［Research Note］

# Phenolic Off Flavor Characterization of Commercially Available Wine Yeasts and Selection of the Yeast for Koshu Winemaking

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**Abstract**

The grape Koshu is a traditional, Japan specific *Vitis vinifera*. As the Koshu wine has a relatively flat taste, the *sur lies* method and the small barrel fermentation have been employed to add richness to the wine. However, the Koshu grape contains relatively high amount of phenolic compounds. Therefore, the screening of phenolic off flavor (POF) negative strains has been carried out using mainly dried yeast products obtained from Lallemand. Of 47 yeast strains tested, the Uvaferm 228, Lalvin ICV Opale, Lalvin V1116 were found to be POF negative. We further examined the performance of winemaking using the yeasts, and the Uvaferm 228 was found to produce Koshu wine having rich aromas with many esters. The results of screening of POF negative yeasts and the GC-MS profile of the Uvaferm 228 are reported.

**Key words:** Aromas, Koshu grape**,** Phenolic off flavor, Yeast selection, Uvaferm 228

## Introduction

The Koshu grape is indigenously grown in Japan and has been traditionally cultured as table and wine grapes in the Katsunuma region, located 100 km west of Tokyo. Goto-Yamamoto genetically analyzed the grape origin, and found that the Koshu grape is a kind of oriental varieties of *Vitis vinifera* (Goto-Yamamoto et al. 2006). The Koshu wine contains relatively high levels of phenolics (Okamura et al. 1981) and it shows a relatively flat taste. Sometimes, the Koshu wine exhibits phenolic off flavors (POF) because of the phenolics (Kobayashi et al. 2006). In white wine, volatile phenols such as 4-vinylphenol and 4-vinylguaiacol are produced by the action of cinnamate decarboxylase produced by genus *Saccharomyces* and *Brettanomyces* (Chatonnet et al. 1993, Edlin et al. 1998). The defective aromas are called as the phenolic off flavor (POF). The precursor of 4-vinylphenol and 4-vinylguaiacol are *p*-coumaric acid and ferulic acid, respectively (Fig. 1). In

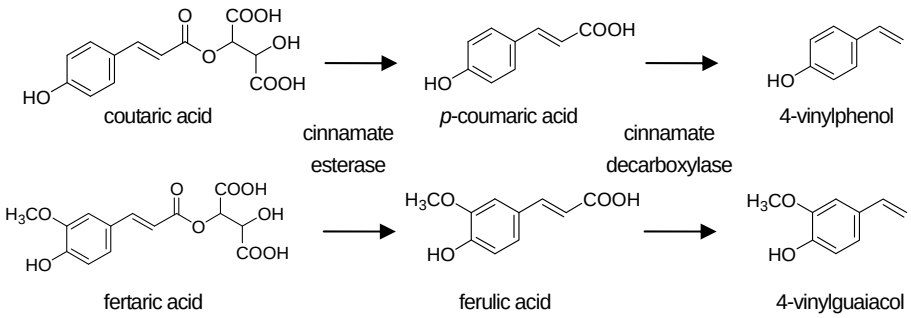


Fig. 1 Formation pathways of vinylphenol by enzyme reactions involved in winemaking.

grapes, those hydroxycinnamates are bonded with tartaric acid, forming coutaric acid and fertaric acid. Unfortunately, Koshu grape contains a relatively high level of the

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hydroxycinnamic tartrates (Yokotsuka et al. 1993). In making wine, pectinase preparations are normally added to grape juice for its clarification. If pectinase preparations contain the cinnamate esterase activity, the precursor of POF such as *p*-coumaric acid and ferulic acid are formed in the grape juice. Unfortunately the popular pectinase preparation such as Scrase®N contains cinnamate esterase. Therefore, not only the selection of pectinase preparations but also the selection of POF negative strain is important to prevent POF formation in Koshu winemaking. We added the Scrase®N in Koshu juice to form POF precursors in our screening system of the POF characterization.

We had developed the Koshu wine made by the s*ur lie* method for overcoming the flatness. The contact of wine with yeast lees for about 6 months resulted in the wine with high levels of amino acids, fruity and crispy flavor (Ari'izumi et al. 1994, Sato et al. 1997). Fermentation in a small oak barrel gives the wine richness with vanillin and oak flavors. The barrel fermentation is another typical style of Koshu wine. Recently, Dr. Tominaga et al. (Tominaga et al. 2003), found a thiol aroma, 3-mercaptohexane-1-ol (3MH) in Koshu grapes by collaboration with a Japanese winery (Kobayashi et al. 2004), and developed the wine with aromas of grapefruits and passion fruits. It developed a new type Koshu wine; however the level of 3MH was not a sufficient level to maintain aromas over years.

There is an exception, *Isehara*, the wine has rich aromas, and made only from Koshu grapes grown in a vineyard at the Isehara region, Katsunuma, Japan. Therefore, we thought that it will be still possible to make quality Koshu wine by examination of other factors to be tested. There had been a few reports on the selection of wine yeasts for domestic winemaking (Hikawa et. al. 2004, 2005, Iino 1991). Those reports dealt with relatively a small number of wine yeast, and there is no large report on the selection of wine yeast appropriate for Koshu winemaking, especially on the POF formation. Therefore, we screened the POF negative strains and selected yeast strains making Koshu wine with a rich aroma using commercially available wine yeasts.

## Materials and Methods

**Reagents.** The phenolic standard compounds such as 4-vinylphenol, 4-vinylguiacol, and the internal standard, 1-octanol, for GC-MS were purchased from Wako Pure Chemical Industries, Ltd., Japan. Sucrose is a commercial product, and a pectinase (Scrase®N, Mitsubishi-Kagaku Foods Co., Ltd., Japan) was used for the juice clarification. Other reagents were all analytical grade.

**Grapes**. Koshu grapes were grown at the experimental vineyard of University of Yamanashi, and grapes were harvested at early October 2007 and 2008.

**Wine yeast**. Most yeast strains were provided as dried products by Lallemand, and W-3 developed in the University of Yamanashi was used as the reference. The strains used were as follows: Cross Evolution, Enoferm AMH, BDX, BGY, CSM, L2226, L2323, M1, M2, Simi White, QA23, T306, VQ15, (12 strains), Lalvin 71B, BA11, BM45, BM 4 x 4, BRL97, CY3079, DV10, EC1118, ICV D21, ICV D47, ICV D80, ICV D254, ICV GRE, ICV Opale, QD145, R2, RC 212, Rhone 2056, Rhone 4600, R-HST, S6U, T73, V1116, W15, (24 strains), Uvaferm 228, CEG, CM, CS2, HPS, SVG, VN, VRB, (8 strains), AWRI 1176 (test sample), W-3 (University of Yamanashi). The strain VL-3 (Laffort) was also examined to compare the performance.

**HPLC analysis for Phenolic Off Flavor (POF)**. The HPLC conditions are as follows: column, Atlantis™ T3 4.6 × 150 mm, 3 μm (Waters); mobile phase, A =0.4% $H_3PO_4$, B = $CH_3CN$; Flow Rate, 1.0 mL/min; injection volume, 10 μL; column temperature, 40ºC; detection, 1ch: diode array (190-600 nm), 2ch: fluorescence (*ex*. 225 nm, *em*. 320 nm); instrument, HITACHI L-2000 System; detection limit, 10 μg/L.

**Aroma Analysis by GC-MS.** One mg of 1-octanol as an internal standard was added to 500 mL of wine followed by extraction twice with 50 mL of pentane/ether (1:1). The solvent layer was dehydrated on anhydrous $Na_2SO_4$ for overnight. The extracted solvent was concentrated *in vacuo* to 20 μL, and it was injected to GC-MS. The GC-MS conditions were as follows: GC-MS system, Shimadzu QP5050; column, TC-Wax (60 m x 0.25 mm, GL Science); split ratio, 1:20; temperature program, 9 min (50ºC), 50→ 230ºC (4ºC/min), 230ºC 20 min hold; sample injection volume, 1 μL.

## Results and Discussion

Koshu grapes were harvested October 10 (2007), the grapes were pressed with a small water-press followed by addition of a pectinase (Scrase®N, 200 mg/L), and placed in a cold room (5ºC) for over night. Clear juice (16.8ºBrix, yield 65%) was obtained by decantation and recovered the juice by centrifugation of the sediment. Each dried yeast was rehydrated at 40 ºC and inoculated (250 mg/L) to the 200 mL of grape juice (21ºBrix with addition of sucrose) in a 300 mL flask, and the juice was fermented at 25ºC for 10 to 14 days. Wine was obtained by centrifugation followed by addition of $SO_2$ (100 mg/L). Resulted wine was subjected to the sensory evaluation by members of the Institute of Enology and Viticulture, University of Yamanashi (n = 15). The aromas, bitterness, mouth feel, and general impression were scored. Phenolics of the wine were analyzed by HPLC and aromas were analyzed by GC-MS.

Table 1 The levels of 4-vinylphenol (4VP) and 4-vinylguaiacol (4VG) in Koshu wine.

| Strains | 4VP | 4VG |
|---|---|---|
| Cross Evolution | 0.15 ± 0.01 | 0.19 ± 0.01 |
| Enoferm AMH | 0.80 ± 0.05 | 0.86 ± 0.09 |
| BDX | 1.15 ± 0.09 | 1.14 ± 0.25 |
| BGY | 1.07 ± 0.09 | 0.84 ± 0.08 |
| CSM | < 0.01 | < 0.01 |
| L2226 | 0.31 ± 0.02 | 0.31 ± 0.04 |
| L2323 | 1.36 ± 0.11 | 1.27 ± 0.17 |
| M1 | 1.18 ± 0.10 | 1.05 ± 0.23 |
| M2 | 1.02 ± 0.10 | 0.94 ± 0.07 |
| Simi White | 1.88 ± 0.12 | 2.73 ± 0.12 |
| QA23 | 1.06 ± 0.18 | 1.09 ± 0.16 |
| T306 | 0.13 ± 0.02 | 0.16 ± 0.01 |
| VQ15 | 1.25 ± 0.15 | 1.00 ± 0.08 |
| Lalvin 71B | 1.20 ± 0.17 | 1.25 ± 0.10 |
| BA11 | 1.20 ± 0.14 | 1.30 ± 0.23 |
| BM4*4 | < 0.01 | < 0.01 |
| BM45 | 0.42 ± 0.03 | 0.53 ± 0.09 |
| BRL97 | 0.82 ± 0.07 | 0.92 ± 0.10 |
| CY3079 | 1.04 ± 0.15 | 1.04 ± 0.05 |
| DV10 | 1.06 ± 0.06 | 0.87 ± 0.08 |
| EC1118 | 0.94 ± 0.09 | 0.76 ± 0.05 |
| ICV D21 | 0.95 ± 0.10 | 0.73 ± 0.03 |
| ICV D47 | 1.14 ± 0.16 | 1.20 ± 0.06 |
| ICV D80 | 0.93 ± 0.07 | 0.73 ± 0.08 |
| ICV D254 | 0.97 ± 0.05 | 0.97 ± 0.16 |
| ICV GRE | 0.73 ± 0.06 | 0.80 ± 0.10 |
| ICV OPALE | 0 | 0 |
| QD145 | 0.79 ± 0.09 | 0.67 ± 0.07 |
| R2 | 0.42 ± 0.04 | 0.25 ± 0.01 |
| RC212 | 1.06 ± 0.06 | 0.97 ± 0.05 |
| Rhone 2056 | 1.39 ± 0.11 | 1.16 ± 0.07 |
| Rhone 4600 | 0.99 ± 0.07 | 0.59 ± 0.09 |
| R-HST | 1.44 ± 0.11 | 1.38 ± 0.19 |
| S6U | 1.00 ± 0.12 | 1.60 ± 0.22 |
| T73 | 0.81 ± 0.04 | 1.01 ± 0.08 |
| V1116 | 0 | 0 |
| W15 | < 0.01 | < 0.01 |
| Uvaferm 228 | 0 | 0 |
| CEG | 0.80 ± 0.05 | 0.70 ± 0.09 |
| CM | 0.97 ± 0.07 | 0.66 ± 0.06 |
| CS2 | 1.40 ± 0.20 | 2.17 ± 0.19 |
| HPS | 0.90 ± 0.07 | 0.99 ± 0.11 |
| SVG | 0.65 ± 0.02 | 0.61 ± 0.03 |
| VN | 0.46 ± 0.03 | 0.71 ± 0.07 |
| VRB | 0.20 ± 0.01 | 0.18 ± 0.02 |
| AWRI 1176 | 0.81 ± 0.04 | 0.76 ± 0.08 |
| W-3 | 0.93 ± 0.07 | 0.95 ± 0.08 |

The values are average of triplicated analysis in mg/L concentration.

The results on the POF formation are shown in Table 1. The typical HPLC analytical profiles of the wine produced with Lalvin EC 1118 are shown in Fig. 2. The profile of Fig. 2-A was analyzed with a UV spectrophotometer (Abs. 260 nm) and 2-B was analyzed with a fluorescence detector (*ex*. 225 nm, *em*. 320 nm). The peaks of 4-vinylphenol and 4-vinylguiacol were clearly detected in Fig. 2-B. On the other hand, the Uvaferm 228 did not produce POF peaks.

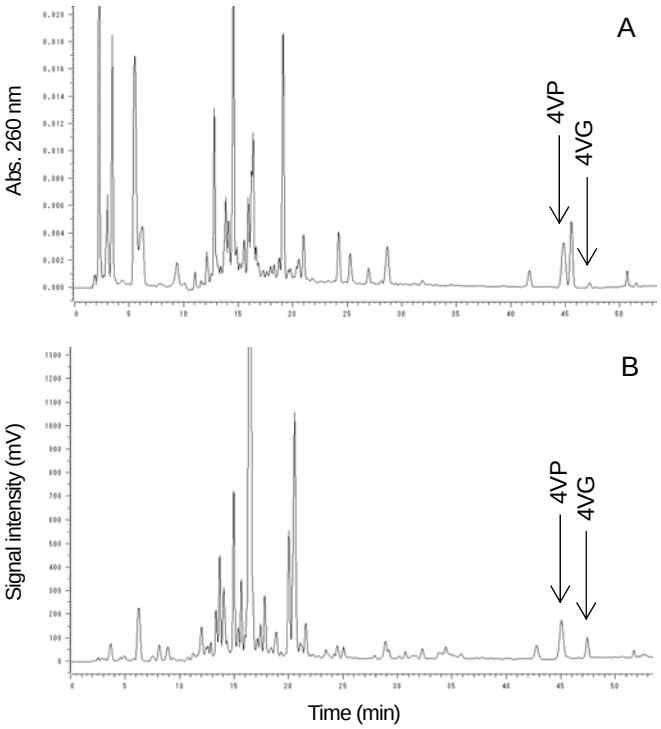


Fig. 2 The phenolic HPLC profile of the wine produced with Lalvin EC 1118. 2-A shows the analytical profile with a UV spectrophotometer (Abs. 260 nm), and 2-B was detected by fluorescence (*ex*. 225 nm, *em*. 320 nm).

The detective sensitivity was higher in fluorescence detector than the UV spectrophotometer. The detection limit of the HPLC system with the fluorescence detector was 0.01 mg/L (data not shown). Of the yeast strains tested, Cross

Evolution, Lalvin ICV Opale, Lalvin V1116, Lalvin BM 4×4, Uvaferm 228, Uvaferm VN, Uvaferm VRB, Enoferm CSM did not produce POF more than 0.1 mg/L (Table 1). Boutou and Chatonnet (Boutou et al. 2007) reported the sensory threshold of 4-vinylphenol (1.5 mg/L) and 4-vinylguaiacol (0.38 mg/L) in wine. As the strains Enoferm L2226, Lalvin R2, and Enoferm T306 produced the phenolics below the threshold, they may be also good for making Koshu wine. Of the low POF strains, Lalvin ICV Opale, Lalvin V1116, and Uvaferm 228 did not produce any POF compound.

In the preliminary fermentation test (Nakagawa 2010), evaluation of aromas (5 points) shows that the average value of POF negative strains was 2.85±0.16, and it was higher than that of POF positive strains of 2.61±0.30 ($p < 0.05$). POF negative strains got higher points than POF positive strains in the evaluation of aromas.

Unfortunately, the number (n = 15) of panelists was too small to select significantly good strains from yeast strains tested. Five strains were selected by evaluations of aromas and the total impression (11). The strains were as follows: Lalvin QD145, Uvaferm 228, Lalvin T73, Uvaferm CEG, and W-3. The 5 strains were fermented in 7 L Koshu grape juice/10 L bottle at 18ºC for about 4 weeks. The results of general analysis are shown in Table 2. In the sensory evaluation, average values (n = 15) were shown in 5 points evaluation; 5, high and 1, low. From the result (Table 3), the Uvaferm 228 was selected as the best one for the Koshu winemaking. As the Uvaferm 228 is POF negative, the high evaluation in aromas of the strain may be related to the POF negative trait.

Of the 5 strains tested, as aromas and general impression of the Uvaferm 228 showed excellent evaluations, and the aromas were analyzed by GC-MS. Fig. 3 shows the GC-MS profile of the wine made from Uvaferm 228. EC1118 was the reference strain, and its aroma was not well balanced.

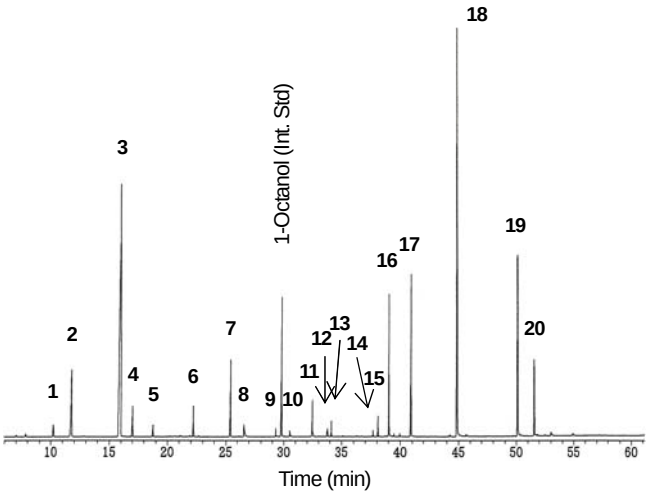


Fig. 3 The GC-MS profile of wine made with Uvaferm 228.

The aromas of isoamyl alcohol and phenethyl alcohol were too intensive (Table 4). The aroma of W-3 wine was highly evaluated as fresh and fruity. The W-3 wine was a traditional style of Koshu, and GC-MS profile showed that isoamyl acetate was very intensive like sake (rice wine). Sake has high levels of isoamyl alcohol and its ethyl ester. The aroma of the wine made with Uvaferm 228 was well balanced with many kinds of esters, and there were several comments such as fresh, citrus, grape fruits, blue grass, etc., similar to that of Sauvignon blanc. However, we could not detect the thiol compounds such as 3-sulfanylhexan-1-ol by GC-MS. The comparison of the profiles shows clearly that many peaks derived from esters appeared in the wine of Uvaferm 228 (Table 4). The complex profile reflected the high level aroma score of Uvaferm 228 by the sensory evaluation.

As Koshu grapes contain relatively high levels of hydroxycinnamic tartrates (Okamura et al. 1981), the selection of POF negative strain seems to be important to produce Koshu wine without POF. This time we firstly

Table 2 General analysis of wine made with selected 5 yeast strains.

| Strains | Specific gravity | pH | Alcohol % (v/v) | Titratable acidity (g/L) | Total Phenols (mg/L) |
|---|---|---|---|---|---|
| Lalvin QD145 | 0.994 | 3.17 | 13.7 | 6.48 | 319 |
| T73 | 0.994 | 3.14 | 13.9 | 7.98 | 284 |
| Uvaferm 228 | 0.995 | 3.15 | 13.8 | 7.91 | 303 |
| CEG | 1.015 | 3.16 | 12.2 | 7.47 | 316 |
| W-3 | 0.993 | 3.16 | 14.1 | 7.09 | 297 |

Table 3 Sensory evaluation of wine made with selected 5 yeast strains.

| Strains | Bitterness | Aromas | Taste | General Impression |
|---|---|---|---|---|
| Lalvin QD145 | 2.3 | 2.9 | 2.5 | 2.6 |
| T73 | 2.3 | 2.4 | 2.7 | 1.8 |
| Uvaferm 228 | 2.6 | 3.3 | 2.5 | 2.9 |
| CEG | 2 | 2.4 | 2.7 | 1.3 |
| W-3 | 2.9 | 2.9 | 2.8 | 1.9 |

The figures are average of 5 point evaluation (n = 15).

Table 4 Contents of volatile compounds in wine made with selected 3 yeast strains by GC-MS.

| peak | $t_R$ (min) | compound | Concentration (mg/L) Uvaferm 228 | Lalvin EC1118 | W-3 |
|---|---|---|---|---|---|
| **1** | 10.1 | Isobutanol | 0.36 | 18.71 | 0.82 |
| **2** | 11.8 | Isoamyl acetate | 2.26 | 0.47 | 9.66 |
| **3** | 15.7 | Isoamyl alcohol | 4.59 | 25.12 | 11.73 |
| **4** | 16 | Ethyl caproate | 0.43 | 0.22 | 0.88 |
| **5** | 18.8 | Hexyl acetate | 0.36 | - | 0.71 |
| **6** | 22.2 | 1-Hexanol | 0.75 | 0.3 | 0.43 |
| **7** | 25.3 | Ethyl caprylate | 0.92 | 0.54 | 1.44 |
| **8** | 25.5 | Acetic acid | 0.37 | 0.68 | 0.25 |
| **9** | 29.4 | 2,3-Butanediol | 0.51 | 0.5 | - |
| **10** | 30.5 | Isobutyric acid | 0.23 | - | - |
| **11** | 32.4 | Ethyl caprate | 0.47 | 0.33 | 0.62 |
| **12** | 33.8 | Diethyl succinate | 0.15 | - | - |
| **13** | 34.1 | 1-Undecenoic acid | 0.19 | - | - |
| **14** | 37.7 | 3-Hydroxybutyl butyrate | 0.06 | - | - |
| **15** | 38 | Phenethyl acetate | 0.72 | 0.43 | 0.6 |
| **16** | 39 | Caproic acid | 4.34 | 1.39 | 2.64 |
| **17** | 40.9 | Phenethyl alcohol | 4.51 | 10.1 | 1.12 |
| **18** | 44.9 | Capric acid | 8.36 | 3.58 | 11.02 |
| **19** | 50 | Caprylic acid | 2.85 | 0.99 | 2.9 |
| **20** | 51.6 | Undecanoic acid | 1.12 | - | 0.25 |

reported the POF characters of commercially available yeasts, and the POF negative strain, Uvaferm 228, was selected appropriate for Koshu winemaking.

Although we could select good wine yeast for making Koshu wine, the winemaking scale was very small. Large scale winemaking tests are necessary for the rigid evaluation of the yeast, Uvaferm 228.

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［Research Note］

# 市販酵母のフェノール性異臭産生能と甲州ワイン醸造用酵母の選抜

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**要　約**

日本固有の品種である甲州種ブドウ（*Vitis vinifera*）から製造したワインは比較的平坦な味わいで、現在までシュール・リーや小樽発酵などいろいろな醸造方法が検討されてきた。我々はさらに高品質な甲州種ワインを醸造するために、香りに着目して最適な酵母を選抜した。特に甲州種ブドウは他の品種と比較し、シンナム酸酒石酸エステルが多く含まれることが知られており、これらの化合物はフェノール性異臭（Phenolic Off Flavor: POF）の原因となる揮発性フェノール類の前駆体である。そこで、市販酵母47種類の中から揮発性フェノール類を生成しない酵母を選抜した結果、Uvaferm 228, Lalvin ICV Opale, Lalvin V1116 の 3 株が揮発性フェノール非産生株であった。また、香りを重視した官能評価試験から甲州ワインの醸造に適した酵母として Uvaferm 228 が選抜された。GC-MS による分析の結果、Uvaferm 228 で醸造したワインは、他の市販酵母と比較し、多くの芳香性エステル類を含んでいた。以上より、Uvaferm 228 は揮発性フェノール類を生成せず、芳香性エステル類を多く産生する酵母という点で甲州種ワインの醸造に優れていた。