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Characteristic odour compounds in *shochu* derived from rice *koji*

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***Shochu*, a traditional Japanese distilled liquor, makes use of rice *koji*, which is *koji* mould grown on rice grain. Rice *koji* is an essential ingredient of Japanese liquors such as *shochu*, and it plays a role as a source of the enzyme to degrade starch. However, there has been no research on the effect of rice *koji* on the flavour of *shochu*. Therefore, in this study, the volatile compounds in *shochu* derived from rice *koji* were investigated. Two *shochu* samples were prepared to assess the contribution of rice *koji* to the flavour. One *shochu* sample was prepared from rice *koji*, yeast and water (rice *koji-shochu*). The other *shochu* sample was obtained from steamed rice and various enzymes instead of rice *koji* (enzyme-*shochu*), along with yeast and water. The volatile compounds were analysed by gas chromatography–mass spectrometry (GC-MS) and GC-MS/olfactometry with aroma extract dilution analysis. The results showed that enzyme-*shochu* had a higher flavour dilution value of dimethyl trisulphide and hexanal, whereas rice *koji-shochu* had a higher flavour dilution value of some ester compounds that imparted aromatic odours such as fruity. Some unknown peaks representing compounds that impart characteristic odours such as soda, potato, lavender, and tea-like were specifically detected in rice *koji-shochu*. The concentrations and calculated odour active values of 14 compounds were measured. These results showed that isovaleraldehyde, ethyl caprylate, ethyl caproate and ethyl 2-methylbutyrate played an important role in imparting the specific odour of rice *koji-shochu*.**

Keywords: gas chromatography–mass spectrometry; gas chromatography–olfactometry; rice *koji*; *shochu*; volatile compounds

Introduction

Shochu, a traditional Japanese distilled liquor, is predominantly produced in the southern part of Japan and is one of the most important products that drive the economy in these prefectures. The annual production volume of *shochu* in Japan from April 2013 to March 2014 was approximately 500 million litres, according to the public data of the National Tax Administration Agency, Japan *(1)*. The manufacture of *Shochu* has some differences from the manufacture of whisky, which is distilled liquor made from grain in Europe and America. There are two points that are the most important aspects of *shochu* manufacture. First, the *shochu* is fermented using mould as the source of various enzymes. Second, in *shochu* manufacture, the distillate of the *shochu moromi*-mash is not separated and is thus collected together throughout the distillation process.

Mould is previously cultured on steamed rice or barley grains so that enzymes such as α-amylase, glucoamylase, protease and lipase required for the fermentation are produced *(2–4)*. Rice grain is mainly used as a culture for brewing. This type of solid culture is referred to as rice *koji*. Rice *koji* plays a role similar to that of malt in whisky production and is commonly used for making traditional Japanese fermented foods such as *shochu*, soy sauce and *sake* (rice wine). Moulds are commonly used as sources of enzymes for fermentation in East Asian countries such as China and Korea; however, in Japan, the scenario is different since *Aspergillus* sp. is used exclusively for the preparation of rice *koji* as the starter mould. Specifically, yellow-*koji* mould, *Aspergillus oryzae*, has been used for the production of various fermented foods, including *sake*, soy sauce and *miso*. White-*koji* mould (*A. luchuensis* mut. *kawachii*) and black-*koji* mould (*A. luchuensis*) have been used only in the manufacture of *shochu*. The *shochu* manufacturing process is briefly described below. Rice *koji* prepared using *koji* mould starter is mixed with water and yeast for the preparation of the first *moromi*-mash. The starch value in rice *koji* is almost the same as that in rice grain, even though the former is mould-grown *(5)*. Thus, rice *koji* can also be used as an ingredient for fermentation; starch saccharification and alcohol fermentation proceed simultaneously in the *moromi*-mash. After 5 days, water and steamed sweet potato, barley, rice or buckwheat (known as the main material) are added to the first *moromi*-mash (second *moromi*-mash). Fermentation of the second *moromi*-mash is continued for an additional 10 days. Alternatively, if the main material is not added and the fermentation of the first *moromi*-mash is continued, it becomes the mash of a kind of *shochu* called *awamori*. The fermented mash is finally distilled using a pot still. Therefore, the use of rice *koji* for brewing is one of the main reasons the characteristics of *shochu* are distinct from those of other distilled liquors available around the world.

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Shochu is characterized by the flavour associated with the ingredients. The odour compounds derived from the main material in the second *moromi*-mash have been identified and quantified in previous research *(6,7)*. To date, rice *koji* and *koji* mould have attracted attention because of the mass production and secretion of various enzymes outside of fungus bodies. Therefore, much research has been devoted to understanding the molecular biological mechanism of the secretion of enzymes and the application to the mass production of beneficial enzymes *(8–10)*. The role of rice *koji* is recognized to be supplementary to that of the enzymes. On the other hand, the effect of rice *koji* on the flavour of Japanese liquor has not been researched in detail. Rice *koji* manufacture requires ~45 h after inoculating the *koji* mould on the steamed rice grain. During this period, the odour becomes different from that of steamed rice. Finally, rice *koji* assumes a distinctive flavour of chestnuts, mushrooms and Indian ink. The quality of rice *koji* depends on the growth of the *koji* mould – in other words, the enzyme activity – and this directly affects the fermentation. The flavours play a role in determining the quality of the rice *koji*. Therefore, research was carried out to identify the key volatile compounds in rice *koji*, and isobutyraldehyde, isovaleraldehyde, 1-octen-3-ol and phenylacetaldehyde were identified as the main contributors to the typical odours of rice *koji (11–13)*. The term '*koji*' was used in the sensory evaluation of *sake* by the National Research Institute of Brewing, Japan in 2006 *(14,15)*. It is strongly suggested that rice *koji* has a marked effect on the flavour of Japanese liquors such as *sake* and *shochu*.

To study the effect of rice *koji* on the flavour of *shochu*, two types of *shochu* were prepared; one was made exclusively from rice *koji* with yeast and water (rice *koji-shochu*), whereas the other was made from steamed rice and some enzymes instead of rice *koji* (enzyme-*shochu*). The odours and volatile compounds in the *shochu* were analysed by gas chromatography–mass spectrometry (GC-MS) and GC-MS/olfactometry with aroma extract dilution analysis (AEDA) and sensory evaluation.

Materials and methods

Chemicals and strain

White-*koji* mould starter (*A. luchuensis* mut. *kawachii*) and polished rice were purchased from a local company and market in Japan, respectively. The yeast strain Kagoshima-5 *(16)* was supplied by the Kagoshima Prefectural Brewing Association (Kagoshima, Japan). Dextrozyme-glucoamylase (glucoamylase), Sumizyme *Shochu* (α-amylase) and Orientase 20A (protease) were supplied by Nippon Denpun Kogyo Corp. (Kagoshima, Japan), Shin Nihon Chemical Co. Ltd (Aichi, Japan) and HBI Enzymes Inc. (Hyogo, Japan), respectively.

Rice *koji* preparation. Rice *koji* was prepared according to the method adopted in one of our previous studies *(11)*. Polished rice was washed and soaked in water for 1 h. Then, the water was drained off, and the rice was steamed for 1 h and subsequently cooled to ~43°C. White-*koji* mould starter was inoculated onto the steamed rice, mixed well, and incubated for 45 h at 35°C and a relative humidity of 90%. During the preparation of rice *koji*, the temperature of the inoculated rice was measured using a thermometer at a central point of the rice mass. The inoculated rice was repeatedly stirred and cooled when the temperature increased beyond 38°C. Then, the rice *koji* was stored at −80°C until further use.

***Moromi*-mash of rice *koji-shochu*.** Approximately 1.6 kg of the prepared rice *koji* (corresponding to 1.3 kg of polished rice) was added to 2.4 L of water and 40 mL of yeast seed medium and fermented for 11 days at 30°C.

***Moromi*-mash of enzyme-*shochu*.** Approximately 1.8 kg of steamed rice (corresponding to 1.3 kg of polished rice) was added to 2.2 L of 80 mM citrate buffer (pH 4.0). Glucoamylase, α-amylase and protease corresponding to the enzyme activity in rice *koji* were added to the *moromi*-mash. Yeast seed medium (40 mL) was added to the *moromi*-mash and fermented for 12 days at 30°C.

Monitoring of the alcohol fermentation. The alcohol fermentation was monitored by measuring the amount of CO_2 gas generated. The initial total weight of the containers was measured after preparing the *moromi*-mash. The total weight of the *moromi*-mash container was measured each day. The difference between the initial weight and the weight after incubation (the decrease in weight) was used to determine the amount of CO_2 gas generation. The integration curve for weight reduction was plotted on a graph.

Distillation of *moromi*-mash. *Shochu* was made according to the method used in our previous studies *(7)*. *Shochu* was obtained from single-batch distillation in a glass distillation apparatus (a glass pot still coupled to a glass column). Approximately 1 kg of *moromi*-mash was distilled using the steam generated from water in a round-bottomed flask heated by a mantle heater. The distillate was then water-cooled. The end point of distillation was the point at which the alcohol content in the bundled distillate reached ~38%. The distillate was filtered and diluted to 25% alcohol. The *shochu* samples were stored at room temperature prior to analysis.

Large volume static headspace sampling

Headspace volatile components were collected in a large volume static headspace (LVSH) system (Entech 7100A series; Entech Instruments Inc., Simi Valley, CA, USA). The *shochu* samples (10 mL) were transferred to a 200 mL sample bottle. For quantitative determination, 1 mL of 1-pentanol (10 mg/L) was added to the samples as an internal standard. After incubation at 30°C, 100 mL of headspace gas was vacuum-extracted from the sample bottle. The volatile compounds were adsorbed onto two tandemly arranged commercial traps (Entech Instruments Inc.), which were packed with different stationary phases; trap 1 was packed with a glass beads/Tenax mixture resin and trap 2 was packed with Tenax resin. The volatile compounds were desorbed by thermodesorption using an Entech 7100A preconcentrator and applied to the GC-MS system.

GC-MS/O. Quantification of the volatile compounds was performed using an Agilent 6890 series gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an Agilent 5975B mass-selective detector and a sniffing port (Gerstel, Mülheim an der Ruhr, Germany). One half of the column flow was directed to the MS system, while the other half was directed to a heated sniffing port. The GC-MS system was equipped with a DB-WAX column (60 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies Inc.). The GC operation conditions were an injector temperature of 220°C, transfer line temperature of 250°C, quadrupole ion trap temperature of 150°C and ion source temperature of 250°C. Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 mL/min with the following temperature programme: 40°C for 5 min then increased at 3° C/min to 240°C. The injector and detector were held at 250°C and 300°C, respectively. All mass spectra were acquired in the electron impact mode. Quantitative determinations were obtained using 1-pentanol (*m/z* 55). The volatile compound content was calculated from the GC-peak areas relative to the GC-peak area of the internal standard. The panellists recorded the retention time by using an olfactory detector port recorder software (Gerstel) and as per the description of the aroma compounds. Each sample was sniffed four times. When a volatile compound was detected at least three times, this analyte was determined to be a declared aroma compound.

Qualitative and quantitative analysis

For compound identification, the mass spectra and retention times were compared with those of authentic standards or the retention index in the database of the Aromaoffice software (Nishikawa Keisoku Co. Ltd, Tokyo, Japan). Retention indices were calculated by analysing a series of *n*-alkanes (C7–C33; Shimadzu GLC Ltd, Tokyo, Japan). All standards were prepared and diluted with a 25% ethanol solution to obtain the standard solutions. The peak areas were used for quantification. The calibration curves for individual compounds were constructed by plotting the area ratio of the target compounds to the internal standard. The odour active value (OAV) for each volatile compound was calculated from the formula below:

OAV = concentration in *shochu*/odour threshold value *(17,18)*

AEDA. The flavour dilution (FD) values of the odour-active compounds were determined by AEDA *(19)*. Each extract was serially diluted 3-, 9-, 27-, 91- and 273-fold using 25% ethanol as the diluent. Each sample was sniffed four times. When a volatile compound was detected three or more times, the odorant was defined as having been detected. FD is defined as the ratio of the concentration of a compound in the initial extract to that in the most diluted extract in which the odour was detected by GC-MS/O.

Sensory evaluation. Rice *koji-shochu* and enzyme-*shochu* were evaluated in terms of odour and taste. Sensory profile analysis was conducted by nine assessors (six females and three males) from Kagoshima University. Most of the assessors were previously trained using sensory evaluation techniques. The intensity of each characteristic was evaluated on a scale of 0–5 (0 = not detected, 1 = very weak intensity, 2 = weak intensity, 3 = moderate intensity, 4 = strong intensity, 5 = very strong intensity). The results of the sensory profile analysis were averaged for each odour and plotted in a spider web diagram.

Results and discussion

Preparation of rice *koji-shochu* and enzyme-*shochu*

The white-*koji* mould was selected to prepare rice *koji* since this mould is popular for *shochu* manufacture. Although most types of *shochu* are produced by adding a steamed main material such as sweet potato, barley or rice, the *shochu* was prepared without addition of the main material. This manufacturing method was

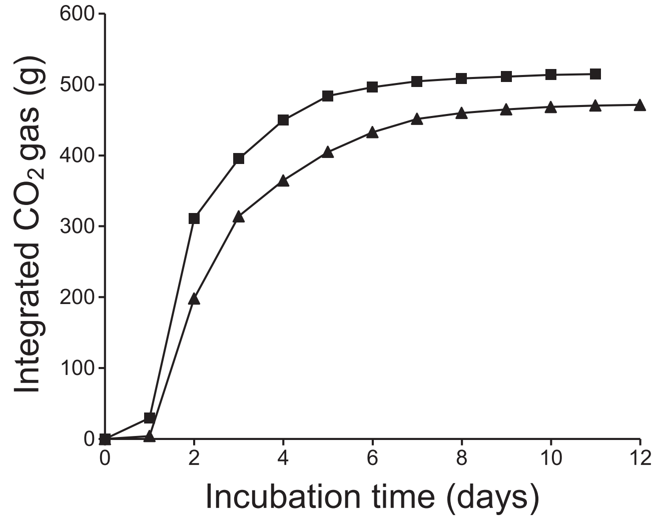

Figure 1. Time course of the alcohol fermentation. The alcohol fermentation was monitored by observing the amount of CO_2 gas generated. The total weight of the *moromi*-mash container was measured each day. The difference between the initial weight and the weight after incubation (the decrease in weight) was used to determine the amount of CO_2 gas generation. The integration curve for weight reduction was plotted on a graph. The squares and triangles represent the *moromi*-mash of rice *koji*- and enzyme-*shochu*, respectively.

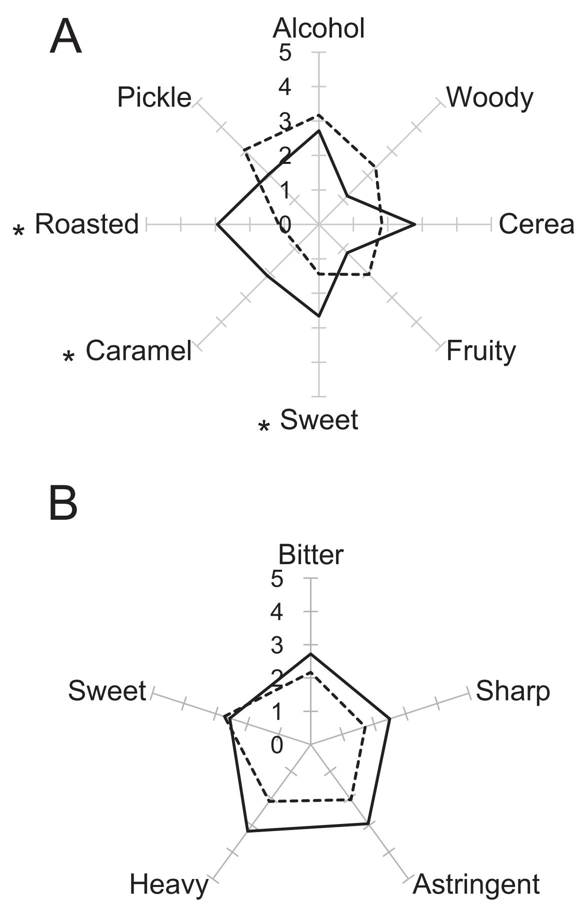

Figure 2. Spider plot for sensory evaluation of (A) odours and (B) tastes of rice *koji-shochu* and enzyme-*shochu*. Rice *koji-shochu* and enzyme-*shochu* are indicated by the solid line and dotted line, respectively. Asterisks indicate the significant differences between rice *koji-shochu* and enzyme-*shochu* as calculated by the *t*-test ($p < 0.05$).

Table 1. Odour intensity and description of odour active compounds in rice *koji-shochu* or *enzyme-shochu*

Compounds	RI*	RI[ref¶]	Identification[†]	Odour description	FD		Reference
					Rice *koji*-made	Enzyme-made	
Alcohols							
Isoamyl alcohol	1217	1224	MS, RI	Characteristic odour	27	81	*(20)*
Ester compounds							
Ethyl acetate	858	892	MS, RI	Sweet	3	3	*(20)*
Ethyl propionate	939	999	MS, RI	Fruity	9	3	*(20)*
Ethyl isobutyrate	949	1007	MS, RI	Fruity	81	243	*(20)*
Isobutyl acetate	1009	1034	MS, RI	Raisin, pear	3	9	*(20)*
Ethyl butyrate	1030	1055	MS, RI	Fruity	27	27	*(20)*
Ethyl 2-methyl butyrate	1047	1069	MS, RI	Strawberry, pineapple	81	81	*(20)*
Ethyl isovalarate	1062	1083	MS, RI	Apple	81	81	*(20)*
Isoamyl acetate	1114	1137	MS, RI	Banana, pear	3	1	*(20)*
Ethyl caproate	1235	1251	MS, RI	Sweet	27	27	*(20)*
Ethyl caprylate	1435	1441	MS, RI	Fruity, soapy	1	ND	*(21)*
Ethyl nonanoate	1537	1541	MS, RI	Cereal	3	1	*(21)*
Ethyl caprate	1635	1643	MS, RI	Fruity	1	ND	*(21)*
Diethyl succinate	1658	1655	MS, RI	Baked apple, roasted	9	9	*(22)*
Ethyl laurate	1872	1847	MS, RI	Soapy	1	ND	*(21)*
Aldehydes							
Acetaldehyde	766	713	MS, RI	Fruity, acrid	1	1	*(23)*
Acetal	865	889	MS, RI	Sweet,vinyl	27	243	*(23)*
Isovaleraldehyde	890	915	MS, RI	Acrid	3	1	*(22)*
Hexanal	1072	1094	MS, RI	Green, grass	3	27	*(21)*
Sulphuric compounds							
Dimethyl trisuphide	1374	1385	MS, RI	Pickles	3	3	*(24)*
Unknown compounds							
Unknown 1	1024	-	-	Fruity	81	ND	
Unknown 2	1116	-	-	Fruity	1	3	
Unknown 3	1126	-	-	Vinyl	3	ND	
Unknown 4	1173	-	-	Potato, roasted	243	243	
Unknown 5	1285	-	-	Soda	3	ND	
Unknown 6	1301	-	-	Perilla	9	27	
Unknown 7	1320	-	-	Sweet	ND	1	
Unknown 8	1464	-	-	Fried potato	9	ND	
Unknown 9	1559	-	-	Lavender	1	ND	
Unknown 10	1660	-	-	Floral	1	1	
Unknown 11	1825	-	-	Tea-like	1	ND	

* RI, retention index calculated on a DB-WAX capillary column with a series of *n*-alkanes (C7–C33).
¶ RI[Ref], retention index from each reference *(21–27)*. ND, not detected.
† Identification proposal is indicated by the following: MS, identification by comparing the mass spectrum with Nist05a spectral database; RI, identification by retention indexes with literature data.

the same as that for *awamori*. It was thought that this would make the identification of key volatile compounds in *shochu* from rice *koji* clearer, as it would not contain the volatile compounds from the main materials. Furthermore, another *shochu* was prepared from steamed rice and enzymes instead of rice *koji* as a control. Since white-*koji* mould produces and secretes a large amount of citric acid, the pH of the mash becomes low during fermentation of the *shochu moromi*-mash. Thus, a citrate buffer (pH 4.0) was also used to adjust the concentration of the citric acid and the pH in accordance with that of rice *koji*. This *shochu* was expected to contain volatile compounds from rice and the metabolites of yeast. It was confirmed that the *moromi*-mash made from steamed rice and enzymes was nearly fermented with the desired outcome, although it was inferior to the *moromi*-mash made from rice *koji* (Fig. 1). These *shochu* samples were referred to as rice *koji-shochu* and enzyme-*shochu*, respectively, in this study.

Flavour characterization of rice *koji-shochu* and enzyme-*shochu*

Rice *koji-shochu* and enzyme-*shochu* were compared by sensory evaluation. The assessors selected a perceived term for each *shochu* from the 21 odour and seven taste terms that were selected from the flavour wheel of *sake (14,15)*. The odour terms were fruity, alcohol, woody, green, spicy, floral, cereal, rice bran, rice *koji*, steamed rice, sweet, caramel, roasted, sulphuric, mushroom, rubber, mouldy, earthy, soapy, oily and sour, while the taste terms were sour, sweet, salty, bitter, astringent, sharp and heavy. Sulphuric and rubber were not selected by any of the assessors. Many assessors selected seven odour and five taste terms. Furthermore, many assessors pointed out the unlisted odour of 'pickle-like' for the enzyme-*shochu*. Therefore, eight odour terms were compared by adding 'pickle-like' and five taste terms for each *shochu* (Fig. 2). Rice *koji-shochu* had strong sweet, caramel and roasted odours, and strong heavy tastes. Significant differences were found in sweet, caramel, and roasted odours between rice *koji-shochu* and enzyme-*shochu*. On the other hand, the enzyme-*shochu* had a strong woody and pickle-like odour. Although rice *koji-shochu* prepared in this study was not identified as 'rice *koji*' by most of the assessors, three out of the nine assessors identified it as having a 'rice *koji*' odour. It is considered that these differences were derived from the odour of the rice *koji* and the effects of the small amount of enzymes in the rice *koji*.

GC-MS/O and AEDA

To identify the key volatile compounds in rice *koji-shochu*, GC-MS/O and AEDA analyses were performed. Rice *koji-shochu* and enzyme-*shochu* exhibited 31 and 22 odour peaks, respectively (Table 1) *(20–24)*. Eleven odour peaks could not be identified by mass spectrometry. These peaks were referred to as unknowns 1–11. Isoamyl alcohol, ethyl isobutyrate, isobutyl acetate, acetal, hexanal, unknown 2, unknown 6 and unknown 7 were detected at higher dilution in enzyme-*shochu* than in rice *koji-shochu*. In contrast, ethyl propionate, isoamyl acetate and isovaleraldehyde were detected at higher dilution in rice *koji-shochu* than in enzyme-*shochu*. Ethyl caprylate, ethyl caproate, ethyl laurate and unknowns 1, 3, 5, 8, 9 and 11 were detected in rice *koji-shochu* only. Among them, unknowns 5, 8, 9 and 11 that impart characteristic odours such as soda, potato, lavender and tea-like were specifically detected in rice *koji-shochu*. These unknown odour peaks do not have higher FD values but may contribute the characteristic odours. It has been reported previously that *shochu* prepared using a saccharification enzyme as an alternative to rice *koji* has a dry taste *(25,26)*. Although that *shochu* was prepared by adding sweet potato as the main material, it is consistent with our results. Isovaleraldehyde has been previously identified and regarded as a characteristic compound in rice *koji (11)*. Therefore, it was confirmed that isovaleraldehyde also affects the flavour of *shochu*.

Quantification and OAV

To obtain insight into the contribution of each volatile compound to the aroma, a total of 14 aroma-active compounds were quantified. Isovaleraldehyde, ethyl 2-methylbutyrate, ethyl caproate (C6), ethyl caprylate (C8) and ethyl laurate (C12) were detected at higher concentrations in rice *koji-shochu* than in enzyme-*shochu* (Table 2). These results showed that ethyl esters of medium-chain fatty acids (C6–C12) had a higher content in the rice *koji-shochu*. The lipase activity of rice *koji* has been detected in the extract of intact rice *koji (27)*. In that study, it was considered that the lipase in rice *koji* contributed to the production of ethyl esters of long-chain fatty acids. It was revealed that the enzyme from rice *koji* may affect the production of ethyl esters of medium-chain fatty acids directly or indirectly. Therefore, most fatty acid-ethyl ester compounds imparted a strong sweet odour to rice *koji-shochu*. The concentrations of isoamyl alcohol and isoamyl acetate were higher in the enzyme-*shochu* than in the rice *koji-shochu*. It is considered that isoamyl acetate facilitates the production of high concentrations of isoamyl alcohol *(28)*. All compounds, except dimethyl trisulphide, had an OAV >1. Isovaleraldehyde and ethyl caprylate showed the highest OAVs and concentrations in both *shochus*. It is suggested that these compounds contribute to the flavour of *shochu* made from rice *koji*. This result also supports the assertion that isovaleraldehyde is one of the key flavour compounds in *shochu* made from rice *koji*. Isovaleraldehyde has also been

Table 2. Odour intensity and description of odour active compounds in rice *koji-shochu* or enzyme-shochu

Compounds	Concentration		OAV¶		Threshold* (μg/L)	Reference
	Rice *koji-shochu*	Enzyme-*shochu*	Rice *koji-shochu*	Enzyme-*shochu*		
Isovaleraldehyde	230	80	1150	400	0.2	*(17)*
Ethyl caprylate	350	230	175	115	2	*(18)*
Acetal	2,600	3,800	52.0	76.0	50	*(18)*
Ethyl caproate	90	40	18	8	5	*(18)*
Ethyl 2-methyl butyrate	16	6	16	6	1	*(18)*
Ethyl isobutyrate	110	90	7.3	6.0	15	*(18)*
Ethyl acetate	39,000	30,000	5.2	4.0	7,500	*(18)*
Hexanal	21	25	4.7	5.6	5	*(18)*
Isoamyl alcohol	139,000	278,000	4.6	9.3	30,000	*(18)*
Isoamyl acetate	130	310	4.3	10.3	30	*(18)*
Ethyl butyrate	70	50	3.5	2.5	20	*(18)*
Ethyl isovalarate	7	9	2.3	3.0	3	*(18)*
Dimethyl trisulfide	0.13	0.16	0.7	0.8	0.20	*(18)*
Ethyl laurate	110	80	-	-	-	-

* Odour threshold were taken from the literature *(17,18)*. *(17)* Odour thresholds were determined in water. *(18)* Odour thresholds were determined in 10% (w/w) ethanol/water solution.
¶ OAV were calculated by dividing the concentration by the odour thresholds.

detected in other liquors such as sherry wine, pear brandy and bourbon whisky *(29–31)*. It was previously revealed that isovaleraldehyde is formed during fermentation as an intermediate of isoamyl alcohol production by yeast *(32,33)*, and formed during distillation through the Maillard reaction *(34,35)*. In addition, in Japanese *sake*, it has been reported that isovaleraldehyde is produced from isoamyl alcohol by the alcohol oxidase of *koji* mould during storage and commercial distribution at room temperature and that the active enzyme in *sake* is inactive during the heating process *(36)*. With respect to the fact that *shochu* is a distilled liquor, the increment of isovaleraldehyde during storage in *shochu* can be eliminated. Therefore, isovaleraldehyde in enzyme-*shochu* might be mostly produced by yeast metabolites and distillation. The Maillard reaction during distillation is also a process that can produce isovaleraldehyde. However, when one considers that distillation is a common process between rice *koji-shochu* and enzyme-*shochu*, it is suggested that the higher concentration of isovaleraldehyde in rice *koji-shochu* is derived from rice *koji* itself and produced during fermentation by enzymes from rice *koji*. The results of this study suggest that the isovaleraldehyde content in rice *koji* affects the concentration in *shochu* and the *shochu* flavour. In the future, it will be necessary to identify the formation mechanisms of isovaleraldehyde during the making of rice *koji* and the effect of isovaleraldehyde on the flavour of *shochu*.

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