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Full Length Research Paper

Volatile compounds of vegetarian soybean kapi, a fermented Thai food condiment

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Kapi is a traditional shrimp paste used as a food condiment in Thailand. Several vegetarian soybean kapi, S1-S5, were fermented from various bacterial starter cultures isolated from commercial shrimp paste. The volatile compounds of S1-S5 were analyzed using SPME coupled with gas chromatography/mass spectrometry and compared to three samples of commercial vegetarian kapi (J1-J3) and commercial shrimp pastes (K1-K3). 124 volatile compounds consisting of aldehydes, alcohols, ketones, acids and esters, N-containing compounds, aromatic compounds, S-containing compounds, miscellaneous, indoles and hydrocarbons were identified. Principle component analysis and cluster analysis separated the volatile profile of the fermented samples into four groups. Vegetarian soybean kapi, S1, S4 and S5 produced from *Bacillus subtilis* IS4, TISTR10 and TISTR1, respectively, were classified into the following groups containing commercial kapi (J1, J2, K2 and K3), that had a predominance of indole, S-containing and N-containing compounds. Sensory evaluation of S1 showed a strong kapi odor with higher scores among the vegetarian soybean kapi and there were no significant differences in evaluation scores between S1 and commercial vegetarian kapi J1-J3. These data demonstrate that *B. subtilis* IS4 can be employed as a starter culture to produce an acceptable vegetarian soybean kapi substitute for shrimp paste kapi.

Key words: Vegetarian kapi, volatile compounds, fermentation, principal component analysis, cluster analysis.

INTRODUCTION

Kapi is a traditional Thai fermented shrimp paste that has a paste-like consistency, dark brown color and strong odor. It is a widely used as food condiment and a salt replacement because of its high (nearly 18%) salt content (Allagheny et al., 1996). Fermented shrimp paste products are commonly consumed in Asia and are known as bagoong (Philippines), shiokara (Japan) and terasi (Indonesia) (Mizutani et al., 1992; Montaño et al., 2001; Kobayashi et al., 2003). The traditional production of Thai shrimp paste (kapi) involves the washing of small sized shrimp, then mixing them with salt, liquid removal and the sun-drying of the shrimp for a day before grinding into paste and ferment in a jar for at least 4 months at an ambient temperature. Moreover, there has been an attempt to use a starter culture consisting of *Staphylococcus*, *Micrococcus* and *Bacillus* for shrimp paste kapi production and it was found that the batches inoculated with *Staphylococcus aureous*, *Staphylococcus epidermidis* and *Micrococcus morrhuae* resulted in a better odor, while *Bacillus* sp. worked well at the beginning of the process but produced a putrid odor in the later stages of fermentation (Chotwanawirach, 1980).

Most vegetarian condiment products are derived from soybeans, such as thua-nao in Thailand (Visessanguan et al., 2005), dawadawa in Ghana (Dakwa et al., 2005), douchi and sufu in China (Zhang et al., 2007; Han et al., 2004), natto and miso in Japan (Sugawara et al., 1985; Yamabe et al., 2007), kinema in India, Nepal and Bhutan (Tamang and Nikkuni 1996) and doenjang in Korea (Choi et al., 2007). The use of starter cultures is widespread in various kinds of fermented foods since they have a significant impact on food quality parameters such as taste, texture, odor and nutritive value. The selection of

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Table 1. Starter culture used in the laboratory fermentation of vegetarian soybean kapi.

Fermentation batch	Starter cultures	Source
S1	*B. subtilis* IS4	Isolate from shrimp paste kapi (Chiang Mai)
S2	*B. amyloliquefaciens* R1	Isolate from shrimp paste kapi (Rayong)
S3	*B. subtilis* N1	Isolate from shrimp paste kapi (Nakornsawan)
S4	*B. subtilis* TISTR10	Obtained from TISTR
S5	*B. subtilis* TISTR01	Obtained from TISTR

starter cultures has been evaluated by both ability of isolates to ferment food substances and the sensory quality of the final product (Visessanguan et al., 2006). There have been many reports describing the attempts to use a starter culture for producing food condiments such as ugba (Sanni et al., 2002) and soy-daddawa (Omafuvbe et al., 2002), which are fermented from different species of *Bacillus* sp. Thua-nao is a Thai traditional fermented soybean that has been prepared by *Bacillus subtilis* as the starter culture (Chantawannakul et al., 2002). Vegetarian kapi in local markets in Thailand is also produced from soybeans and has the characteristic kapi smell and sticky texture similar to shrimp paste kapi. Today, vegetarian kapi has been highly popularized and it is now used in many Thai vegetarian dishes. However, there have been no reports as of yet on the vegetarian kapi fermentation as it could be associated with the selection of particular starter cultures and the respective aroma profile. The main objective of this study was to analyze the volatile compounds resulting from the use of soybeans fermented with various starter cultures and to investigate the strong potential of those starter cultures for use in the production of acceptable vegetarian soybean kapi.

MATERIALS AND METHODS

Commercial vegetarian kapi and kapi samples

Samples of commercial vegetarian kapi (J1-J3) were obtained from a vegetarian store in Chiang Mai, Thailand and samples of commercial shrimp paste kapi were obtained from local market in Nakonsawan (K1), Chonburi (K2) and Chiang Mai (K3), Thailand.

Preparation of vegetarian soybean kapi

Microorganisms

B. subtilis IS4, *Bacillus amyloliquefaciens* R1 and *B. subtilis* N1 were used as representatives of isolates from commercial shrimp paste kapi which was obtained from local markets in Chiang Mai, Rayong and Nakornsawan, respectively (Table1). The selection of these bacteria was carried out according to the previous study (Wittanalai et al., 2010). Genotypic identification involved 16S rDNA sequencing method by BIOTEC Culture Collection (BCC), Thailand. The strains were subjected to 16S rDNA sequence analysis. A homology search was performed using the standard nucleotide BLAST from NCBI web server against previously reported sequences, at the GenBank/EMBL/DDBJ database for determination of the nearest sequences. *B. subtilis* TISTR10 and *B. subtilis* TISRT01, isolated from Thai-fermented soybeans, Thua-nao were obtained from the TISTR Culture Collection, Pathumthani, Thailand. The strains were maintained at 4°C on nutrient agar slants (LAB-SCAN 409101).

Preparation of inocular

The microorganism strains from nutrient agar slants were incubated in nutrient broth for 16 h at 37°C, 180 rpm. The dilution of microorganisms was made in sterile 0.85% (w/w) NaCl solution to obtain 10^8 cells/ml of inoculum for the fermentation of vegetarian soybean kapi.

Fermentation of vegetarian soybean kapi

Dry soybeans (*Glycine max* L Merrill) were washed twice with clean water and then soaked for 12 h. After removing the water, the seeds were boiled for 3 h to soften seeds, the water was drained and the seeds were ground by blender. Thirty grams of cooked soybeans were added to an Erlenmeyer flask which was stopped with cotton wool and aluminum foil and sterilized at 121°C for 20 min. After sterilization, inoculum was added followed by thorough mixing using a sterilized glass rod and incubation for 8 days at 37°C. The vegetarian soybean kapi S1, S2, S3, S4 and S5 were obtained as described in Table1.

Samples were taken at the end of fermentation to determine the volatile compounds using the solid phase microextraction (SPME) with gas chromatography-mass spectrometry (GC/MS) analysis. The odor, color and appearance of the seeds were also noted.

Volatile compound analysis

The extraction of headspace volatile compounds was done using a SPME device (Sipel, U.S.A) with a 100 µm polydimethylsiloxane (PDME) fiber. For each experiment, 10 g of sample was weighed into a 125 ml glass bottle and covered with a cap and SPME fiber. The bottle was left at 50°C in a water bath for 30 min to equilibrate its headspace.

The compounds adsorbed by the fiber were desorbed in the injection port of the gas chromatograph (GC 6890 Agilent Technologies) at 250°C in the split less mode. The compounds were separated on an AT-1MS capillary column (30 m, 0.25 mm i.d., film thickness 0.25 µm). The GC was equipped with an HP 5972 mass selective detector (Hewlett Packard, USA). Helium was used as the carrier gas with a velocity of 1.0 ml/min. The total run time was 30 min. Mass specta were obtained by electron impact at 70 eV. The compounds were identified by comparison with mass spectra from the library database (Nist'98 and Wiley7n).

Table 2. Volatile compounds groups in unfermented soybean (Sc), vegetarian soybean kapi samples (S1-S5), traditional commercial vegetarian kapi (J1-J3) and traditional commercial shrimp paste kapi (K1-K3).

Volatile compound group	Relative amounts of the compound groups (%)[a]											
	Sc	S1	S2	S3	S4	S5	J1	J2	J3	K1	K2	K3
Aldehydes	-	3.48	-	-	-	5.5	1.6	4.16	2.76	25.99	-	1.13
Alcohols	-	-	1.19	7.02	1.55	-	1.53	0.74	10.67	10.75	2.04	6.47
Ketones	-	-	1.80	0.87	0.36	-	1.86	-	-	13.07	1.68	-
Acids and esters	-	68.42	8.81	59.29	79.60	74.52	77.26	47.37	42.66	8.99	3.82	4.80
N-containing compounds	5.78	28.10	2.55	1.95	2.91	11.8	3.64	26.40	10.09	2.64	58.59	38.51
Aromatic compounds	-	-	50.97	-	5.88	5.53	2.13	2.55	2.33	12.11	7.91	17.87
S-containing compounds	-	-	-	-	4.46	-	4.21	4.17	-	-	8.66	9.45
Miscellaneous compounds	6.24	-	-	21.11	-	-	6.61	10.97	25.96	8.28	4.37	7.41
Indole	-	-	-	-	-	-	0.87	1.11	-	-	2.06	3.35
Hydrocarbon compounds	87.98	-	34.68	9.76	5.24	2.65	0.29	2.53	5.53	18.17	10.87	11.01

[a]%, concentration of volatile compounds relative to the total concentration of volatile compounds identified; -, compounds not detected. Sc, unfermented soybean; S1, vegetarian soybean kapi of *B. subtilis* IS4; S2, vegetarian soybean kapi of *B. amyloliquefaciens* R1; S3, vegetarian soybean kapi of *B. subtilis* N1; S4, vegetarian soybean kapi of *B. subtilis* TISTR10; S5, vegetarian soybean kapi of *B. subtilis* TISTR01; J1-J3, commercial vegetarian kapi; K1-K3, commercial shrimp paste kapi

Sensory analysis

The sensory analysis of vegetarian soybean kapi (S1-S5) with diffe-rent types of starter culture and commercial vegetarian kapi (J1-J3) was carried out by a panel of 12 regular consumers of kapi using a 5-point hedonic scale (Watts et al., 1989) from 1 (dislike extremely) to 5 (like extremely) for overall acceptability according to appea-rance, color and odor.

Statistic analysis

Principal component analysis was performed based on the relative amounts of different volatile compound according to groups. The fermented samples were classified into 4 groups by K-mean cluster analysis using the SPSS package version 13.0 (SPSS 13.0 for window, SPSS Inc., Chicago, Ill., U.S.A.). The data obtained in sensory analysis were subjected to analysis of statistical differences by Duncan's multiply range tests using the SPSS package version 13.0 (SPSS 13.0 for window, SPSS Inc., Chicago, Ill., U.S.A.). Statistical significance was accepted at P value less than 0.05.

RESULTS AND DISCUSSION

Volatile compounds analysis

The volatile compounds from unfermented soy-beans (Sc), vegetarian soybean kapi (S1-S5), commercial vegetarian kapi (J1-J3) and com-mercial kapi (K1-K3) were identified by the solid phase microextraction (SPME) extraction process followed by GC/MS analysis. The relative amounts of volatile compounds in the resulting groups are shown in Table 2. Ten major groups were found, aldehydes, alcohols, ketones, acids and esters, N-containing compounds, aromatic compounds, S-containing compounds, miscellaneous compounds, indoles and hydrocarbons. Acids and esters represent over 50% of the total amount of com-pounds in the fermented samples S1, S3, S4, S5 and J1, while J2 and J3 had 47.37 and 42.66% acids and esters, respectively. N-containing com-pounds were found in high amounts in S1 (28.10%), J2 (26.40%), J3 (10.09%), K2 (58.59%) and K3 (38.51%). While hydrocarbons were found in higher amounts in Sc: unfermented soybeans (87.98%), S2 (34.68%) and K1 (18.17%). The identified volatile compounds in unfermented and fermented samples are detailed in Table 3. A total of 121 compounds including 8 aldehydes, 8 alco-hols, 11 ketones, 21 acids and esters, 18 N-containing compounds, 9 aromatic compounds, 3 S-containing compounds, 3 miscellaneous com-pounds, 1 indole and 39 hydrocarbon compounds were found. The unfermented soybeans (Sc) yielded 3 groups of volatile compounds including N-containing compounds, hydrocarbons and miscellaneous compounds. Compared to Sc, more volatile compounds were found in the S1-S5 samples, which could be the result of the fermentation abilities of the starter cultures. The S1 sample produced from *B. subtilis IS4* (isolated

Table 3. Volatile compounds in unfermented soybean (Sc), vegetarian soybean kapi samples (S1-S5), traditional commercial vegetarian kapi (J1-J3) and traditional commercial shrimp paste kapi (K1-K3).

Compound	Relative amount of compound (%)[a]											
	Sc	S1	S2	S3	S4	S5	J1	J2	J3	K1	K2	K3
Aldehydes (8)												
3-Methyl butanal	-	-	-	-	-	-	0.38	1.39	0.56	3.24	-	-
2-Methyl butanal	-	-	-	-	-	-	0.46	1.59	-	1.92	-	-
Benzaldehyde	-	3.28	-	-	-	4.94	0.76	1.18	1.52	11.89	-	1.13
5-Methyl-2-phenyl-2-hexenal	-	-	-	-	-	-	-	-	0.68	-	-	
Hexanal	-	-	-	-	-	0.56	-	-	-	4.68	-	-
4-Heptanal	-	-	-	-	-	-	-	-	-	2.06	-	-
2,6-Nonadienal	-	-	-	-	-	-	-	-	-	0.97	-	-
Heptanal	-	-	-	-	-	-	-	-	-	1.23	-	-
Alcohols (8)												
Dimethyl-silanediol	-	-	-	-	-	-	0.76	-	1.08	-	-	-
3-Methyl-1-butanol	-	-	-	-	-	-	-	-	2.94	5.36	1.28	2.60
2-Methyl-1-butanol	-	-	-	-	-	-	0.77	-	-	-	-	-
Ethanol	-	-	1.19	-	-	-	-	-	4.49	-	-	-
1-Octene-3-ol	-	-	-	-	-	-	-	-	2.16	3.66	0.76	1.77
2-Phenylethanol	-	-	-	5.24	1.55	-	-	0.74	-	-	-	2.10
3-Methyl-3-cyclohexan-1-ol	-	-	-	1.78	-	-	-	-	-	-	-	-
1-Ethylcyclopropanol	-	-	-	-	-	-	-	-	-	1.73	-	-
Ketones (11)												
3-Octanone	-	-	-	-	-	-	0.99	-	-	-	-	-

Table 3. Continued.

Compound	Relative amount of compound (%)[a]											
	Sc	S1	S2	S3	S4	S5	J1	J2	J3	K1	K2	K3
5-Methyl-3-heptanone	-	-	-	-	-	-	0.49	-	-	-	-	-
2-Undecanone	-	-	-	-	-	-	0.38	-	-	-	0.30	-
2-Propanone	-	-	-	-	0.36	-	-	-	-	-	-	-
6-Methyl-2-heptanone	-	-	0.75	-	-	-	-	-	-	0.97	-	-
3,5-Octadiene-2-one	-	-	-	-	-	-	-	-	11.17	-	-	-
2-Octanone	-	-	-	-	-	-	-	-	-	0.49	-	-
2-Heptanone	-	-	-	-	-	-	-	-	-	0.45	-	-
2-Nonanone	-	-	-	-	-	-	-	-	-	0.44	-	-
3-Undecen-2-one	-	-	-	-	-	-	-	-	0.93	-	-	-
2,6-Di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	-	1.80	1.91	0.87	-	-	-	-	-	-	-	-
Acid and esters (21)												
Acetic acid	-	7.10	-	-	4.74	1.71	1.07	2.70	6.12	-	-	-
Propanoic acid	-	-	-	-	8.35	1.43	-	-	-	-	-	-
2-Methyl-propanoic acid	-	12.79	1.22	11.51	30.45	12.55	11.82	4.81	2.32	-	-	-
Butanoic acid	-	0.71	-	0.61	-	-	1.60	4.94	-	-	0.92	-
3-Methyl butanoic acid	-	9.97	-	26.42	3.70	-	39.64	14.90	-	-	-	-
Pentanoic acid	-	28.30	5.37	-	18.40	33.55	-	-	10.66	-	1.63	-
2-Methyl butanoic acid	-	9.45	2.22	15.05	13.96	23.93	22.14	15.12	5.81	-	1.27	-
Methyl pentanoate	-	-	-	-	-	-	-	-	2.11	-	-	-
4-Methyl pentanoic acid	-	-	-	-	-	-	0.99	2.72	2.08	-	-	-

Table 3. Continued.

Compound	Relative amount of compound (%)[a]											
	Sc	S1	S2	S3	S4	S5	J1	J2	J3	K1	K2	K3
Methyl hexanoate	-	-	-	-	-	-	-		1.11	-	-	-
Ethyl hexanoate	-	-	-	-	-	-	-	-	2.68	-	-	-
Methyl 3-phenylpropanoate	-	-	-	-	-	-	-	-	2.21	-	-	-
Ethyl 3-phenylpropanoate	-	-	-	-	-	-	-	-	1.72	-	-	-
2-Methyl hexanoic acid	-	-	-	-	-	-	-	-	-	0.80	-	2.12
Diethyl 5-hydroxybenzene-1,3-dicarboxylate	-	-	-	5.70	-	1.35	-	-	-	-	-	-
Benzoic acid	-	-	-	-	-	-	-	-	-	3.04	-	-
Iso-valeric acid	-	-	-	-	-	-	-	-	-	1.45	-	-
Ethyl benzoate	-	-	-	-	-	-	-	-	5.84	1.45	-	-
Methyl benzoate	-	-	-	-	-	-	-	-	-	2.25	-	-
Hexanoic acid	-	-	-	-	-	-	-	2.18	-	-	-	-
Pentanoic acid	-	-	-	-	-	-	-	-	-	-	-	2.68
N-contianing Compounds (18)												
Acetamide	5.78	17.89	-	-	-	-	-	-	-	-	-	-
2,5-Dimethyl pyrazine	-	10.06	-	1.95	1.94	8.62	1.08	4.26	1.54	-	-	-
2-Ethyl-6-methyl pyrazine	-	-	-	-	-	-	-	1.02	-	-	2.26	2.01
Trimethyl pyrazine	-	-	-	-	0.97	2.19	1.59	8.71	4.03	-	4.02	4.23
3-Ethyl-2,5-dimethyl pyrazine	-	-	-	-	-	-	0.56	1.72	0.70	-	12.11	8.28
2,3,5,6-Tetramethyl pyrazine	-	-	-	-	-	-	-	8.14	-	-	-	-
Tetramethyl pyrazine	-	-	-	-	-	0.99	-	-	2.91	-	-	-

Table 3. Continued.

Compound	Relative amount of compound (%)[a]											
	Sc	S1	S2	S3	S4	S5	J1	J2	J3	K1	K2	K3
2,3,5-Trimethyl-6-ethylpyrazine	-	-	-	-	-	-	0.41	2.55	0.91	-	2.61	-
2,3,4,5-Tetrahydro-6-propylpyridine	-	-	2.55	-	-	-	-	-	-	-	-	-
N,N-Dimethyl methylamine	-	-	-	-	-	-	-	-	-	2.64	5.75	5.93
2,6-Dimethyl pyrazine	-	-	-	-	-	-	-	-	-	-	28.28	12.59
2-Methyl-5-isopropyl pyrazine	-	-	-	-	-	-	-	-	-	-	0.68	-
2-Ethyl-3,5-dimethyl pyrazine	-	-	-	-	-	-	-	-	-	-	0.25	0.16
2,3-Diethyl-5-methyl pyrazine	-	-	-	-	-	-	-	-	-	-	0.80	-
4-Ethyl-2,5,6-trimethyl pyrazine	-	-	-	-	-	-	-	-	-	-	0.72	3.23
2,5-Dimethyl-3-butyl pyrazine	-	-	-	-	-	-	-	-	-	-	1.11	-
2-Methyl-5- (1-methylehyl) pyrazine	-	-	-	-	-	-	-	-	-	-	-	0.89
2-Butyl-3,5-dimethyl pyrazine	-	-	-	-	-	-	-	-	-	-	-	1.19
Aromatic compounds (9)												
Phenol	-	-	-	-	-	-	0.70	-	-	-	6.05	11.48
1-Methyl-3-(1-methylethyl)-benzene	-	-	-	-	-	-	-	-	2.33	-	0.89	-
1-Methyl-4-(1-methylethyl)-benzene	-	-	-	-	-	-	0.48	-	-	-	-	0.82
2,6-Bis(1,1-dimethylethyl)-4-methyl phenol	-	-	34.5	-	5.88	5.53	0.95	2.55	-	5.79	0.97	-

from shrimp paste kapi in Chiang Mai) could yield the satisfactory fermented soybean with a strong kapi odor. This may be due to the high content of volatile compounds in S1 (acids and esters and N-containing compounds such as 2-methyl propanoic acid, pentanoic acid, acetamide and 2, 5-dimethyl pyrazine) which contains some N-containing compounds similar to K1-K3 shrimp past kapi (3-ethyl-2, 5-dimethyl pyrazine, trimethyl pyrazine and 2, 6-dimethyl pyrazine). While the other samples have the undesirable odor described as being beany, putrid and/or ammonia-like. The differences in volatile compounds between the vegetarian soybean kapi

Table 3. Continued.

Compounds	Relative amounts compound (%)[a]											
	Sc	S1	S2	S3	S4	S5	J1	J2	J3	K1	K2	K3
Diethyl phthalate	-	-	16.47	-		-	-	-	-	-	-	-
1-methyl-2-(1-methylethyl)-benzene	-	-	-	-	-	-	-	-	-	-	-	4.82
Methyl benzene	-	-	-	-	-	-	-	-	-	4.44	-	0.75
Diphenyl	-	-	-	-	-	-	-	-	-	0.71	-	-
4-methyl-1,1’-biphenyl	-	-	-	-	-	-	-	-	-	1.17	-	-
S-containing compound (3)												
Dimethyl disulfide	-	-	-	-	2.72	-	0.74	2.32	-	-	4.52	3.87
Dimethyl trisulfide	-	-	-	-	1.38	-	2.60	1.85	-	-	4.14	4.38
Dimethyl tetrasulfide	-	-	-	-	0.36	-	0.87	-	-	-	-	1.20
Miscellaneous (3)												
Chloroform	6.24	-	-	-	-	-	6.61	9.33	15.65	8.28	3.59	4.90
Toluene	-	-	-	-	-	-	-	1.64	5.45	-	0.78	-
Butyl hydroxy toluene	-	-	-	21.11	-	-	-	-	4.86	-	-	2.51
Idole (1)												
1H-Indole	-	-	-	-	-	-	0.87	1.11	-	-	2.06	3.35
Hydrocarbon compounds (39)												
Tetrahydro-thiophene	-	-	-	-	-	-	-	-	1.53	-		-

Table 3. Continued.

Compound	Relative amount of compound (%)[a]											
	Sc	S1	S2	S3	S4	S5	J1	J2	J3	K1	K2	K3
dl-Limonene	-	-	-	-	-	-	0.29	-	1.03	-	-	2.42
γ-Terpinene	-	-	-	-	-	-	-	-	0.74	-	-	2.46
2,4,6-Trimethyl octane	1.86	-	-	-	-	-	-	-	-	-	-	-
Hexadecane	4.02	-	-	-	1.24	-	-	-	-	-	0.84	-
3,4,5,6-Tetramethyl octane	7.92	-	-	-	-	-	-	-	-	-	-	-
4-Methyl undecane	7.46	-	-	-	-	-	-	-	-	-	-	-
5-Methyl undecane	8.81	-	-	-	-	-	-	-	-	-	-	-
Dodecane	5.07	-	-	-	-	-	-	-	-	-	0.59	-
3,8-Dimethyl decane	3.67	-	-	-	-	-	-	-	-	-	-	-
Decane	16.44	-	-	-	-	-	-	-	-	-	-	-
2,2,4,6,6-Penta-ethyl-heptane	-	-	4.18	-	0.72	-	-	-	-	-	-	-
2,2-Dimethyl decane	-	-	2.67	1.64	0.44	-	-	-	-	-	-	-
2,2,4,4,6,8,8-Heptamethyl nonane	-	-	4.39	2.28	-	-	-	-	-	-	-	-
2,6,7-Trimethyl dodecane	-	-	8.95	-	-	-	-	-	-	-	-	-
3,5-Dimethyl octane	-	-	5.30	-	-	-	-	-	-	-	-	-
2,2,5-Trimethyl hexane	-	-	7.29	-	1.01	0.96	-	-	-	-	-	-
3,3-Demethyl hexane	-	-	1.90	-	-	-	-	-	-	-	-	-
4-Ethyl-2,2,6,6-tetramethyl heptane	-	-	-	1.72	-	-	-	-	-	-	-	-
2,5-Dimethyl octane	-	-	-	-	-	0.56	-	-	-	-	-	-
2,2,4,4-Tetramethyl octane	-	-	-	-	0.60	-	-	-	-	-	-	-
1-Ethyl-1-methyl cyclohexane	-	-	-	-	0.51	-	-	-	-	-	-	-

Table 3. Continued.

Compound	Relative amount of compound (%)[a]											
	Sc	S1	S2	S3	S4	S5	J1	J2	J3	K1	K2	K3
7,9-Dimethyl hexadecane	8.90	-	-	-	-	-	-	-	-	-	-	-
3-Methyl undecane	2.92	-	-	-	-	-	-	-	-	-	-	-
Heptadecane	2.48	-	-	-	-	-	-	-	-	-	-	-
Dodecane	13.2	-	-	-	-	-	-	-	-	-	-	-
4-Methyl tetradecane	5.23	-	-	-	-	-	-	-	-	-	-	-
2,6,7-trimethyl decane	-	-	-	-	-	1.13	-	-	-	-	-	-
2,6,10-Trimethyl dodecane	-	-	-	0.95	0.72	-	-	-	-	-	-	-
Pentadecane	-	-	-	-	-	-	-	2.53	2.23	3.80	3.67	3.09
2,5,5-Trimethyl-2-hexene	-	-	-	-	-	-	-	-	-	4.56	-	-
1,3-Cyclooctadiene	-	-	-	-	-	-	-	-	-	1.32	-	-
1,4-Cyclooctadiene	-	-	-	-	-	-	-	-	-	1.89	-	-
1-Methyl-2-methylenecyclohexane	-	-	-	-	-	-	-	-	-	2.56	0.49	-
3-Methyl-1-octene-3-yne	-	-	-	0.94	-	-	-	-	-	4.04	-	-
2-Methyl-naphthalene	-	-	-	-	-	-	-	-	-	-	0.53	-
Tridecane	-	-	-	-	-	-	-	-	-	-	1.67	1.05
1,6-Dimethyl-naphthalene	-	-	-	-	-	-	-	-	-	-	0.76	-
Tetradecane	-	-	-	-	-	-	-	-	-	-	2.32	1.99

[a]%, Concentration of volatile compounds relative to the total concentration of volatile compounds identified; -, compounds not detected Sc, unfermented soybean; S1, vegetarian soybean kapi of *B. subtilis* IS4; S2, vegetarian soybean kapi of *B. amyloliquefaciens* R1; S3, vegetarian soybean kapi of *B. subtilis* N1; S4, vegetarian soybean kapi of *B. subtilis* TISTR10; S5, vegetarian soybean kapi of *B. subtilis* TISTR01; J1-J3, commercial vegetarian kapi; K1-K3, commercial shrimp paste kapi.

samples produced from different starter cultures may be related to the ability of the microorganisms to hydrolyze the proteins, lipids and carbohydrates in soybeans. The higher amounts of volatile compounds identified in commercial vegetarian soybean kapi (J1-J3) and shrimp paste kapi (K1-K3) when compared with (S1-S5) kapi may be due to the long fermentation time for the traditional kapi which may increase the degradation of the substrate producing higher concentrations of volatile aroma compounds. Pyrazines were found to be among the volatile compounds that were the major compounds in the N-containing group and were previously reported to be good precursors of volatile compounds (Allagheny et al., 1996). The formation of pyrazines is associated with heating and metabolic activities of microorganisms (Owens et al., 1997), and pyrazines are the major volatile compounds in various fermented foods (Sugawara et al., 1985; Azokpota et al., 2010). For example, pyrazines which formed during the fermentation of soya beans into natto are the main contributors to the characteristic natto odor (Sugawara et al., 1985). The volatile compounds present at the highest concentration levels in natto are tetramethyl pyrazine, trimethyl pyrazine and 2, 5-dimethyl pyrazine. These compounds have also been detected in high concentration levels in three Beninese condiments, afitin, iru and sonru (Azokpota et al., 2010). However, acid and ester, the other main group of volatile compound is probably produced from the chemical reactions between microbial acidic and alcoholic metabolites. Volatile compounds present in lower concentration levels, such as aldehydes, ketone and alcohol, are generally produced from the oxidative cleavage of the lipids and the degradation of amino acids and saccharides (Leejeerajumnean et al., 2001; Ouoba et al., 2005; Azokpota et al., 2008).

Principal component analysis (PCA) was performed on 10 groups of volatile compound as variables to evaluate their contribution in the fermented samples (S1-S5, J1-J3 and K1-K3). The PCA analysis separated the data into 4 groups with eigenvalues exceeding 1 (Kaiser's rule) that accounted for 93.85% of the total variance (Table 4). The 3D-plot of factor score (Figure 1) showed 4 clusters of the volatile compound group from various fermented samples. The first component explained the highest percent of variance (28.77%) and was characterized as having relatively high amounts of indole, S-containing compounds and N-containing compounds. Group 1 included vegetarian soybean kapi S1, S4 and S5, commercial vegetarian kapi J1, J2 and commercial shrimp kapi K2, K3. The second component explained 24.89% of the variance and the important volatile compounds in this group were aromatic compounds, hydrocarbon compounds and acids and esters. Group 2 comprised only vegetarian soybean kapi S2. The third component explained 22.04% of the variance with ketones and aldehydes as the important volatile compounds in this group. Group 3 comprised of only commercial shrimp paste K1. Although a smaller variation was assigned to Group 4 (18.14%), it was characterized as having relatively high

Table 4. Loading scores for principal component analysis (PCA) of relative amounts of volatile compound groups.

Compound group	Component			
	1	2	3	4
Indole	0.950	0.079	-0.102	0.053
S-containing compounds	0.916	-0.029	-0.149	-0.098
N-containing compounds	0.887	-0.077	-0.097	-0.118
Hydrocarbons compounds	-0.099	0.973	0.159	0.029
Aromatics compounds	-0.047	0.965	-0.046	-0.207
Acids and esters	-0.531	-0.716	-0.330	-0.248
Aldehydes	-0.167	-0.005	0.968	0.083
Ketones	-0.122	0.214	0.945	0.093
Miscellaneous compounds	-0.097	-0.164	-0.095	0.961
Alcohols	-0.028	0.114	0.434	0.863
Eigenvalue	3.259	2.905	1.986	1.234
% variance	28.77	24.89	22.04	18.15
Cumulative % of variance	28.77	53.66	75.70	93.85

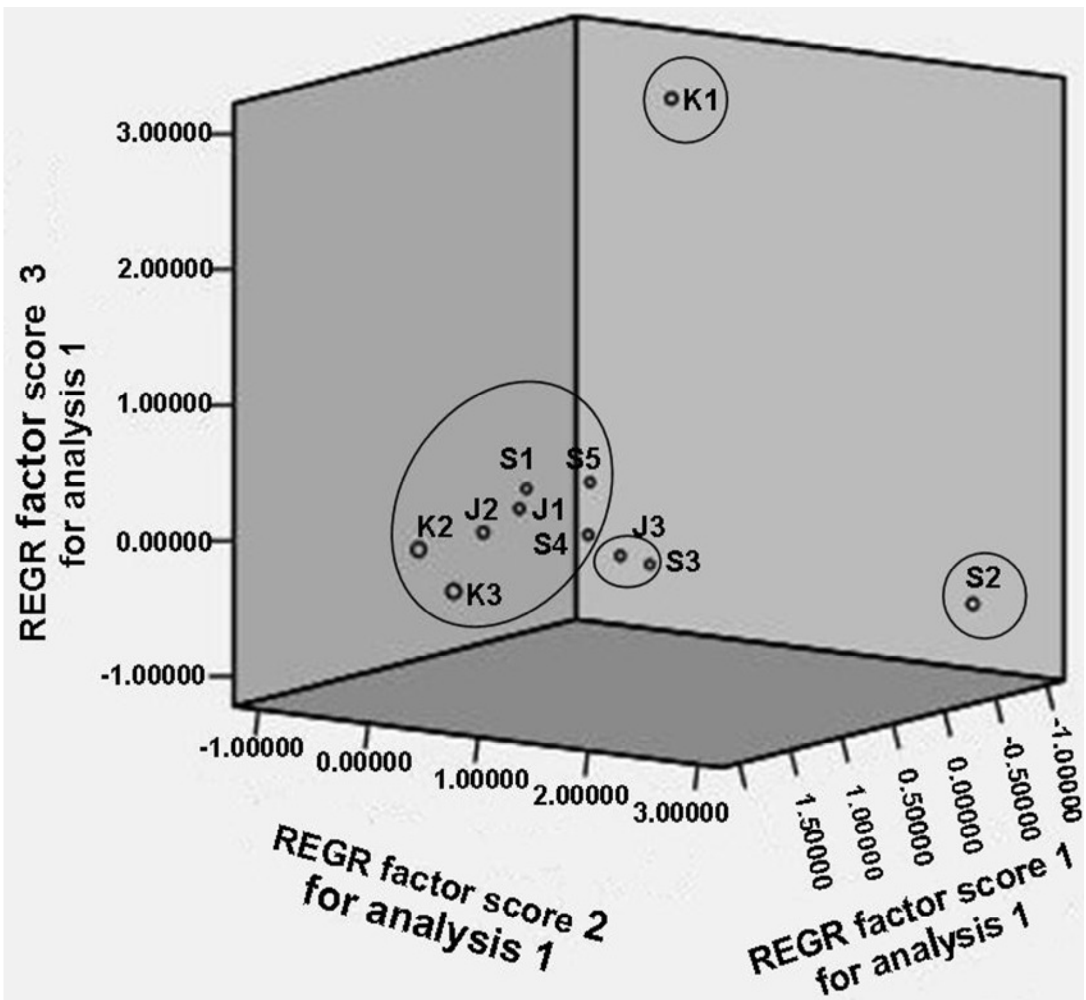

Figure 1. 3D-plot from principal component analysis scores (REGR factor score 1, 2 and 3) of relative amounts of volatile compound groups from various fermented samples: S1, vegetarian soybean kapi of *B. subtilis* IS4; S2, vegetarian soybean kapi of *B. amyloliquefaciens* R1; S3, vegetarian soybean kapi of *B. subtilis* N1; S4, vegetarian soybean kapi of *B. subtilis* TISTR10; S5, vegetarian soybean kapi of *B. subtilis* TISTR01; J1-J3, commercial vegetarian kapi; K1-K3, commercial shrimp paste kapi.

Table 5. Sensory evaluation of various samples of vegetarian kapi.

Samples of vegetarian kapi	Characteristic of vegetarian soybean kapi samples (appearance, color, odor)	Overall acceptability (scores*)
S1	Very sticky, yellow-brown, strong kapi odor	3.92 ± 0.54
S2	Slightly stickly, yellow, kapi with bean odor	3.12 ± 0.71
S3	Slightly stickly, yellow-brown, kapi with ammonia odor	3.45 ± 0.57
S4	Slightly stickly, yellow brown, kapi with ammonia odor	3.32 ± 0.79
S5	Slushy, yellow, slightly kapi odor	3.60 ± 0.81
J1	Very stickly, dark brown, strong kapi odor	4.40 ± 0.43
J2	Very stickly, brown, strong kapi odor	4.10 ± 0.68
J3	Very stickly, dark brown, strong kapi odor	3.94 ± 0.82

*Scores from five to one (5 like extreamly; 1 dislike extreamly). Data presented as means value ± SD. S1, vegetarian soybean kapi of *B. subtilis* IS4; S2, vegetarian soybean kapi of *B. amyloliquefaciens* R1; S3, vegetarian soybean kapi of *B. subtilis* N1; S4, vegetarian soybean kapi of *B. subtilis* TISTR10; S5, vegetarian soybean kapi of *B. subtilis* TISTR01; J1-J3, commercial vegetarian kapi.

amounts of alcohols and miscellaneous compounds. Group 4 included vegetarian soybean kapi S3 and commercial vegetarian kapi J3. The fermented samples obtained with S1, S4 and S5 were classified into the same group as commercial vegetarian kapi (J1, J2) and commercial shrimp paste kapi (K2, K3) in group 1.

Sensory evaluation

The results of sensory evaluation of vegetarian soybean kapi samples are shown in Table 5. Products with the highest scores were most preferred and those with the lowest scores were least preferred. Comparing the mean scores of S1-S5 samples, S1 earned high scores for overall acceptability. Comparing the mean scores of this sample (S1) with those of commercial vegetarian kapi (J1-J3), the different scores that have been observed could be attributed mainly to the fermentation process. However, evaluation scores showed no significant difference. S1 fermented from *B. subtilis* IS4, had desirable sticky texture, yellow-brown color and strong kapi odor, while S2-S5 had undesirable odors described as beany, putrid and ammonia. Therefore, the starter culture *B. subtilis* IS4 has good potential for use in the production of accep-table vegetable soybean kapi.

Conclusion

In this study, the volatile compounds of the vegetarian soybean kapi samples produced from various starter cultures were identified and compared with those of commercial vegetarian kapi (J1-J3) and shrimp paste kapi (K1-K3). The volatile profile of vegetarian soybean kapi S1 produced from *B. subtilis* IS4 had a similar pattern to the volatile compound groups (indole, S-containing compounds and N-containing compounds) when compared with the commercial vegetarian kapi J1, J2 and commercial shrimp paste kapi K2, K3, from the same group. Furthermore, the sensory evaluation of S1 showed a high score for overall acceptability with a strong kapi odor with no significant difference ($P < 0.05$) between S1 and commercial vegetarian kapi. From this study, the *B. subtilis* IS4 may be regarded as a potential starter culture due to its ability to ferment soybean into vegetarian soybean kapi and hence, it can be used effectively as a shrimp paste kapi substitute. This strain also provides the possibility for modernization of traditional fermentation in the commercial production of this vegetarian condiment.

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