ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (4): 718-724, 2008
ISSN 1682-296X
© 2008 Asian Network for Scientific Information

Quantitative Analysis of Main Volatile and Other Compounds in Traditional Distilled Spirits from Thai Rice

[1]K. Wechgama [1,2]L. Laopaiboon and [1,2]P. Laopaiboon
[1]Fermentation Research Center for Value Added Agricultural Products,
Khon Kaen University, Khon Kaen 40002, Thailand
[2]Department of Biotechnology, Faculty of Technology, Khon Kaen University,
Khon Kaen 40002, Thailand

Abstract: This research aims to identify and quantify main volatile and other compounds in Thai traditional distilled spirits from Thai rice using capillary gas chromatography. The optimal chromatographic condition for volatile compound separation was the initial oven temperature of 40°C and the oven temperature programme was as follows: 6 min at 40°C, 10°C min^{-1} to 55°C, 1 min at 55°C, 10°C min^{-1} to 120°C, 15 min at 120°C, 10°C min^{-1} to 129°C, 3 min at 129°C, 10°C min^{-1} to 150°C and 22 min at 150°C. At the optimal conditions, the compounds were separated within 54 min. For acid and vanillin separations, the optimal conditions were the initial oven temperature of 70°C, 12°C min^{-1} to 220°C and 26 min at 220°C. Acids and vanillin were separated within 40 min. The results showed that 12 commercial Thai traditional distilled spirit samples from Thai rice contained at least 28 compounds at various concentrations. From all compounds identified in the Thai traditional distilled spirits, 11 compounds were determined to be the most powerful odourants (aroma index > 1): ethyl acetate, ethyl butyrate, ethyl decanoate, acetaldehyde, ethyl laurate, ethyl caprylate, 2-phenethyl acetate, 1-hexanol, phenethyl alcohol, isoamyl alcohol and 2-furaldehyde.

Key words: Aroma index, gas chromatography, thai traditional distilled spirits, volatile compounds

INTRODUCTION

Aroma substances e.g., alcohols, aldehydes, esters and acids are important in alcoholic beverages as they make a major contribution to the quality of the products (Valero *et al*., 2002; Mallouchos *et al*., 2003; Mamede *et al*., 2005). Most of them appear during fermentation process as main volatile compounds and their concentrations vary over a wide range.

Thai distilled spirits are traditional alcoholic beverages using either glutinous rice or sugar cane molasses as a substrate for bio-ethanol production. The spirits have been the most popular alcoholic drinks for Thais since ancient times especially in the North, Northeast and Central of Thailand. The spirits are popular among labors of the native population. They consume in very large quantities during festivals. However, the aroma compounds of the Thai traditional distilled spirits from Thai rice have not been characterized. Similar Thai traditional distilled spirits from rice, they have equivalent appellations, for example as Japanese Shôchu, Chinese Shui Jji, Sheung Jing and San Jing, Malaysian Bambarayon, Indonesian Arak and Vietnamese Quoc Lui.

In this research, the attention has been principally focused on identification and quantification of the main volatile and other compounds of 12 commercial Thai traditional distilled spirit samples in order to determine potent aroma compounds in the Thai traditional distilled spirits from Thai rice on the basis of the aroma index (I) (Moreno *et al*., 2005). The contents of the main volatile compounds in the Thai traditional distilled spirits were also compared with those of other alcoholic beverages.

MATERIALS AND METHODS

Standard and sample preparations: All standards (chromatographic grade) were purchased from Sigma (Sigma Chemical, USA). For alcohol, ester and aldehyde analyses, the standard mixtures contained ethyl acetate, ethyl butyrate, ethyl decanoate, acetaldehyde, ethyl L-lactate, isobutyl acetate, diethyl succinate, ethyl laurate, ethyl caprylate, propyl acetate, ethyl caproate, hexyl acetate, isopentyl acetate, phenyl acetate, 2-phenethyl acetate, methyl acetate, 1-hexanol, butanol, 2-butanol, 1-octanol, 3-octanol, 4-penten-1-ol, isobutyl alcohol, phenethyl alcohol, isoamyl alcohol, methanol, 4-methyl-1-pentanol, 4-methyl-2-pentanol, 3-methyl-1-pentanol,

Corresponding Author: Pattana Laopaiboon, Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand Tel: +66 43 362121 Fax: +66 43 362121

propanol, isovaleic acid, caproic acid, 2-furaldehyde, acetone and acetoin. Each component of the standard mixtures was prepared at concentrations of 13.75, 27.50, 55, 110 and 220 mg L^{-1} using 80% ethanol as a solvent. Pentanol was prepared at the concentration of 800 mg L^{-1} in 80 % ethanol as an internal standard.

For vanillin and acid analyses, the standard mixtures contained vanillin, octanoic, dodecanoic, butyric and acetic acids. Each component of the standard mixtures was prepared at concentrations of 40, 400, 800 and 1,600 mg L^{-1} using 80% ethanol as a solvent. 1-Octanol at concentration of 50 mg L^{-1} (in 80% ethanol) was used as an internal standard.

Twelve commercial Thai traditional distilled spirits made from Thai glutinous rice produced Year 2006 were collected from Roi-et, Srisaket, Yasothorn, Nakonsawan, Lopburi, Supanburi, Lumpang and Chiangmai provinces in Thailand. Then they were transported to the Biotechnology Laboratory for analysis at the Department of Biotechnology, Faculty of Technology, Khon Kaen University. The samples were placed into 1.0 mL microtube and stored in the dark at 4°C. Each sample was filtered through 0.45 μm nylon membrane filter before direct injection into a Gas Chromatography (GC).

The standard mixtures at each concentration and the internal standard were mixed in the ratio of 1:10 (v/v). Then 1 μL of the mixtures obtained was injected into the GC. The standard calibration between concentrations of the standard mixtures and the ratios of peak area (standard peak area per internal standard peak area) was established.

Each distilled spirit sample and the internal standard were mixed in the ratio of 1:10 (v/v). One microliter of the mixtures was injected into the GC. The peak areas of the samples were compared with those of the standard calibration curve to determine the concentrations of the volatile and other compounds. The analyses were carried out in triplicate.

Gas chromatographic conditions: GC analysis of alcohol, ester and aldehyde compounds of the Thai traditional distilled spirits was performed using a GC-17A (Shimadzu, Japan) equipped with a fused capillary column coated with Stabiwax (60 m×0.25 mm diameter, 0.25 μm thickness, Restex, USA). The flow rate of carrier gas, helium, was 2.0 mL min^{-1}. One microliter of sample was injected with split/splitless mode (split ratio, 1:10) by direct injection into the GC, using pentanol as an internal standard. The oven temperature programme was varied. The oven temperature was started at 40, 55 and 70°C and ended at 150°C. Injector and detector temperatures were maintained at 150 and 160°C, respectively.

For acids and vanillin, GC analysis was performed using the GC-17A (Shimadzu, Japan) equipped with a fused capillary column coated with Stabiwax-DA (60 m×0.25 mm diameter, 0.25 μm thickness, Restex, USA). The flow rate of carrier gas, helium, was 2.0 mL min^{-1}. One microliter of sample was injected with split/splitless mode (split ratio, 1:10) by direct injection into the GC, using 1-octanol as an internal standard. The oven temperature programme was varied. The oven temperature was started at 60, 70 and 80°C and ended at 220°C. Injector and detector temperatures were maintained at 210 and 230°C, respectively.

RESULTS AND DISCUSSION

When the conditions of GC analysis were varied, the results showed that the optimal chromatographic condition for alcohol, ester and aldehyde separations was the initial oven temperature of 40°C and the oven temperature programme was as follows: 6 min at 40°C, 10°C min^{-1} to 55°C, 1 min at 55°C, 10°C min^{-1} to 120°C, 15 min at 120°C, 10°C min^{-1} to 129°C, 3 min at 129°C, 10°C min^{-1} to 150°C and 22 min at 150°C. At the optimal conditions, the compounds were separated within 54 min (Fig. 1). For acid (octanoic, dodecanoic, butyric and acetic acids) and vanillin separations, the optimal conditions were the initial oven temperature of 70°C, 12°C min^{-1} to 220°C and 26 min at 220°C. At the optimal conditions, the compounds were separated within 40 min (Fig. 2). The results of volatile and other compounds in the 12 samples of Thai traditional distilled spirits from Thai rice were shown in Table 1. The results showed that all samples exhibited ethanol contents ranging from 30 to 43% (v/v) which might be justified by the distillation system used (units using entrainment with steam or directed heat) or an instrument used for ethanol measurement. In addition, it was found that alcohols and esters were the largest groups in all distilled spirit samples.

Higher alcohols and esters, produced during alcoholic fermentation, played an important role in the flavour of wines and distilled spirits depending on types of compounds and their concentrations (Selli *et al*., 2004). In this study (Table 1), the important higher alcohols were isobutyl alcohol and isoamyl alcohol. They were in the range of 148.7-472.1 and 37.3-1117.7 mg L^{-1}, respectively. It was indicated that isoamyl alcohol was a contributor to wine aroma and it was characterized by fruity attribute whereas isobutyl alcohol was characterized by candy (Selli *et al*., 2004). In addition, Table 1 showed that 2-butanol, 1-octanol, 3-octanol, 4-methyl-2-pentanol and 4-pentan-1-ol were not detectable in all samples. 1-hexanol was not an alcoholic fermentation product

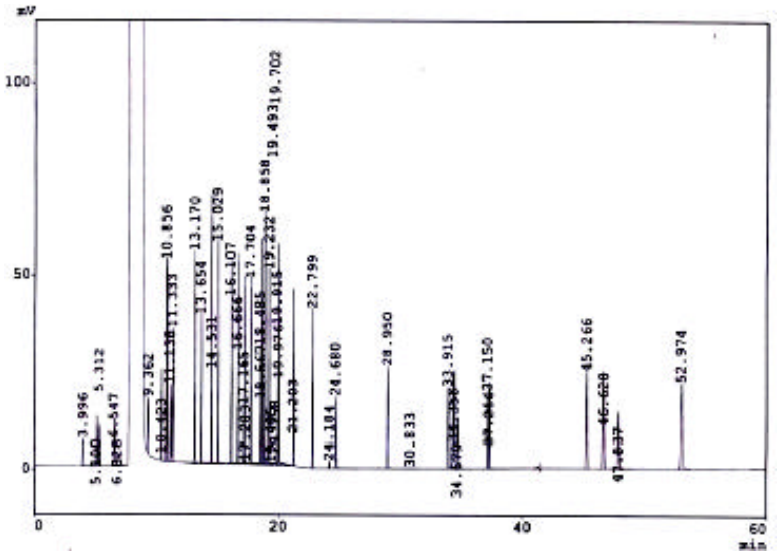

Fig. 1: Chromatogram of the standard mixtures at the optimum chromatographic conditions (Stabiwax capillary column) (3.996, acetaldehyde; 5.100, acetone; 5.312, methyl acetate; 6.547, ethyl acetate; 6.828, methanol; 9.362, propyl acetate; 10.423, isobutyl acetate; 10.856, 2-butanol; 11.138, ethyl butyrate; 11.333, propanol; 13.170, isobutyl alcohol; 13.654, isopentyl acetate; 14.531, butanol; 15.029, 2-methyl-2-pentanol; 16.107, isoamyl alcohol; 16.66, ethyl caproate; 17.165, pentanol; 17.704, hexyl acetate; 18.485, acetoin; 118.567, 4-penten-1-ol; 18.858, 4-methyl-1-pentanol; 19.232, 3-methyl-1-pentanol; 19.915, 1-hexanol; 19.976, ethyl L-lactate; 21.203, butyric acid; 22.799, ethyl capryrate; 24.680, 2-furaldehyde; 28.950, 1-octanol; 33.915, 3-octanol; 34.358, ethyl decanoate; 34.679, phenyl acetate; 37.150, isovaleic acid; 37.256, diethyl succinate; 45.266, 2-phenethyl acetate; 46.628, ethyl laulate; 47.837, caproic acid and 52.974, phenethyl alcohol)

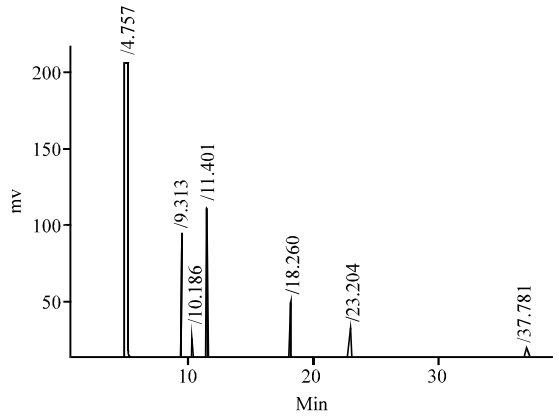

Fig. 2: Chromatogram of the standard mixtures at the optimum chromatographic conditions (Stabiwax-DA capillary column) (9.313, butyric acid; 10.186, acetic acid; 11.401, 1-octanol; 18.260, dodecanoic acid; 23.204, octanoic acid and 37.781, vanillin)

(Apostolopoulou *et al*., 2005). It was an alcohol originating only from raw material and unfavourable to wine quality. However, it was considered to be a favourable compound if its concentration was above 5 mg L^{-1} but not higher than 100 mg L^{-1} (Soufleros *et al*., 2001). Its concentration in kiwi wine ranged from 5.2-463.9 mg L^{-1} (Soufleros *et al*., 2001), whereas butanol content in the Thai distilled spirits was in the range of 0.3-15.5 mg L^{-1}. Butanol was an important higher alcohol in Mouro distillate and it was found in the range of 1.6-11.5 mg L^{-1} (Soufleros *et al*., 2004). Low concentrations of phenethyl alcohol had a positive influence on the wine aroma (Apostolopoulou *et al*., 2005). It contributed to the typical flavour characteristics of Mouro fruit to distillate (Soufleros *et al*., 2004). The content of phenethyl alcohol in Mouro distillate was in the range of ND-12.7 mg L^{-1} whereas that in this study was in the range of 3.3 - 78.6 mg L^{-1}. Methanol is not a normal product of alcoholic fermentation. The control of methanol content in alcoholic beverages is important because of its toxicity. The methanol level of 200 mg L^{-1} is considered the maximum safe concentration to be inhaled for continuous eight-hour working day (Cabaroglu *et al*., 2003). In this study, the content of methanol varied between ND and 40 mg L^{-1}. The lower levels of methanol indicated that glutinous rice as a raw material had little pectin. Propanol concentration in wines was influenced by yeast strains responsible for the

Table 1: Volatile and other compounds in the Thai distilled spirit samples

Compounds	Distilled spirit samples* 1	2	3	4	5	6	7	8	9	10	11	12	Threshold** (mg L^{-1})	Odour descriptor**
Esters, acetates and aldehydes (mg L^{-1})														
Ethyl acetate	297.1	194.7	124.2	1444.3	102.4	150.0	58.2	71.2	114.1	76.5	81.5	222.7	7.5	Bubble-gum, pineapple
Ethyl butyrate	195.4	300.0	440.0	201.0	79.9	276.0	141.5	131.7	149.8	199.6	237.0	227.7	0.2	Pineapple
Ethyl decanoate	5.2	ND	2.3	ND	0.7	3.4	0.1	0.9	2.3	ND	0.7	ND	1.1	Sweety, fruity, dry fruit
Acetaldehyde	452.9	182.4	94.7	530.2	127.0	81.0	42.9	130.4	58.1	36.5	36.5	98.2	10.0	Sherry
Ethyl L-lactate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	14.0	Fruity, buttery
Isobutyl acetate	ND	ND	ND	0.4	ND	ND	ND	ND	ND	ND	ND	ND	3.4	Fig
Diethyl succinate	3.4	2.2	ND	2.6	ND	4.3	ND	ND	9.0	0.7	1.5	2.8	100.0	Cheese, earthy, spicy and fruity
Ethyl laurate	3.5	ND	ND	ND	ND	ND	ND	ND	8.3	ND	ND	ND	0.6	Sweaty, waxy, floral and soapy
Ethyl caprylate	1.9	ND	1.6	ND	ND	2.3	ND	ND	ND	ND	ND	ND	0.2	Candle-wax
Propyl acetate	ND	8.7	1.5	ND	4.6	ND	2.1	ND	ND	ND	ND	ND	65.0	Celery
Ethyl caproate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.1	Candle-wax
Hexyl acetate	8.2	8.4	ND	ND	7.0	ND	9.7	4.5	ND	ND	ND	ND	-	Pear
Isopentyl acetate	1.3	0.9	3.3	1.9	0.9	4.2	ND	ND	ND	ND	0.5	0.3	-	-
Phenyl acetate	3.6	1.5	1.4	4.4	3.0	7.7	ND	3.9	ND	3.9	3.7	3.6	-	-
2-phenethyl acetate	ND	0.4	0.7	ND	ND	1.9	ND	0.8	4.6	ND	ND	ND	0.3	Flowery
Methyl acetate	13.8	5.4	ND	5.0	ND	ND	ND	ND	ND	ND	ND	ND	-	-
Ethanol (% v/v)	39.4	36.3	43.1	36.1	33.6	34.8	30.0	34.9	31.5	31.2	37.4	32.8	-	-
Other alcohols (mg L^{-1})														
1-hexanol	5.2	74.3	60.7	265.6	5.5	48.7	34.6	25.3	134.9	321.1	309.5	463.9	8.0	Herbaceous
Butanol	8.8	7.3	15.5	12.9	0.3	11.8	6.4	5.0	5.2	3.3	6.1	12.7	1000.0	Medicinal
2-butanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1000.0	-
1-octanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.0	-
3-octanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	Mushroom
4-penten-1-ol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
Isobutyl alcohol	261.2	175.4	472.1	377.4	343.7	385.1	148.7	319.0	219.7	206.6	402.8	263.6	-	Candy
Phenethyl alcohol	38.5	16.1	37.0	21.5	78.6	75.5	3.3	16.1	18.4	29.3	40.2	34.1	10.0	Rose
Isoamyl alcohol	1117.7	432.2	37.3	538.9	768.8	581.4	319.4	482.3	360.0	445.9	670.0	487.4	30.0	Red-fruit, framboise
Methanol	25.8	24.0	ND	12.8	25.7	7.2	11.0	14.6	3.7	8.0	10.3	7.5	668.0	Manure
4-methyl-1-pentanol	2.4	ND	ND	200.3	1.4	24.1	4.2	0.0	0.3	1.9	6.7	12.8	50.0	-
4-methyl-2-pentanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
3-methyl-1-pentanol	ND	1.7	ND	ND	ND	ND	ND	ND	1.3	ND	ND	ND	50.0	Potato
Propanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
Acids (mg L^{-1})														
Acetic acid	93.8	402.5	78.3	1537.2	222.2	260.9	99.7	94.0	2.5	50.4	72.6	240.8	-	Acetic acid
Isovaleic acid	ND	0.9	1.3	5.2	3.9	1.9	0.8	1.0	5.5	3.3	2.0	1.8	-	Sweaty
caproic acid	7.4	ND	ND	3.7	2.1	3.3	ND	2.3	9.6	2.8	2.4	ND	8.6	Sweaty
Butyric acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.4	Fatty-rancid, cheesy
Dodecanoic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
Octanoic acid	16.2	1.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.0	Fatty-rancid, cheesy
Others (mg L^{-1})														
Vanillin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	Vanilla
2-furaldehyde	1.5	4.3	42.2	6.5	6.1	1.5	7.9	2.6	ND	3.2	2.2	2.1	0.1	Almond
Acetone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
Acetoin	4.5	43.0	21.1	ND	7.6	ND	ND	13.9	17.4	ND	ND	ND	150.0	Fatty, toasted bread, butter

ND: Not detected, *Distilled spirit number 1, 2, 3 from Roi-et; 4 from Yasothorn; 5, 6, 7 from Nakonsawan; 8 from Lopburi, 9 from Supanburi; 10 from Lumpang and 11, 12 from Chiangmai, **Selli *et al.* (2004), Apostolopoulou *et al.* (2005) and Rocha *et al.* (2004)

fermentation (Giudici *et al.*, 1993). The content of propanol in spirit samples from Greek might be the result of a possible microbial spoilage during storage under unfavourable conditions of the raw material before distillation (Apostolopoulou *et al.*, 2005). However, propanol was not detected in all Thai distilled spirits. The contents of 4-methyl-1-pentanol and 3-methyl-1-pentanol from the Thai distilled spirits were in the range of ND-200.3 and ND-1.7 mg L^{-1}, respectively. Their concentrations of the spirits were higher than those of wines (Selli *et al.*, 2004).

Ethyl acetate had a significant effect on the organoleptic characteristics of wines. It was frequently described as having fingernail polish remover properties

and at levels of 150-200 mg L^{-1}, it could add spoilage notes to wine. At lower concentrations, ethyl acetate might contribute to fruity properties of the wine (Apostolopoulou *et al*., 2005). Ethyl acetate content in the studied samples ranged from 58.2-1444.3 mg L^{-1}. Ethyl butyrate (ethyl butanoate) concentration ranged from 79.9-440 mg L^{-1} in the Thai distilled spirit samples. Ethyl butyrate which contributed fruity notes to wine aroma was mainly produced during the alcoholic fermentation. Ethyl decanoate was produced during the raw materials fermentation and increases during aging (Soufleros *et al*., 2004). It was reported that ethyl decanoate was impact odourants of Kalecik Karasé wines (Selli *et al*., 2004). In this study, ethyl decanoate concentrations ranged between ND and 5.2 mg L^{-1}.

Acetaldehyde was a by-product from direct alcoholic fermentation. Sensory descriptors for acetaldehyde ranged from classic, nutty and sherry-like to be reminiscent of overripe bruised apples (Fugelsang, 1997). Acetaldehyde content in the Thai distilled spirit samples was in the range of 36.5-530.2 mg L^{-1}. The sensory threshold in wines ranged from 100-125 mg L^{-1}. Above this concentration acetaldehyde could impart an odour to the wines (Zoecklein *et al*., 1995). A similar value of acetaldehyde (448.4 mg L^{-1}) was detected in the traditional Greek fruit distillate Mouro (Soufleros *et al*., 2004). Ethyl L-lactate, ethyl caproate and isobutyl acetate were not detected in all studied samples suggesting that these compounds were not produced during the alcoholic fermentation. Diethyl succinate, ethyl laurate, ethyl caprylate, hexyl acetate, propyl acetate, 2-phenethyl acetate, isopentyl acetate, phenyl acetate and methyl acetate contents in the Thai distilled spirit samples were in the range of ND-9, ND-8.3, ND-2.3, ND-9.7, ND-8.7, ND-4.6, ND-4.2, ND-7.7 and ND-13.8 mg L^{-1}, respectively. Diethyl succinate was an ester that was mainly produced from bacterial alterations of various wine components (Soufleros *et al*., 2004). It was described as having fruity whereas ethyl laurate is described as having sweaty, waxy, floral and soapy to the wine. In addition, ethyl caprylate, hexyl acetate, propyl acetate and 2-phenethyl acetate were described as having candle-wax, pear, celery and flowery, respectively.

Short chain fatty acids (isovaleric and butyric acids) were usually found at low concentrations but there was an odour similar in strength string smell to acetic acid and consequently, with an important contribution to the aromatic character of distillates (Soufleros *et al*., 2004). The contents of isovaleric and acetic acids in the Thai distilled spirits varied from ND-5.5 and 2.5-1537.2 mg L^{-1} whereas that of butyric acid was not detected in all samples. Long-chain fatty acids, octanoic, caproic and dodecanoic acids, were of smaller flavour effect to distillates (Soufleros *et al*., 2004). Octanoic and caproic acid concentrations in the Thai distilled spirits were ND-16.2 and ND-7.4 mg L^{-1}, respectively whereas dodecanoic acid was not detected in all samples (Table 1).

Compositions of some esters, aldehyde, alcohols and acids of other traditional distilled spirits were shown in Table 2. It was found that the contents of most volatile compounds in the Thai distilled spirits could be comparable to those of the other traditional distilled spirits.

Furfural (2-furaldehyde) is an oily. It was reminiscent of bitter almond and cinnamon and it had a toxic character (Apostolopoulou *et al*., 2005). Furfural content in the studied samples ranged from ND-42.2 mg L^{-1} which was not higher than the maximum level limitation of furfural in the distillates of Thailand (160 mg L^{-1}). Acetone also had a toxic character. However, the content of acetone in the Thai distilled spirit samples was not detected. Acetoin (3-hydroxy-2-butanone) was described as an unpleasant fatty and toasted bread attribute in wine (Selli *et al*., 2004). The content of acetoin in the Thai distilled spirit samples ranged from ND - 43 mg L^{-1} which was far lower than that of acetoin threshold. The results suggested that acetoin was rarely influence to the distillates. Vanillin, the main aroma compound in natural vanilla, was also present in raw oak. The quantities of vanillin reported in wine varied with oak species and seasoning. Vanillin increased with medium toast levels, but decreased with very high toast. Vanillin was partially transformed to non-aromatic vanillyl alcohol by yeast during barrel fermentation (Pérez-Prieto *et al*., 2002). However, this compound was undetected in all Thai distilled spirit samples implying that the spirits were not incubated in oak after alcoholic fermentation.

Of all volatile and other compounds, one way to quantify the odour activity of a compound is to determine aroma index (I), also so-called Odour Activity Value (OAV) or Number of Olfactory Units (NOU). The value is calculated by dividing the concentration of the compound in the spirit into its perception threshold (Moreno *et al*., 2005). Therefore, the odour impact of a substance increases in proportion to its I when this value is more than one. Based on these criteria, the above mentioned compounds (particularly those exhibiting the highest I) can be assumed to be those with the strongest odour impact, thereby contributing to a great extent to the aroma of the spirits and largely reasonably responsible of the sensory profile of these spirits. Aroma indices of the would-be impact odorants of the Thai distilled spirit samples were shown in Table 3.

Table 2: Volatile compounds in other traditional distilled spirits

Compounds	Koumaro (Soufleros *et al.*, 2005)	Portuguese grape Marcs (Silva *et al.*, 1996)	Mouro (Soufleros *et al.*, 2004)	Bagaceiras (Silva and Malcata, 1998)	Rum (Lehtonen *et al.*, 1999)	Whiskey (Fitzgerald *et al.*, 2000)	Aguardiente (Rogerson and Freitas, 2001)	Melon fruit distillates (Hernández-Gómez *et al.*, 2003)
Esters and aldehyde (mg L^{-1})								
Ethyl acetate	34,000	200	1,856	3,147-4,940	0.6-1.2	15.9-206	-	40.8-154
Ethyl decanoate	4.0	11	14	-	-	-	-	-
Acetaldehyde	1,000	300	448.4	699-863	-	-	-	153-243
Ethyl L-lactate	-	-	-	105-479	-	15.8-31.7	80-335	274-902
Diethyl succinate	3.3	5	-	4.1-5.6	-	-	5.6-14.6	-
Ethyl laurate	-	2.3	-	-	-	-	-	-
Ethyl caprylate	-	11	12.9	-	-	0.3-1.3	6.9-15.7	-
Ethyl caproate	-	-	-	1.8-2.4	-	0.4-2.2	1,020-3,260	-
Hexyl acetate	1.4	0.3	-	0.9-2.1	-	-	-	-
Isopentyl acetate	5.0	6	3.2	-	-	-	-	-
2-Phenethyl acetate	-	0.3	-	0.04-0.5	-	0.2-0.8	100-490	-
Ethanol (% v/v)	39.4	45	42.5	-	-	-	-	-
Other alcohols (mg L^{-1})								
1-Hexanol	9.0	60	5.6	114-219	-	-	-	-
1-Butanol	70.0	23	74.1	5.6-22.7	-	10.9	-	0-3.4
2-Butanol	-	50	-	-	-	-	-	-
Isobutyl alcohol	-	-	248.5	-	-	-	-	-
2-Phenethyl alcohol	26.0	10	63.9	11-13.1	-	14.4	5.5-18	-
Isoamyl alcohol	-	-	1,451.8	-	-	-	-	-
Methanol	7,440	3,400	1,457.4	10,210-10,310	0.2	-	-	618-4,750
Propanol	-	250	-	-	-	-	-	-
Acids (mg L^{-1})								
Isovaleic acid	2.4	-	3	1.9-2	-	-	0-5,560	-
Caproic acid	5	2	9.8	2.1-3.6	-	-	0-5,180	-
Butyric acid	17	-	0.7	-	-	-	-	-
Dodecanoic acid	1.3	1	8.3	1.6-3.6	-	-	0-1,170	-
Octanoic acid	3.8	4	4.1	0.8-1.4	-	-	455-16,630	-

Table 3: Aroma index and odour descriptor of volatile compounds having aroma index more than one in the Thai distilled spirits

Compounds	Aroma index	Odour descriptor
Ethyl acetate	31.05	Bubble-gum, pineapple
Ethyl butyrate	1096.35	Pineapple
Ethyl decanoate	1.2	Sweety, fruity, dry fruit
Acetaldehyde	11.51	Sherry
Ethyl laurate	6.98	-
Ethyl caprylate	5.22	Candle-wax
2-phenethyl acetate	7.49	Flowery
1-hexanol	20.21	Herbaceous
Phenethyl alcohol	3.27	Rose
Isoamyl alcohol	5.61	Red-fruit, framboise
2-furaldehyde	45.87	Almond

CONCLUSIONS

The proposed methodology in this study seems to be adequate as a preliminary step to quantify main volatile and other compounds of the Thai distilled spirits and to establish the would-be impact odorant of the spirits. From all compounds identified in the 12 commercial Thai distilled spirits, 11 compounds were determined to be the most powerful odourants (aroma index >1): ethyl acetate, ethyl butyrate, ethyl decanoate, acetaldehyde, ethyl laurate, ethyl caprylate, 2-phenethyl acetate, 1-hexanol, phenethyl alcohol, isoamyl alcohol and 2-furaldehyde (Table 3). The results obtained from this study indicated that the major aroma contributors in the Thai distilled spirits originated from both microbial metabolism and raw material. Further steps, such as GC-olfactometric studies may be necessary to confirm the impact of the identified odor-active compounds in the distilled spirits.

ACKNOWLEDGMENTS

The authors would like to thank the Fermentation Research Center for Value Added Agricultural Products (FerVAAP) and the National Research Council of Thailand (NRCT) for financial support (Grant: Year 2005) and Department of Biotechnology, Faculty of Technology, Khon Kaen University, Thailand for instrumentation support.

REFERENCES

Apostolopoulou, A.A., A.I. Flouros, P.G. Demertzis and K. Akrida-Demertzi, 2005. Differences in concentration of principal volatile constituents in traditional Greek distillates. Food Cont., 16: 157-164.

Cabaroglu, T., S. Selli, A. Canbas, J.P. Lepontre and Z. Gunata, 2003. Wine flavor enhancement through the use of exogenous fungal glyoxidase. Enzyme Microbiol. Tech., 33: 581-587.

Fitzgerald, G., K.J. James, K. Macnamara and M.A. Stack, 2000. Characterization of whiskeys using solid-phase micro extraction with gas chromatography-mass spectrometry. J Chromatogr. A, 896: 351-359.

Fugelsang, K.C., 1997. Wine Microbiology. 1st Edn. Chapman and Hall, New York, USA., pp: 274.

Giudici, P., C. Zambonelli and R.E. Kunkee, 1993. Increased production of n-propanol in wine by yeast strains having an impaired ability to form hydrogen sulfide. Am. J. Enol. Viticult., 44: 17-21.

Hernández-Gómez, L.F., J. Úbeda and A. Briones, 2003. Melon fruit distillates: Comparison of different distillation methods. Food Chem., 82: 539-543.

Lehtonen, P.J., A. Keller LaDena and E.T. Ali-Matila, 1999. Multi-method analysis of matured distilled alcoholic beverages for brand identification. Zeitschrift fuer Lebensmittel-Untersuchung und-Forschung A, 208: 413-417.

Mallouchos, A., M. Komaitis, A. Koutinas and M. Kanellaki, 2003. Wine fermentations by immobilized and free cells at different temperatures: Effect of immobilization and temperature on volatile by-products. Food Chem., 80: 109-113.

Mamede, M.E.O., H.M.A.B. Cardello and G.M. Pastore, 2005. Evaluation of an aroma similar to that of sparking wine: Sensory and gas chromatography analyses of fermented grape musts. Food Chem., 89: 63-68.

Moreno, J.A., L. Zea, L. Moyano and M. Medina, 2005. Aroma compounds as markers of the changes in sherry wines subjected to biological ageing. Food Control, 16: 333-338.

Pérez-Prieto, L.J., J. López-Roca, A. Martínes-Cutillas, F. Pardo-Mínguez and E. Gómez-Plaza, 2002. Maturing wines in oak barrels: Effects of origin, volume, and age of the barrel on the wine volatile composition. J. Agric. Food Chem., 50: 3272-3276.

Rocha, S.M., F. Rodrigues, P. Coutinho, I. Delgadillo and M.A. Coimbra, 2004. Volatile composition of Baga red wine: Assessment of the identification of the would-be impact odourants. Anal. Chim. Acta, 53: 257-262.

Rogerson, F.S.S. and V.A.P. Freitas, 2001. Quantitative analysis of volatiles present in wine spirits used for port production. American Society for Enology and Viticulture 52nd Annual Meeting, June 28-30, San Diego, CA., PP: 60-60.

Selli, S., T. Cabarglu, A. Canbas, H. Erten and C. Nurgel *et al* 2004. Volatile compositions of red wine from cv. Kalecik Karasi grown in central Anatolia. Food Control, 85: 207-213.

Silva, M.L., F.X. Malcata and G. De Revel, 1996. Volatile contents of grape marcs in Portugal. J. Food Compos. Analysis, 9: 72-80.

Silva, M.L. and F.X. Malcata, 1998. Relationships between storage conditions of spirits obtained hereform. Am. J. Enol. Viticult., 49: 56-64.

Soufleros, E.H., I. Pissa, D. Petridis, M. Lygerakis and K. Mermelas *et al*., 2001. Instrumental analysis of volatile and other compounds of Greek kiwi wine; sensory evaluation and optimization of its composition. Food Chem., 75: 487-500.

Soufleros, E.H., A.S. Mygdalia and P. Natskoulis, 2004. Characterization and safety evaluation of the traditional Greek fruit distillate Mouro by flavor compounds and mineral analysis. Food Chem., 86: 625-636.

Soufleros, E.H., A.S. Mygdalia and P. Natskoulis, 2005. Production process and characterization of the traditional Greek fruit distillate Koumaro by aromatic and mineral composition. J. Food Compos. Analysis, 18: 669-716.

Valero, E., L. Moyano, M.C. Millan, M. Medina and J.M. Ortega, 2002. Higher alcohols and esters production by *Saccharomyces cerevisiae*: Influence of the initial oxygenation of the grape must. Food Chem., 78: 57-61.

Zoecklein, B.W., K.C. Fugelsang, B.H. Gump and F.S. Nury, 1995. Alcohol and Extract. In: Wine Analysis and Production, Zoecklein, B.W., K.C. Fugelsang, B.H. Gump and F.S. Nury (Eds.). Chapman and Hall, New York, USA, ISBN: 0-412-98921-2, pp: 97-114.