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Volatile flavour compounds analysis of solid state fermented Thai rice wine (*Ou*)

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ABSTRACT: Solid-state fermented Thai rice wines (*Ou*) were analysed to determine their chemical components. Determined parameters were pH (4.5–5.5), protein (0.45–0.99 g/100g), ash (0.10–0.30 g/100g), total solid (1.72–14.34 g/100g), glucose (4.07–7.91 mg/ml) contents and volatile compounds. The ethanol concentration was in the range of 12.15 to 104.60 mg/ml. Profiles of volatile compounds were analysed by dynamic headspace coupled with gas chromatography mass spectrometry and gas chromatography olfactometry. The potent odours were alcoholic and solvent-like, sweet, fruity, buttery, and pungent aromas. The concentration in *Ou* of *n*-propanol, *iso*-butyl alcohol, *iso*-amyl alcohol, furfuryl alcohol, benzene ethanol, acetol, 2,3-butanediol, glycerol, ethyl lactate, acetoin, furfural, 5-methyl furfural, 5-hydroxymethyl-2-furfural, and acetic acid were also determined by a direct injection technique. From principal component analysis, *Ou* samples could be categorized into two groups based on the concentration of ethanol and their profiles of volatile compounds.

KEYWORDS: Thai rice wine, *Ou*, flavour profile, dynamic headspace, gas chromatography-olfactometry, principal component analysis

INTRODUCTION

Rice wines are widely produced in Asian countries such as Japan (*Sake*), China (*Jiu*), Korea (*Yakju*), Philippines (*Tapuy*), Vietnam (*Ruou nep than*), Malaysia (*Tapai*), and Thailand (*Sato*, *Krachae* and *Ou*)[1]. The varieties of rice wines depend on raw materials, inocula and brewing processes. Generally, rice wine fermentation can be categorised into submerged and solid state process. In the case of submerged process, barley and rice are used as substrates for the fermentation[2]. The inocula for rice wine brewing are fungi, such as *Aspergillus oryzae*. *A. sojae*, and *Rhizopus* spp., and yeasts, such as *Saccharomyces cerevisiae*, *S. sake, S. fibuligera*, *Hansenula mrakii* and *Pichia polymorpha*[1]. Traditionally, microbial starters are used for saccharification. However, in Japanese rice wine (*Sake*) sprouting rice is also used for this purpose[3]. Following saccharification of rice, the sugar liberated, especially glucose, is then converted to ethanol by submerged fermentation of yeasts.

Ou is a Thai rice wine produced from solid state fermentation. This kind of rice wine is also produced in other Southeast Asian countries such as Cambodia, Laos and Vietnam[1]. In *Ou* fermentation, solid state fermentation is employed for both saccharification and ethanol production. To make *Ou*, glutinous rice is first soaked in water overnight. Then, the glutinous rice and washed rice husk are mixed together in a ratio of 1:1 and cooked by steaming. The rice husk is added to maintain the moisture during solid–state fermentation. The mixture is cooled, mixed with *Loog-paeng* powder (a dry starter culture) and incubated at room temperature for 24 hours. This step is similar to *Koji* production in Japanese *Sake* fermentation except for the strains of microorganisms used[4]. The dry starter culture consists of fungi (*Aspergillus* sp. and *Rhizopus* sp.), yeast (*S. cerevisiae*) and herbs[5]. The mixture is then transferred into an earthenware jar, covered and tightly sealed with either banana leaves or a plastic sheet. The jar is left at room temperature to ferment for more than a week. To drink *Ou*, water is poured into the opened jar to elute the fermented content. A narrow bamboo stem is then inserted into the rice mixture to allow the sucking of *Ou* from the jar. The elution can be made several times in this manner.

In Thailand, all rice wines are produced by various traditional methods. This was practiced illegally until the government relaxed its liquor production laws. This has resulted in an incentive for legal mass production. This work is a report on chemical analyses based on proximate analysis and volatile flavour compounds of *Ou*. These data are expected to be valuable for standard setting of this product and other related products.

MATERIALS AND METHODS

Samples

Ou samples were obtained from three regions in Thailand where the wines are traditionally produced, namely, the northern, north-eastern, and central regions (Table 1). Three earthenware jars were purchased from each manufacturer. One litre of deodourised water[6] was poured into the earthenware jar. *Ou* was then extracted by a peristaltic pump equipped with a silicone tube and kept at -20 °C until further analysis.

Chemicals

All standard volatile compounds with purity grade higher than 98% were purchased from Aldrich Chemical (St.Louis, MO) and Fluka (Buchs, Switzerland). Other chemicals were HPLC grade or of the best grade available from Merck (Darmstadt, Germany). The helium gas was ultra high purity.

Analysis for protein, total solid, and ash contents

Protein content of *Ou* samples was determined by Kjeldahl's method following AOAC Official[7] No. 981.10 with a conversion factor of 6.25. Total solid and ash content was determined according to AOAC Official[7] No. 925.10 and 920.67, respectively. These analyses were performed with triplicate measurements.

Glucose content

One ml of *Ou* was centrifuged at 5,000 *g* for 10 min. (Sigma 202 centrifuge, Harz, Germany) to precipitate insoluble particles. To deproteinize, 0.5 ml of supernatant was added to 0.5 ml of cold methanol, which was then filtered through a cellulose acetate membrane (0.45 μm). A high performance liquid chromatograph (Water 2690 Separation module, MA, USA) equipped with an Hypersil APS2 column (250 × 4.6 mm, 5 μm; Hypersil, UK) was used to analyse the glucose of *Ou* samples. An isocratic elution was performed by using a mixture of acetonitrile and water (80:20; v/v) as mobile phase with a flow rate of 1 ml/min at 40 °C. A refractive index detector was used[8].

Table 1. The origin of *Ou* samples

Regions of Thailand	City, Province	Sample
Middle	Muang, Ratchaburi	A
	Muang, Lop Buri	B
Northern	Weang Nong Long, Lamphun	C
	Muang, Nan	D
North-eastern	Ranu Nakhon, Nakhon Phanom*	E, F, G
	Phimai, Nakhon Ratchasima	H
	Muang, Ubon Ratchathani	I
	Reung Nok Ta, Yasothon	J

* Nakhon Phanom samples were obtained from different manufactures in the same village.

Determination of ethanol by static headspace technique

Determination of ethanol concentration was conducted according to the method of Otero *et al*[9] with modification. One ml of *Ou* saturated with sodium chloride was put into a 20-ml headspace vial with 50 μl of 1,4-dioxane added as an internal standard. The vial was sealed with PTFE-coated rubber septum, and placed in a headspace autosampler (HP 7694E, Agilent, USA). Headspace gas was analysed by GC-flame ionization (HP 6890A, Agilent, CA, USA) equipped with an HP-5 capillary column (5% phenylmethylpolysiloxane; 30 m × 0.32 mm i.d. × 25 μm film thickness; Hewlett-Packard). Sample injection was performed with a split ratio of 10:1. The initial oven temperature was 35 °C holding for 2 min, then programmed to 200 °C at a rate of 20 °C/min. Helium gas was used as mobile phase (1 ml/min). The flame ionization temperature was 250 °C.

Dynamic headspace analysis

Dynamic headspace analysis (DHA) was conducted according to Wanakhachornkrai and Lertsiri[6] with modification. One ml of *Ou* saturated with sodium chloride was placed in a dynamic headspace tube (15.2 ×1.6 cm i.d.) and then installed in a Tekmar Dohrmann 3100 purge and trap concentrator (Tekmar, OH,USA). Helium gas at flow rate of 40 ml/min over the headspace for 30 min was used to purge a Tenax TA trap (part no. 12-0083-303, Tekmar) and the trap was dry-purged for 2 min to remove moisture. Volatiles trapped were desorbed at 220 °C for 2 min and then directly introduced onto gas chromatography mass spectrometer (HP 6890A, Agilent, CA, USA) with split ratio of 10:1. The transfer line was maintained at 220 °C with a trap pressure of 4 psi. Volatile compounds were separated on an HP-FFAP capillary column (polyethylene glycol modified nitroterephthalic acid, 25 m × 0.32 mm i.d. × 0.50 μm film thickness, Hewlett-Packard). The initial oven temperature was 50 °C holding for 1 min, then programmed to 100 °C at a rate of 20 °C/min. The carrier gas was helium gas at a constant flow rate of 1.5 ml/min. The ionization energy of the mass spectrometer detector (HP 5973 Mass Selective Detector, Agilent, CA, USA) was 70 eV, and the mass range was 20–350 a.m.u.

Compound identification

Positive identification of a component was

performed by comparing its retention index (RI) and mass spectrum to that of authentic standard compounds. RI values of each compound were calculated from the retention times of *n*-alkanes[10]. Integration of peaks was performed using HP Chemstation software (Hewlett-Packard). The minimum peak area for detection was 100,000 counts.

Gas chromatography-olfactometry coupling dynamic headspace analysis

Gas chromatography-olfactometry (GCO) was conducted according to the method of Lee *et al*[11] with modification. One ml of *Ou* was placed into a 25-ml headspace sampling tube and purged with helium gas at 40 ml/min to a Tenax TA trap. After desorption, volatiles were directly transferred to the gas chromatography-olfactometer. The system consisted of an HP 6890A GC, a flame ionization detector (FID), and a sniffing port (Olfactometer ODO II; SGE Incorporation, USA) that was supplied with humidified air at 40 °C. High purity nitrogen was supplied to the column end at a flow rate of 70 ml/min to split each fraction into a ratio of 1:10 to both sniffing port and FID. Other GC conditions were the same as described above.

Quantitative analysis of volatile compounds

Volatile compounds were quantified by using their correction factors[12]. Direct injection was used to determine the concentrations of 14 compounds: *n*-propanol, *iso*-butyl alcohol, *iso*-amyl alcohol, furfuryl alcohol, benzene ethanol, furfural, 5-methyl furfural, 5-hydroxy methyl furfural, acetic acid, acetol, acetoin, ethyl lactate, glycerol, and 2,3-butanediol. The 14 standard compounds were diluted in absolute ethanol. Correction factors of authentic standard compounds were determined by triplicate injection of 1 µl of a mixture of standard and internal standard (1,4-dioxane) into a GCMS (HP 6890A, Agilent, USA) equipped with an HP-FFAP capillary column. The injection was performed in split mode with a split ratio of 10:1. The injector temperature was 220 ºC. The initial oven temperature was 35 °C holding for 1.25 min, then programmed to 120 °C at a rate of 20 °C/min and held for 1 min. Finally, the temperature was raised to 220 ºC at a rate of 20 ºC/min and held for 3.50 min. The carrier gas was helium at a constant flow of 2.0 ml/min. A mass selective detector was used as described above 5 ml of 1,4-dioxane was added into 500 µl of each *Ou* sample by a microsyringe, and centrifuged at 5,000 *g* for 30 min. A supernatant was collected and filtered through a cellulose acetate membrane (0.45 µm) prior to the GC analysis.

Table 2. The pH, protein, ash, total solid, glucose, and ethanol content in *Ou* samples

Sample	pH	Protein (g/100 g)	Ash (g/100 g)	Total solid (g/100 g)	Glucose (mg/ml)	Ethanol (mg/ml)
A	5.51 (0.01)	0.73 (0.00)	0.10 (0.00)	14.34 (2.08)	4.79 (0.12)	12.15 (0.15)
B	4.50 (0.00)	0.71 (0.05)	0.26 (0.11)	6.76 (0.05)	4.07 (0.32)	99.57 (0.44)
C	4.53 (0.06)	0.45 (0.00)	0.21 (0.00)	2.71 (0.31)	4.71 (0.20)	37.01 (0.54)
D	4.63 (0.06)	0.66 (0.01)	0.29 (0.00)	3.69 (0.16)	4.55 (0.09)	32.06 (0.12)
E	4.52 (0.03)	0.99 (0.00)	0.25 (0.00)	6.43 (0.34)	4.94 (0.25)	92.66 (0.64)
F	4.55 (0.05)	0.79 (0.01)	0.20 (0.00)	1.72 (0.23)	4.64 (0.05)	104.60 (0.92)
G	4.49 (0.02)	0.94 (0.03)	0.21 (0.00)	8.27 (2.62)	7.91 (0.51)	66.60 (0.18)
H	4.50 (0.00)	0.67 (0.00)	0.29 (0.00)	6.22 (1.47)	4.21 (0.31)	57.58 (2.16)
I	4.58 (0.03)	0.80 (0.03)	0.28 (0.16)	5.95 (0.14)	4.51 (0.57)	57.19 (0.76)
J	5.43 (0.06)	0.66 (0.00)	0.30 (0.00)	3.63 (0.21)	4.14 (0.29)	92.61 (0.89)

Numbers in parentheses are standard deviation of three measurements.

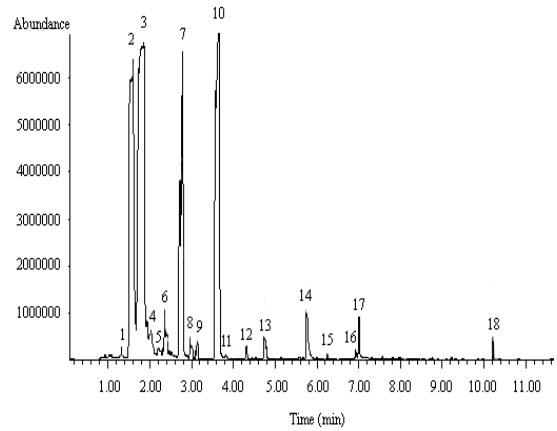

Fig. 1 Typical GC chromatogram of volatile flavour compounds analysed by DHA (Sample B).

Data analysis

Principal component analysis (PCA) was conducted by XLSTAT 2006 version 2006.3 (license ID: 0006747). The PCA was applied to the analytical data based on the Pearson correlation matrix. Factors with values greater than 1 were selected. The verimax rotation method was applied.

RESULTS AND DISCUSSION

Proximate analyses and glucose and ethanol determinations in *Ou* samples showed that most of the analytical values varied slightly except for totalsolid and ethanol contents (Table 2). In the case of Japanese *Sake*, the ethanol content exceeded 109–121 mg/ml[13]. After one month of fermentation, the ethanol concentration of sample F reached 104.6 mg/ml, while sample A gave the lowest amount (12.15 mg/ml). Based on the Thai Community Product Standard, the

Ministry of Industry requires that the ethanol concentration of *Ou* is lower than 118 mg/l[14]. The ethanol concentration of all *Ou* samples was within specification. Ethanol in *Ou* was generated anaerobically by glucose metabolism of *Saccharomyces cerevesiae* after starch from the rice was firstly hydrolysed by fungal amylase. The amylase was produced during the early stage of the fermentation when aerobic conditions were still available in air gaps between the rice husks[5]. Hence the low amylolytic activity during fermentation could result in high total solid content in the sample due to the high level of starch remaining. Protein content of *Ou* samples was as low as that in Japanese *Sake* (0.3–0.5 g/100g)[15]. This was lower than the protein content in wine (1.5–2.3 g/100g)[16]. This protein was possibly liberated from yeast cells and raw materials such as rice during the fermentation.

Volatile compounds in *Ou* samples were studied using both DHA coupled with gas chromatography mass spectrometry (GCMS) and GCO together with direct injection of *Ou* into the spectrometer. Using DHA, volatile analytes were continuously purged from the sample and were trapped on the adsorbent. This analysis provided qualitative data of volatile compounds in terms of compound identification by GCMS and odour characterization by GCO (Table 3). Similar profiles of volatile flavour compounds from 10 *Ou* samples were obtained. Eighteen compounds were commonly found in all samples (Fig. 1): 7 kinds of alcohols, 5 esters, 3 ketones, 2 acids, and 1 furan. These compounds were also detectable in Japanese *Sake*[15]. Ethanol, *iso*-butyl alcohol, *iso*-amyl alcohol, and ethyl acetate predominated on the GC chromatogram because their volatilities were higher than the others[7]. Among the compound detected, GCO was used to recognise the odour-active compounds in *Ou* samples. Their odour attributes were similar as described previously[17]. As a result (Table 3), the odour active compounds in *Ou* samples were categorized into 4 groups based on their odour characteristics, namely, alcohol and solvent-like, sweet and fruity, buttery, and pungent aromas. Alcoholic compounds including ethyl acetate and acetone presented alcohol and solvent-like aromas. Ester compounds mostly showed sweet and fruity aromas. In Japanese *Sake*, esters give rise to a fruity aroma[2]. Furfural also produced a sweet aroma. Diacetyl and acetoin generated a buttery aroma. Pungent aromas resulted from acetic and *iso*-butyric acids. Apart from ethanol, higher alcohols including furfurals, acetic acid, acetoin, and ethyl lactate were quantified by GCMS. The components of higher alcohol in *Ou* were similar to those of Japanese *Sake*, white wine and red wine[15,18,19] (Table 4). The amounts of these higher alcohols in *Ou* were at the level of 35–270 mg/l which was lower than the amounts in Japanese *Sake* (68–633 mg/l)[2], wine (87–564 mg/l) and beer (54–715 mg/l)[20]. The content of these compounds are influenced by raw materials and fermentation conditions[21]. Such higher alcohols were probably formed from both branched-chain amino acid

Table 3. Volatile flavour compounds in *Ou* samples analysed by DHS technique and their attributes from GC-O analysis

Compounds		Peak number	RI	Attributes from GC-O analysis	Peak area (counts)									
					A	B	C	D	E	F	G	H	I	J
Alcohols	Ethanol	3	946	Alcohol-like	8138328 (19.69)	69819090 (31.62)	64294627 (34.62)	76774381 (37.58)	64084493 (31.25)	67490727 (33.83)	71455151 (35.50)	70943785 (31.81)	76324259 (30.73)	65987731 (34.26)
	n-Propanol	6	1055	Solvent-like	4546279 (11.00)	5244536 (2.38)	3075673 (1.66)	8084747 (3.96)	2897271 (1.41)	4661533 (2.34)	7782851 (3.87)	5185709 (2.32)	4539283 (1.83)	5274175 (2.74)
	iso-Butyl alcohol	7	1107	Wine-like	4725272 (11.43)	28422592 (12.87)	14278768 (7.69)	20422770 (10.00)	16494870 (8.04)	27152698 (13.61)	30478796 (15.14)	27155091 (12.17)	24203824 (9.74)	29793038 (15.47)
	n-Butanol	9	1156	Solvent-like	465521 (1.13)	1718983 (0.78)	3836669 (2.07)	1176205 (0.58)	572055 (0.28)	945204 (0.47)	1598518 (0.79)	1689560 (0.76)	1806543 (0.73)	1107603 (0.57)
	iso-Amyl alcohol	10	1215	Whiskey-like	5197258 (12.57)	47345183 (21.44)	26168705 (14.09)	29381670 (14.38)	30674689 (14.96)	38253510 (19.18)	39984924 (19.86)	43928418 (19.69)	42347092 (17.05)	39962564 (20.75)
	2,3-Butanediol	17	>1493	Solvent-like	725288 (1.75)	5344444 (2.42)	5009029 (2.70)	2941458 (1.44)	7960517 (3.88)	1592969 (0.80)	3222549 (1.60)	6693514 (3.00)	7710295 (3.10)	2814380 (1.46)
	Benzene ethanol	18	>1493	Sweet	800527 (1.94)	2229156 (1.01)	426802 (0.23)	107112 (0.05)	187483 (0.09)	910706 (0.46)	996124 (0.49)	1434074 (0.64)	759758 (0.31)	1114040 (0.58)
Esters	Ethyl acetate	2	904	Solvent-like	6386544 (15.45)	45657194 (20.68)	36871840 (19.85)	43543300 (21.31)	49932871 (24.35)	45168530 (22.64)	30555513 (15.18)	47655649 (21.36)	47384404 (19.08)	35969871 (18.67)
	iso-Butyl acetate	5	1028	Sweet	222377 (0.54)	1224143 (0.55)	859187 (0.46)	922503 (0.45)	2055729 (1.00)	1166998 (0.59)	1105893 (0.55)	1615695 (0.72)	1735265 (0.70)	956397 (0.50)
	iso-Amyl acetate	8	1139	Fruity	127034 (0.31)	1985373 (0.90)	615034 (0.33)	752041 (0.37)	3678487 (1.79)	1471016 (0.74)	1510382 (0.75)	2901675 (1.30)	2162928 (0.87)	1149745 (0.60)
	Ethyl pyruvate	11	1283	Sweet	107587 (0.26)	135378 (0.06)	106234 (0.06)	116802 (0.06)	159948 (0.08)	207256 (0.10)	101846 (0.05)	100213 (0.04)	104822 (0.04)	98350 (0.05)
	Ethyl lactate	13	1353	Fruity	3509422 (8.49)	2521525 (1.14)	7342743 (3.95)	700216 (0.34)	8440922 (4.12)	2548078 (1.28)	1286249 (0.64)	2392704 (1.07)	2723701 (1.10)	4481067 (2.33)
Ketones	Acetone	1	<904	Solvent-like	327850 (0.79)	539209 (0.24)	<100000 (<0.05)	395671 (0.19)	557875 (0.27)	470917 (0.24)	659400 (0.33)	601334 (0.27)	11681531 (4.70)	<100000 (<0.05)
	Diacetyl (2,3-Butanedione)	4	1002	Buttery	1505551 (3.64)	1712424 (0.78)	4883717 (2.63)	1871777 (0.92)	1524381 (0.74)	864037 (0.43)	4571571 (2.27)	2104958 (0.94)	5831300 (2.35)	1148562 (0.60)
	Acetoin (3-Hydroxy-2-butanone)	12	1306	Buttery	2298028 (5.56)	631829 (0.29)	5723387 (3.08)	226599 (0.11)	328084 (0.16)	135919 (0.07)	1613369 (0.80)	925383 (0.41)	2363315 (0.95)	178133 (0.09)
Acids	Acetic acid	14	1464	Pungent	1210239 (2.93)	5733694 (2.60)	11584408 (6.24)	10815017 (5.29)	14788733 (7.21)	6121064 (3.07)	3799164 (1.89)	7207078 (3.23)	14684189 (5.91)	2027131 (1.05)
	iso-Butyric acid	16	>1493	Pungent	199606 (0.48)	418029 (0.19)	408659 (0.22)	148347 (0.07)	509388 (0.25)	209443 (0.10)	375594 (0.19)	419882 (0.19)	1358791 (0.55)	255295 (0.13)
Furans	Furfural	15	1493	Sweet	840947 (0.03)	107862 (0.05)	144943 (0.08)	5935919 (0.91)	205138 (0.10)	<100000 (<0.05)	185558 (0.09)	101394 (0.05)	656314 (0.26)	216181 (0.11)

Numbers in parentheses are percentage of peak areas.

precursors and glucose metabolism of yeast [22]. Higher alcohols give flavouring aroma, but high concentrations of *iso*-amyl alcohols are toxic[4]. These compounds are derived from *iso*-amyl alcohol and acetyl coenzyme A by alcohol acetyl transferase in yeast[23]. Benzene ethanol is the only higher alcohol giving pleasant attributes such as rose-like and sweet odours. Table 4 shows benzene ethanol in the amount of 13–95 mg/l. This compound is also detected in sherry wine (52 mg/l)[24] and Pinot noir wine (47.7–53.8 mg/l)[25]. It is derived from L-phenylalanine through metabolic reaction of *S. cerevisiae* during carbonic anaerobiosis[21].

Acetic acid in *Ou* was higher than in Japanese *Sake* (50-350 mg/l)[26]. This indicated the existence of acetic acid-producing bacteria such as *Acetobacter* sp. either in the starter culture or an environment of the brewery as reported by Phithakpol *et al*[5]. This acid is produced by oxidation of ethanol to acetaldehyde followed by oxidation of acetaldehyde to acetate in *Acetobacter* sp[15]. Acetoin, one of the flavour constituents in wine, was also found in *Ou* samples (except in sample C) at the same level as table wine (2 to 32 mg/l)[22]. Concentrations of this compound were reported to be in the range of 1.7–9.4 mg/l in Japanese *Sake*[27]. It is well known that acetoin in wine is produced by *S. cerevisiae* in the early phase of fermentation, and rapidly declines in the final stage of the process as it is reduced to 2,3-butanediol[28]. In *Ou*, the amount of 2,3-butanediol was 7–160 times higher than acetoin.

In order to explain the chemical characteristics and grouping of the samples, the parameters in Tables 2 and 4 were analysed by PCA. Components I and II explained 55.2% and 35.8% of the total variance, respectively. Component I composed of ethanol, *iso*-amyl alcohol, *iso*-butyl alcohol, 2,3-butanediol, 5-hydroxymethyl-2-furfural, 5-methyl furfural, furfural concentrations, ash, glucose, pH, and total solid contents. Acetic acid, furfuryl alcohol, acetol, glycerol, ethyl lactate, benzene ethanol, *n*-propanol, acetoin concentrations, and protein contents predominated in Component II. The Pearson correlations showed strong correlations ($p \leq 0.05$) among ethanol, *iso*-amyl alcohol (0.779), and *iso*-butyl alcohol (0.733). The correlations between these compounds might be explained by their common precursors in biosynthesis pathways. These compounds are generally formed by *S.cerevisiae* in a pathway using pyruvic acid as a precursor[28]. Furthermore, these compounds were important precursors of other odour active compounds in *Ou*. On the other hand, no correlation was found between glucose, which was the substrate of ethanol fermentation, and ethanol. Similarly, there was no correlation found between ethanol, the substrate for acetic acid production, and acetic acid. By Pearson correlations, both significant correlations of 5-hydroxy-2-methyl furfural with furfural and 5-methyl furfural were 0.810 and 0.741, respectively ($p \leq 0.05$). It can be hypothesized that these compounds originated in the same biological pathway. Factor extraction using PCA was applied to define the group of *Ou* samples. *Ou* samples were categorized into two groups (Fig. 2). Group I was composed of samples from the manufacture of A, D, G, and H. This group had low concentrations of ethanol (12.2–66.6 mg/l). High concentrations of

Table 4. Concentrations of volatile compounds in *Ou* samples by direct injection technique

Compounds		RI	Concentration (mg/l) (SD)									
			A	B	C	D	E	F	G	H	I	J
Alcohols	*n*-Propanol	1055	23.52 (0.33)	63.17 (2.34)	17.08 (0.09)	100.97 (2.79)	34.96 (0.14)	58.11 (0.90)	79.79 (0.55)	70.68 (0.80)	33.01 (0.37)	80.50 (0.23)
	iso-Butyl alcohol	1107	25.41 (0.32)	103.55 (0.98)	21.10 (0.36)	44.12 (1.44)	56.41 (1.41)	94.06 (2.80)	78.85 (0.67)	102.46 (1.30)	43.71 (1.09)	144.4 (2.76)
	iso-Amyl alcohol	1215	35.70 (0.46)	226.91 (3.02)	50.48 (1.37)	83.41 (1.85)	126.86 (2.17)	147.84 (5.33)	141.81 (2.24)	174.34 (0.24)	133.23 (1.42)	267.34 (8.35)
	2,3-Butanediol	>1493	228.64 (2.87)	821.77 (22.99)	849.37 (29.15)	743.94 (9.33)	872.84 (19.81)	647.15 (4.63)	853.99 (16.73)	752.56 (22.56)	2035.03 (5.22)	554.15 (6.37)
	Furfuryl alcohol	>1493	286.15 (6.69)	169.16 (2.93)	82.92 (1.44)	277.51 (1.38)	223.96 (5.74)	4.33 (0.02)	466.87 (3.70)	990.50 (6.21)	159.43 (6.49)	137.00 (6.01)
	Benzene ethanol	>1493	27.58 (0.84)	61.98 (2.00)	13.70 (0.37)	33.26 (0.40)	44.07 (1.47)	47.59 (1.04)	59.02 (1.06)	94.84 (2.62)	46.70 (0.93)	80.74 (0.68)
	Glycerol	>1493	928.37 (9.99)	1993.22 (72.93)	879.92 (25.90)	1469.50 (8.40)	2403.50 (126.38)	1816.42 (40.85)	2154.56 (7.03)	2366.99 (97.16)	1752.24 (6.79)	1527.52 (4.64)
Ester	Ethyl lactate	1353	60.20 (4.16)	41.17 (1.78)	70.36 (3.90)	170.55 (4.90)	182.07 (4.61)	30.24 (1.65)	37.10 (1.80)	111.00 (2.38)	25.51 (2.03)	88.37 (5.38)
Acid	Acetic acid	1464	2147.99 (26.76)	2359.78 (43.93)	2112.14 (50.94)	3026.71 (39.58)	4200.20 (36.53)	1878.01 (91.54)	2448.05 (67.04)	5661.65 (112.09)	2616.87 (36.94)	1414.03 (41.66)
Ketones	Acetoin	1306	33.78 (0.70)	15.61 (0.88)	117.02 (1.54)	12.20 (0.30)	7.82 (0.17)	3.87 (0.15)	31.88 (0.86)	33.52 (1.47)	32.38 (1.48)	5.13 (0.27)
	Acetol	>1493	41.07 (0.86)	25.05 (1.07)	9.76 (0.24)	28.46 (0.45)	25.30 (0.49)	1.32 (0.03)	26.41 (1.19)	8.88 (1.73)	19.80 (0.30)	20.67 (1.01)
Furans	Furfural	1493	330.39 (7.62)	62.96 (1.48)	13.90 (1.66)	391.89 (2.27)	92.29 (3.27)	nd	586.09 (4.38)	567.62 (14.30)	84.52 (2.62)	117.93 (2.15)
	5-Methyl furfural	>1493	178.96 (2.64)	70.68 (3.25)	28.31 (1.09)	78.42 (5.80)	66.71 (1.13)	nd	181.32 (3.68)	177.42 (8.85)	165.69 (5.44)	98.63 (1.40)
	5-Hydroxymethyl-2-furfural	>1493	3781.50 (22.41)	314.79 (6.53)	69.23 (2.35)	1499.56 (8.97)	467.83 (15.26)	nd	2469.06 (5.62)	2399.15 (49.99)	518.26 (9.38)	706.80 (0.71)

nd: not detected
Numbers in parentheses are standard deviations of three measurements.

furfuryl alcohol and furan compounds were detected in samples of Group I. Group II included samples from the manufacture of B, C, E, F, I,and J. Group II had high amounts of *iso*-amyl alcohol (50–267 mg/l) and 2,3-butanediol (647–2035 mg/l), but low concentrations of furfuryl alcohol (4–223 mg/l) and benzene ethanol (13-80 mg/l). High concentrations of ethanol were also observed in this group, especially in samples B, E, F and J. From the PCA results, the presence of the samples from Nakhon Phanom in different groups was explained by the different concentrations of ethanol, benzene ethanol, acetoin, furfural, 5-methyl furfural and 5-hydroxymethyl-2-furfural. Furthermore, these samples were produced from different recipes and manufactures.

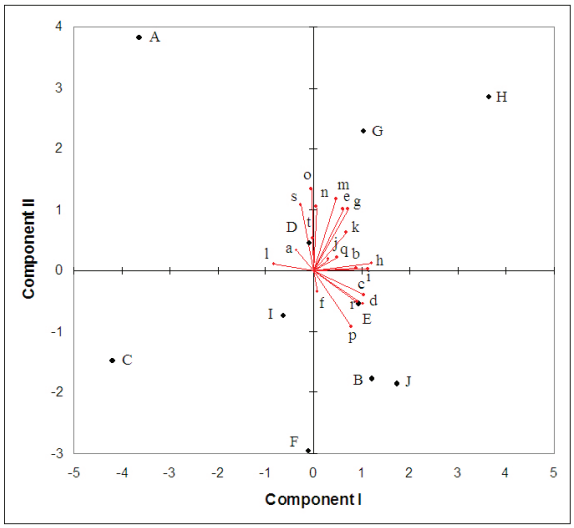

Fig. 2 Bi-plot for Principle Component Analysis (PCA) from *Ou* samples; a: pH, b: *n*-propanol, c: *iso*-butyl alcohol, d: *iso*-amyl alcohol, e: acetol, f: 2,3-butanediol, g: furfuryl alcohol, h: benzene ethanol, i: glycerol, j: ethyl lactate, k: acetic acid, l: acetoin, m: furfural, n: 5-methyl furfural, o: 5-hydroxymethyl-2-furfural, p: ethanol, q: protein, r: ash, s: total solid, and t: glucose (Sample names abbreviated by capital letters are referred in Table 1).

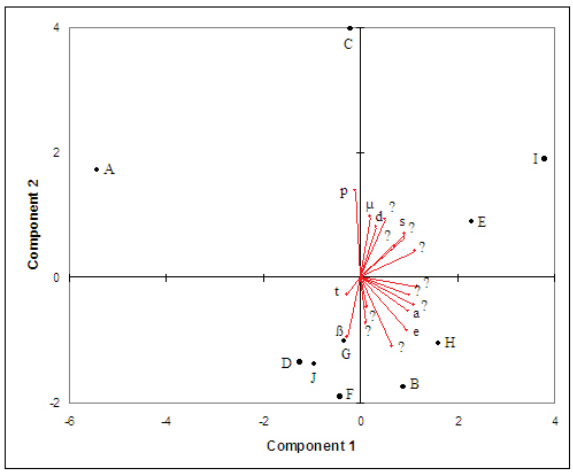

Fig. 3 Bi-plot for PCA of pear areas of volatile compounds from *Ou* samples; α: ethanol, β: *n*-propanol, γ: *iso*-butyl alcohol, δ: *n*-butanol, ε: iso-amyl alcohol, ζ: 2,3-butanediol, η: benzene ethanol, θ: ethyl acetate, ι: iso-butyl acetate, κ: iso-amyl acetate, λ: ethyl pyruvate, μ: ethyl lactate, ν: acetone, ξ: diacetyl, π: acetoin, ς: acetic acid, σ: iso-butyric acid, and τ: furfural (Sample names abbreviated by capital letters are referred in Table 1).

When peak areas of volatile compounds from Table 3 were analysed by PCA, Component 1 and Component 2 explained 82.92% and 9.45% of the variance, respectively. Component 1 was composed of ethanol, *iso*-butyl alcohol, *iso*-amyl alcohol, 2,3-butanediol, ethyl acetate, *iso*-butyl acetate, *iso*-amyl acetate, *iso*-butyric acid and furfural. Component 2 was composed of *n*-propanol, *n*-butanol, benzene ethanol, ethyl pyruvate, ethyl lactate, acetone, diacetyl, acetoin and acetic acid. The Pearson correlation showed that there were correlations ($p \leq 0.05$) among ethanol, *iso*-amyl alcohol (0.847), and *iso*-butyl alcohol (0.764). These were similar to the PCA results when *Ou* samples were tested for the data of proximate analyses and volatile quantification. On the other hand, when the peak areas of volatile flavour compounds were considered for product grouping (Fig. 3). Group 1 was composed of A, E, H, and I while Group 2 was composed of B, C, D, F, G, and J. The grouping result showed that most *Ou* samples were in the same group of PCA by proximate analyses and volatile quantification. Each group composed of all geographic regions of Thailand. It indicated that its origin was unable to be a classification parameter of *Ou*. However, both PCA procedures by all analysed parameters and peak areas showed the same results that the first group was composed of A, B, C, F, H, and J while the second group was composed of D, E, G, and I. The main difference between these groups was the amount of acetic acid. A high concentration of this compound was observed in the second group. Since acetic acid is known as an off-flavour in Japanese *Sake* and wines[26], these samples were possibly unsatisfactory products in terms of flavour perception.

CONCLUSIONS

The chemical components and their flavour attributes of *Ou* were reported in this study. Based on the ethanol concentration of *Ou* of the Thai Community Product Standard, the concentrations of all samples followed the regulations. However,

variation of the ethanol and solid contents indicated that more uniform and systematic production is required in order to ensure good quality of *Ou*. Eighteen volatile compounds were characterized as odour active compounds by GCO. These volatile compounds provided alcohol and solvent-like, sweet, fruity, buttery, and pungent aromas. Furthermore, these compounds are also detectable in Japanese *Sake*. The presence of acetic acid in the samples indicated that there was some bacterial contamination during *Ou* production. This should be of concern to the manufacturers. Further studies should be carried out to investigate the changes of volatile compounds in *Ou* during day fermentation and their flavour release.

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