1-methylethyl) ester, linoleic acid ethyl ester and ethyl oleate were the major compounds detected. Most of the detected esters were previously found in various fermented soybean foods[30,31,32]. A number of high molecular weight fatty acid esters such as pentadecyl heptafluorobutanoate, 1,2-benzenedicarboxylic acid ditridecyl ester and ethyl hexadecanoate were detected, which have also been found in Chinese sufu, miso, and other Korean fermented soybean pastes. These high molecular weight esters were

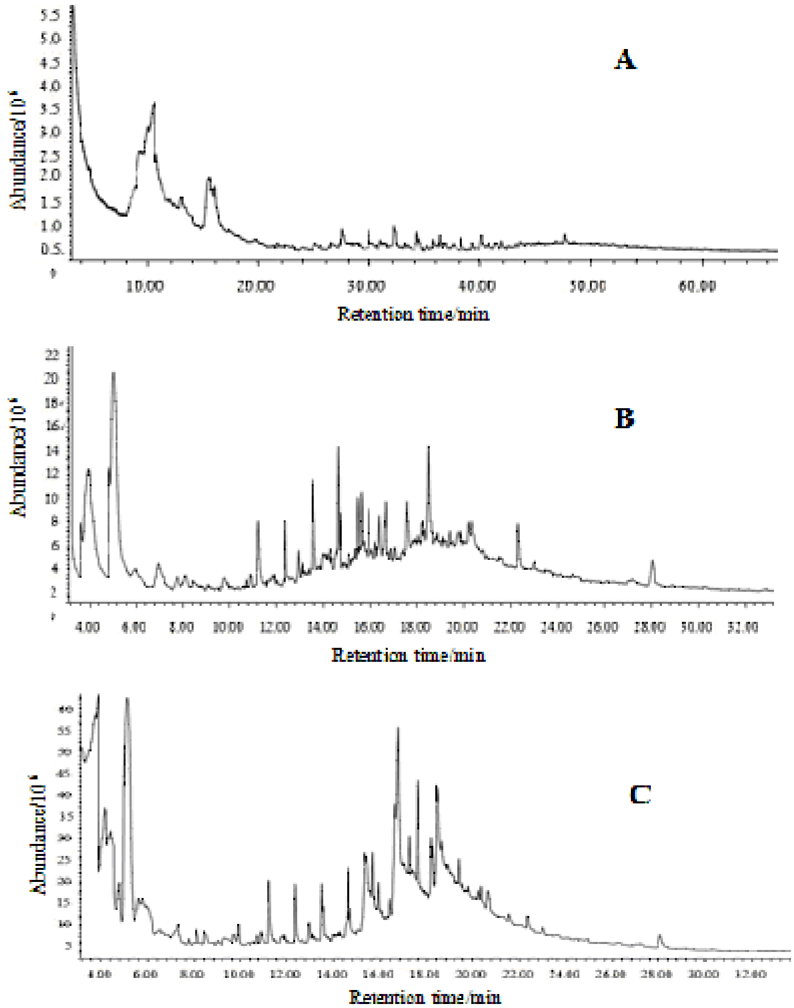

Fig. 1. Representative chromatograms of GC-MS analysis of pure *Aspergillus oryzae* fermented (A), traditionally-fermented (B) and Multi-strainsfermented douchi (C)

probably produced by the action of fungal lipase on soybean lipids[33]. As shown in Table 1, the esters concentration of the pure *Aspergillus oryzae* fermented douchi was lower than traditionally-fermented and Multi-strains fermented douchi samples, only 6.42 μg /g dry weight. The Multi-strains fermented douchi had the highest concentration of esters, i.e. 17.71 μg/g dry weight, This may be attributed to the improved douchi production process and addition of a certain amount of yeast during post-fermentation.

Heterocyclic compounds

A total of 20 compounds were detected in three different douchies, including pyrazines, indenes, furans, pyrrole and other heterocyclic compounds. Pyrazines was the main compound and studies have indicated it was important in fermented soybean products[34,35,36], which could be generated naturally during the aging process, by the condensation of aminoketones formed through the Maillard reaction and Strecker degradation. The methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine and 2-ethyl-6-methylpyrazin have previously been detected in natto and liquid cultures of Bacillus subtilis and sufu, which were also detected in douchi in the present study. Pyrazines have a nutty aroma, especially alkyl pyrazines. 2,5-dimethyl pyrazine, trimethyl pyrazine and 2-ethyl-6-methyl pyrazine contribute to natto odour. Tetramethyl pyrazine has a sweet, chocolate, cocoa, but musty, lard and burnt note. 3-phenyl-furan was detected in traditionally-fermented and Multi-strains fermented douchies, and this compound was previously found in soybean processed products such as sufu, miso, soysauce, and natto. 1-pentyl-1h-pyrrole was detected in pure A*spergillus oryzae* and traditionally-fermented douchies , which has a nutty, sweet and ethereal ordour and may be one of the products of Maillard reaction.

Phenols

Six phenols were identified in three different douchies. 4-methylphenol and mequinol were found in all three samples, and 2-methoxy-phenol and 2-(1,1-dimethylethyl)-phenol were detected in traditionally and Multi-strains fermented douchi. 2-methoxy-4-vinylphenol was detected in pure *Aspergillus oryzae* and Multi-strains fermented douchi. 2-naphthalenol was only detected in traditionally-fermented douchi, most of the above-mentioned phenols were also found in sufu, miso, and soy sauce. Phenols were characterised in cooked soybean with smokey and phenolic odours, and they were considered to be the thermal degradation products of lignin-related phenolic carboxylic acids[37].

Other Compound

A total of 9 compounds were detected in three different douchies, including 3 sulfur-containing compounds. These compounds had a significant contribution to the aroma in foods (Fors, 1983). 5,6-Dihydro-2h-thiopyran and di-tert-dodecyl disulfide were detected in traditionally and multi-strains fermented douchi. Dimethyl trisulfide was detected in pure *Aspergillus oryzae* and traditional fermented douchies. It is one of the major flavour components in natto. 9-octadecenamide and 13-docosenamide were both found in traditional fermented douchi, which were also detected in oatmeal[38].

From the results obtained, the concentrations of volatile compounds were various along with different douchi samples. The situation were feasibly due to the variety of microorganisms and some variations in the proprietary processing steps which used by each manufacturer. Pyrazines were identified in soybean processed products such as sufu, miso, soy sauce, and natto previously, this class of compound together with other common compounds probably contribute more to the characteristic background flavour of all samples investigated. Less predominant groups including esters, acids, alcohols, and aldehydes might play an indispensable role in the flavour of douchies.

The comparison of the results showed that the pure *Aspergillus oryzae* fermented sample had higher hydrocarbons, aldehydes and acids than traditionally-fermented douchi, while these compounds are not the important proups. Therefore the odor of pure *Aspergillus oryzae* fermented sample is not as good as the traditionally-fermented sample. On the other hand, Multi-strains fermented douchi has higher alcohols, esters and pyrazines than the pure *Aspergillus oryzae* fermented sample. But these compounds are the aroma active compounds, so the odor of multi-strains fermented douchi is better than the pure *Aspergillus oryzae* fermented sample. Further research is required on the development of aroma active compounds during the fermentation. The influence of raw materials, microbial community and fermentation conditions on the quality of the products are deserved to discuss.

CONCLUSIONS

In three different types douchi samples, a total of 152 combined volatile compounds were identified. Ten classes of compounds including hydrocarbons, alcohols, aldehydes, ketones, acids, esters, pyrazines, sulfur-containing compound, phenols, and mis-cellaneous compound were detected. The traditional fermented samples had higher contents and classes than the *Aspergillus oryzae* fermented and multi-strains fermented samples. In order to improve the odor of *Aspergillus oryzae* fermented douchi, further research is required on the development of aroma active compound during the fermentation of douchi and optimize the raw materials, microbial community and fermentation conditions worth more attention.

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