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Volatile profile of *khanom jeen*, Thai fermented rice noodles, and the changes during the fermentation process

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ABSTRACT: This study was carried out to determine the volatile compounds profile of *khanom jeen* (Thai fermented rice noodles) and the changes in the volatile compound composition during fermentation. Rice samples were collected at each step of the fermenting process. Identification and quantification of volatiles were performed by evaluating dynamic headspace with gas chromatography mass spectrometry. For *khanom jeen*, a total of 43 compounds were found. Among them, 2-methylpropanoic acid, 3-methylbutanoic acid, diacetyl, ethyl acetate, ethyl valerate, ethyl hexanoate, ethyl heptanoate, heptanal, octanal, nonanal, decanal, and 3-methylbutanal were identified as potent odourants based on their odour activity values. Most of the volatile compounds increased during the first 24–48 h of the rice fermentation process, except for some esters which increased thereafter during the sedimentation step. The concentrations of selected aromatic volatile compounds in *khanom jeen* were affected by the fermentation steps – particularly 2-methylpropanoic acid and 3-methylbutanoic acid, which were not found in the raw material (broken rice). The amount of ethyl acetate in the water elimination process was found to be twice as much as in the first day of rice fermentation. The presence of straight-chain aldehydes changed only slightly during rice fermentation. However, the concentration of 3-methylbutanal increased substantially. The results indicated that the aroma of *khanom jeen* was affected by the fermentation process.

KEYWORDS: volatile compounds, gas chromatography-mass spectrometry, odour activity values

INTRODUCTION

Khanom jeen is a traditional fermented rice noodle widely consumed in Thailand. Broken rice grains, usually stored over a six-month period, are naturally fermented for a few days, wet milled, and then left to precipitate as rice flour. A weight is used to eliminate excess water from the sedimented flour after it is placed in a cotton bag. This water-eliminating process is called the weighting step. Next, the fermented rice flour is pre-cooked before kneading and extruding the noodles through a die into boiling water. The cooked noodles are immediately cooled in tap water and then positioned on racks for drying. *Khanom jeen* made from slightly fermented rice has a characteristic fermented flavour[1]. Natural fermentation of *khanom jeen* includes complex microbiological and enzymatic processes contributing to the appearance of compounds which confer unique flavour and textural characteristics. Like *mifen*, a traditional fermented rice noodle from China, the chewy mouth-feel of the noodles is increased by the natural fermentation process, whereas the protein, lipid, and ash content are decreased[2]. Lactic acid bacteria (LAB) are the major microbial populations found in fermented *khanom jeen*, as well as yeast and mould[3,4].

Recently, much research has been conducted on the volatiles in non-fermented rice products, such as raw rice, cooked rice, aromatic rice, and rice cakes[5–7]. The effects of cultivation times and yeast species on volatile composition were investigated in the yeast-fermented rice products *koji*[8] and *angkak*[9]. However, studies on the determination of volatile compounds in fermented rice noodles have been limited. Thus, this study was conducted to investigate the volatile profile of *khanom jeen* and the influence of fermentation steps on the volatiles.

MATERIALS AND METHODS

Sample preparation

Khanom jeen samples were produced by natural fermentation. Broken rice was soaked in water for 1 h and drained. The rice was fermented for 48 h with water washing at the end of the day. The fermented rice was then wet milled and the slurry was left in the tank for 24 h. The sedimented flour was put into a cotton bag. Excess water was eliminated by weighting for 24 h. The drained wet flour was pregelatinized and kneaded to paste-like consistency with warm water. To make the noodles, the flour was extruded into hot water.

The broken rice, 24 h and 48 h fermented rice, sedimented flour, drained wet flour, and the final product (*khanom jeen*) were collected to determine pH value, titratable acidity, and volatile composition.

Determination of pH and titratable acidity

The samples were analysed for pH and titratable acidity according to the methods given by the AOAC[10]. The titratable acidity was calculated as lactic acid.

Extraction of volatile compounds

Each sample (approximately 0.5 g by a balance with 4 decimals) was weighed into a 40 ml purge vial, and 0.5 µl of internal standard was added. The internal standard used was 2-methyl-3-heptanone (Sigma-Aldrich Chemie, Stenheim) in methanol (0.9 mg/ml). Volatile compounds from the samples were extracted by a purging gas (helium 99.999%) for 20 min, and then trapped in the Tenax TA trap in a purge-and-trap concentrator (Tekmar-3000, Teledyne Technologies). The volatiles were desorbed by heating the trap at 200 °C for 3 min, and subsequently concentrated on a cryofocusing unit at -100 °C with liquid nitrogen. Finally, the collected volatiles were pushed into the analytical column by quickly heating a cryomodule to 180 °C. This procedure made it possible to extract volatile compounds from the samples without interference from the extraction solvent.

Determination of volatile compounds

The volatile compounds were analysed using a HP5890 GC system (Hewlett-Packard) coupled with a 5973 Mass Selective Detector (Hewlett-Packard). The separation was performed using a 007-FFAP capillary column (Quadrex 60 m $\times$ 0.25 mm diameter, 0.25 µm film thickness). The flow rate of the carrier gas (helium) was 2 ml/min. An oven temperature program was used. The column temperature was held at 40 °C for 2 min and then increased at 10 °C/min to 200 °C, which was then held for 10 min. The mass spectra were determined at 70 eV, while the interface was held at 280 °C.

Identification of volatile compounds was achieved by comparing mass spectra with the Wiley library (Hewlett-Packard). The volatile compounds were also identified by matching the retention indices (RI) calculated according to the equation of Van den Dool and Kratz[11] and based on a series of alkanes. The relative concentrations of the investigated compounds were calculated by relating the areas of the internal standard (2-methyl-3-heptanone) to the areas of the compounds of interest. Odour activity values (OAVs) were calculated from the relative concentrations of the volatile compounds divided by their odour-threshold values in water.

Statistical analysis

A completely randomized design consisting of 3 replicates was used. Analysis of variance was conducted on the data, with samples (broken rice, 24 h fermented rice, 48 h fermented rice, sedimentation flour, drained wet flour, and *khanom jeen*) as factors for analysis. Duncan's multiple range test was used to differentiate mean value, with significance defined at $p \leqslant 0.05$. The statistical analysis was conducted using SPSS version 12.0.

RESULTS AND DISCUSSION

pH value and titratable acidity of samples during the natural *khanom jeen* fermentation process

The changes in pH value and titratable acidity during the fermentation process of *khanom jeen* are given in Fig. 1. The pH of broken rice dramatically decreased to 4.06 over 48 h of rice fermentation, and then decreased slightly more, to 3.81, after being made into noodles. This is close to the pH value of the fermented supernatant of *mifen* (around 4)[2,12]. After fermentation for 48 h, the titratable acidity was decreased due to the effect of washing with water. Maximum acidity was reached after 72 h (0.94% as lactic acid). The acidity was greatly decreased by cooking the noodles in boiling water. These results indicated that fermentation of *khanom jeen* might be dominated by LAB. As reported by Tanasupawat and Komagata[13], *Lactobacillus plantarum*, *L. fermentum*, and *Pediococcus acidilactici* were the bacteria predominantly found in *khanom jeen*.

Volatile components of *khanom jeen*

From the volatile analysis of *khanom jeen* samples, 43 compounds were identified using gas chromatography mass spectrometry. These compounds could then be grouped into five major chemical classes (Table 1). Alcohols were the most abundant among the volatiles of *khanom jeen*, constituting 73% of the total volatiles. Acids (18%) and ketones (7%) represented the second and third largest classes.

The most abundant alcohol in the headspace of volatiles in *khanom jeen* samples was 1-propanol. Ethanol, 3-methyl-1-butanol, and 2-butanol were also found. Due to their high odour threshold, these

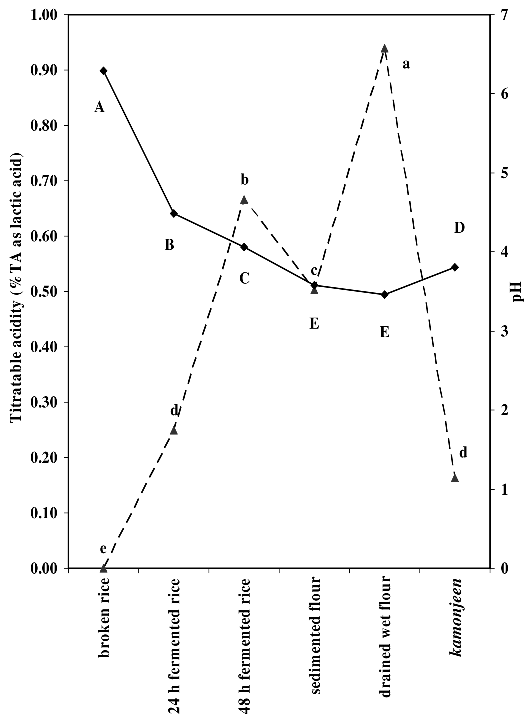

Fig. 1 Evolution of titratable acidity (%TA as lactic acid, --▲--) and pH (–⧫–) value during natural fermentation of *khanom jeen*. Different lower case letters show significant differences ($p \leqslant 0.05$) for TA value. Different upper case letters show significant differences ($p \leqslant 0.05$) for pH value.

Table 1 Quantitative data, odour thresholds and odour activity values (OAV) of *khanom jeen* volatile compounds.

Volatile compounds	RI	OT (ng/g)	Conc. (ng/g)	OAV
2-propanol	913	120 000[14]	5.09	0.00
Ethanol	935	4510[15]	1930	0.43
2-butanol	1028	3300[15]	941	0.29
1-propanol	1049	5700[14]	3630	0.64
2-methylpropanol	1102	8200[14]	21.3	0.00
2-propenol	1125	n.a.	23.5	n.a.
1-butanol	1150	500[16]	12.8	0.03
3-methyl-1-butanol	1208	300[16]	112	0.37
1-pentanol	1250	4000[16]	8.36	0.00
3-methyl-3-buten-1-ol	1252	n.a.	3.13	n.a.
1-hexanol	> 1300	2500[16]	13.5	0.01
2-ethylhexanol	> 1300	830[14]	3.93	0.00
acetic acid	> 1300	22 000[17]	663	0.03
propanoic acid	> 1300	2000[5]	6.52	0.00
butanoic acid	> 1300	240[17]	4.31	0.02
2-methylpropanoic acid	> 1300	50[17]	633	12.7
3-methylbutanoic acid	> 1300	250[17]	335	1.34
acetone	787	120 000[14]	2.14	0.00
2-butanone	891	17 000[14]	251	0.01
diacetyl	977	3[17]	154	51.3
2-pentanone	983	1380[15]	6.38	0.00
3-heptanone	1157	140[17]	1.04	0.01
acetoin	1288	8000[5]	224	0.03
6-methyl-5-hepten-2-one	> 1300	50[16]	14.8	0.30
methyl acetate	806	1500[14]	3.47	0.00
ethyl acetate	871	5[18]	32.6	6.52
ethyl propanoate	951	10[18]	1.41	0.14
propyl acetate	970	4700[19]	10.6	0.00
ethyl valerate	1136	1.5[18]	4.08	2.72
ethyl hexanoate	1234	1[18]	4.94	4.94
ethyl heptanoate	> 1300	2.2[20]	6.58	2.99
ethyl lactate	> 1300	580[19]	11.2	0.02
propyl hexanoate	> 1300	n.a.	4.56	n.a.
Propanal	748	10[16]	1.66	0.17
Butanal	849	9[16]	1.17	0.13
Hexanal	1086	5[16]	4.27	0.85
Heptanal	1189	3[16]	4.23	1.41
Octanal	1293	0.7[16]	6.39	9.13
Nonanal	> 1300	1[16]	16.1	16.1
Decanal	> 1300	2[16]	6.99	3.50
3-methylbutanal	909	0.2[5]	10.1	50.7
Benzaldehyde	> 1300	350[5]	7.48	0.02
sulphur dioxide	826	n.a.	4.29	n.a.
Total			9130	

RI = retention index on capillary FFAP
OT = odour threshold in water
n.a. = not available

volatiles have a low contributory activity to the aroma (OAV $<$ 1). However, they might be important to the overall flavour due to odourant interactions in mixtures[21].

Short-chain aliphatic acids found in *khanom jeen* have been previously reported in rice flavour volatiles as a result of oxidative degradation of lipids and thermal decomposition, except for 2-methylpropanoic acid and 3-methylbutanoic acid[22]. These two compounds with high OAVs probably contribute to the aroma of *khanom jeen*. Their aromas have been described as 'sweaty' and 'cheesy'. The compounds are also found in sourdough bread[23,24] and many cheeses[25]. In addition, acetic acid was found in substantial amounts, but the OAV was less than 1.

Several carbonyl compounds were determined. *Khanom jeen* samples contained high concentrations of some ketone compounds, e.g., 2-butanone, diacetyl, and acetoin. The latter two ketones have a buttery aroma and are produced by microorganisms from various enzymes. Sourdough bread, which is fermented using starter culture and sourdough yeast, typically contains these ketones[26], as does *togwa*, a Tanzanian fermented maize-sorghum gruel[27]. On the basis of the OAV, diacetyl alone is possibly responsible for the flavour of *khanom jeen*. Furthermore, low levels of straight-chain aldehydes were found in *khanom jeen*. The OAVs of the minor components (heptanal, octanal, nonanal, and decanal) were higher than 1. These aldehydes are recognized as producing an 'old rice' aroma[28] and are usually found in stored rice[29–31]. The aldehydes 3-methylbutanal and benzaldehyde, which are probably formed by fermentation, were also found in *khanom jeen*, with 3-methylbutanal having the highest OAV among the aldehydes. This malty compound has been previously found in many fermented cereal products[23,27,32].

Influence of fermentation on volatiles

To establish the effect of fermentation on *khanom jeen* volatiles, we determined the changes in volatile composition during the fermentation process (Fig. 2). The highest increase of volatile components occurred after 24 h of fermentation, especially in the case of alcohols, acids, and ketones. The alcohols and acids slightly decreased during the next day of fermentation due to the water washing effect. Esters developed at every fermentation step, but their presence was lower in *khanom jeen* samples, unlike aldehydes, whose amounts in *khanom jeen* samples were higher.

Potent aroma compounds (OAV > 1) which were used to follow the changes at different fermentation steps are listed in Table 2. In particular, the volatiles 2-methylpropanoic acid and 3-methylbutanoic acid appear to form during the fermentation process, as indicated by the fact that they were not found in broken rice samples, but developed after fermentation for 24 h and then significantly decreased during the sedimentation step. The dominant species identified in *khanom jeen* were similar to *mifen*, in which the primary stage of rice fermentation was dominated by LAB and yeast[12]. Hence, these volatiles may result from the metabolic activities of LAB and yeast. A wide range of esters and aldehydes are synthesized, and these contribute to the flavour of many fermented cereals[33,34]. A high content of 2-methylpropanoic acid and 3-methylbutanoic acid was found in wheat bread made from sourdough fermented with *Lacto-*

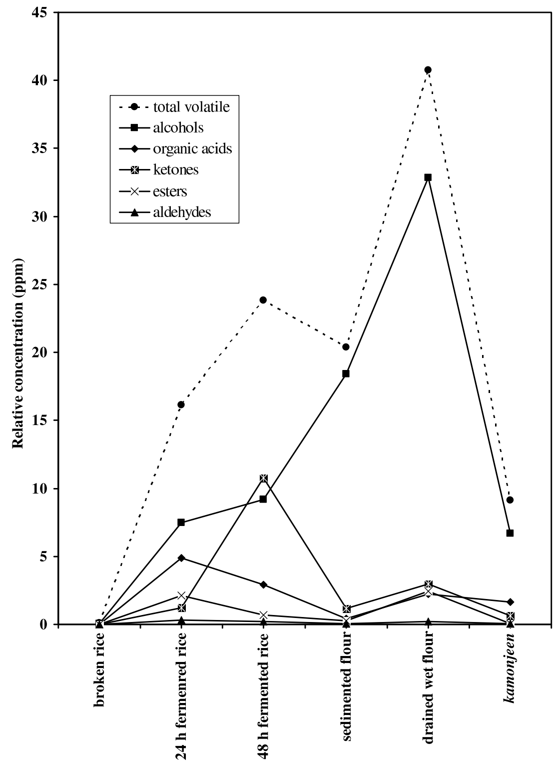

Fig. 2 Changes in concentrations of volatile compounds during the *khanom jeen* fermentation process.

Table 2 Changes in concentrations of selected volatile compounds during the *khanom jeen* fermentation process.

Volatile compounds	Concentration (ng/g)					
	Broken rice	24 h fermented rice	48 h fermented rice	Sedimented flour	Drained wet flour	Khanom jeen
2-methylpropanoic acid	n.d.	443^{b}	578^{b}	65.7^{a}	590^{b}	633^{b}
3-methylbutanoic acid	n.d.	282^{c}	185^{bc}	46.9^{ab}	330^{c}	335^{c}
diacetyl	tr^{a}	311^{b}	2420^{c}	49.0^{a}	325^{b}	154^{ab}
ethyl acetate	8.27^{a}	871^{c}	365^{b}	140^{a}	1860^{d}	32.6^{a}
ethyl valerate	tr^{a}	tr^{a}	tr^{a}	7.84^{b}	22.9^{c}	4.08^{ab}
ethyl hexanoate	tr^{a}	tr^{a}	tr^{a}	15.4^{c}	12.9^{c}	4.94^{b}
ethyl heptanoate	tr^{a}	tr^{a}	tr^{a}	6.78^{c}	4.46^{b}	6.58^{c}
heptanal	2.38^{b}	5.10^{c}	2.75^{b}	tr^{a}	tr^{a}	4.23^{c}
octanal	tr^{a}	tr^{a}	tr^{a}	tr^{a}	tr^{a}	6.39^{b}
nonanal	6.00^{a}	11.0^{a}	14.9^{a}	4.89^{a}	5.69^{a}	16.1^{a}
decanal	tr^{a}	tr^{a}	8.67^{b}	7.17^{b}	tr^{a}	6.99^{b}
3-methylbutanal	tr^{a}	182^{c}	66.2^{b}	tr^{a}	tr^{a}	10.1^{a}

n.d. = not detected; tr = trace (< 0.001); differing superscripts in a row indicate significant differences of the means.

bacillus plantarum[35].

Furthermore, similarities and differences of the volatiles were observed. Diacetyl content significantly increased with the time of rice fermentation, and slightly decreased during the sedimentation step. Diacetyl is a metabolic end product from citrate or amino acid catabolism by LAB[36,37]. Among esters, ethyl acetate was detected in all samples, and underwent changes in concentration similar to 2-methylpropanoic acid and 3-methylbutanoic acid. Its level reached a maximum during the weighting step. Other esters significantly increased during the sedimentation step. The presence of ethyl acetate is probably the result of yeast metabolism[38] and heterofermentative LAB[34].

Straight-chain aldehydes were found in the raw material, and showed slight changes in concentrations during the fermentation process. These aldehydes are usually found in broken rice which has surface lipids and free fatty acids[29]. During the boiling step, lipids in the flour slurry can liberated by thermal decomposition[39] and the dissociation of the inclusion complexes between lipids and starches during cooking might develop the lipid-derived volatiles[40]. An important aldehyde which might be produced from the fermentation process is 3-methylbutanal. The influence of microorganisms on the formation of 3-methylbutanal in sourdough was previously reported by Czerny and Schieberle[23]. The amount of leucine metabolite 3-methylbutanal in fermented sourdough was 5 times greater than in the flour. In *togwa*, the concentration of 3-methylbutanal was detected in different ranges according to different LAB species[27]. Moreover, 3-methylbutanal was found to be an important factor in the aroma of certain cheese products, and is derived from amino acids via α-keto acids by the transaminase pathway[41]. The presence of selected aromatic volatiles indicated that the fermentation steps in the making of *khanom jeen* might play an important role in its aroma (Table 2).

CONCLUSIONS

The results of this study clearly show that the fermentation process of broken rice is necessary to obtain the characteristic aroma of the final product, *khanom jeen*. Several flavour compounds are formed during the fermentation process, including compounds from lipid oxidation. The compounds originate from both microbial metabolism and the raw material itself. According to their OAV and odour description, they seem to have a significant influence on the aroma of *khanom jeen*.

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