Supplementary Information

Liposoluble antioxidative components in Japanese traditional fermented food "amazake" made from brown rice

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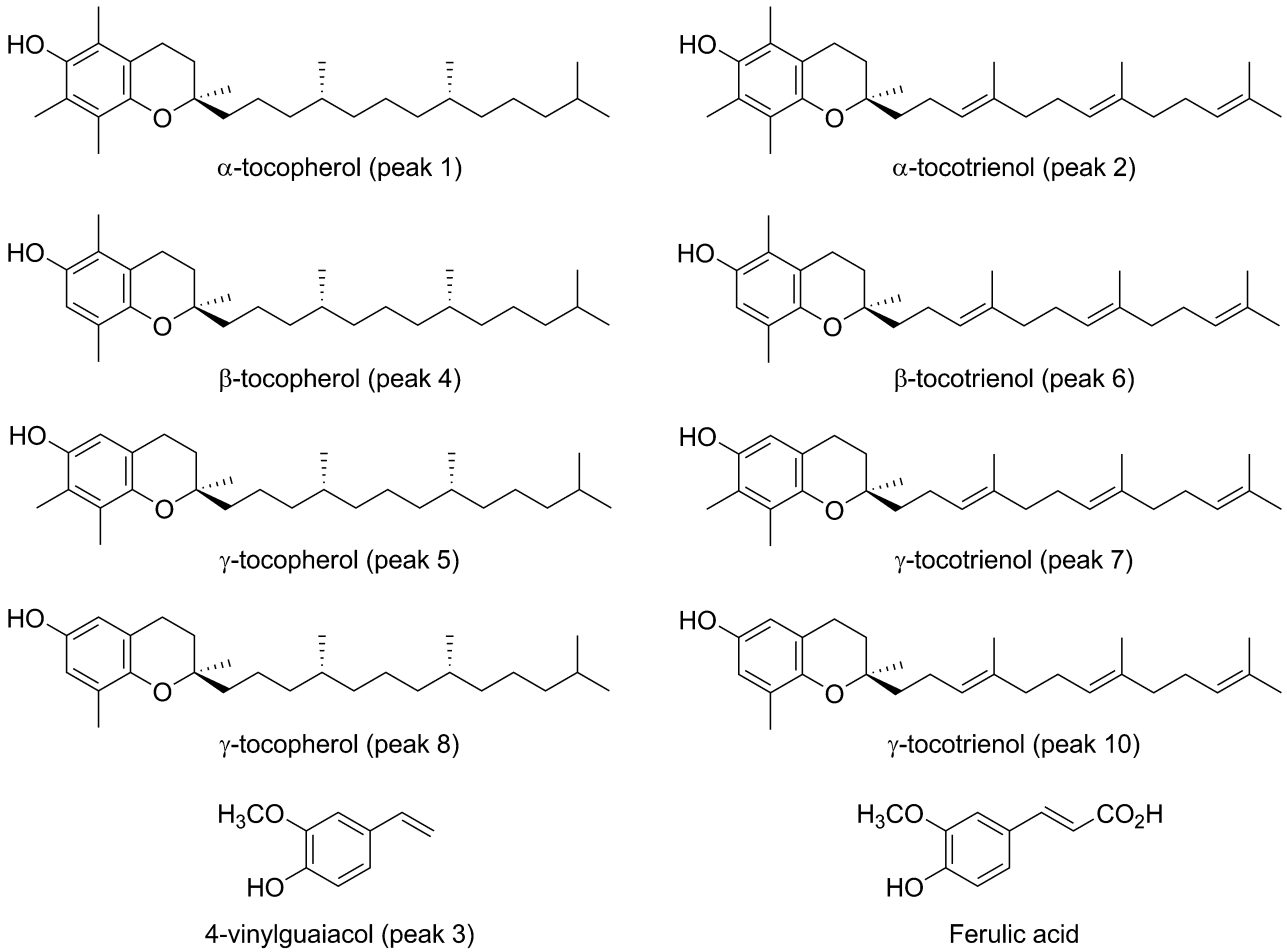
1. Chemicals

Chemicals used in this research except for the typical chemicals listed in the main text were summarized below. Sodium chloride, ethanol, hexane, ethyl acetate, acetic acid were purchased from Wako Pure Chemical Industry (Osaka, Japan). Pyrogallol, methanol and 1,4-dioxane were purchased from Nacalai Tesque (Kyoto, Japan). Chemical structures of the compounds quantitated in this research were listed in scheme S1. Peak numbers in NP-HPLC were written in parentheses.



Scheme S1. Chemical structure of various liposoluble-antioxidants in amazake.

2. HPLC chromatograms of other amazake samples

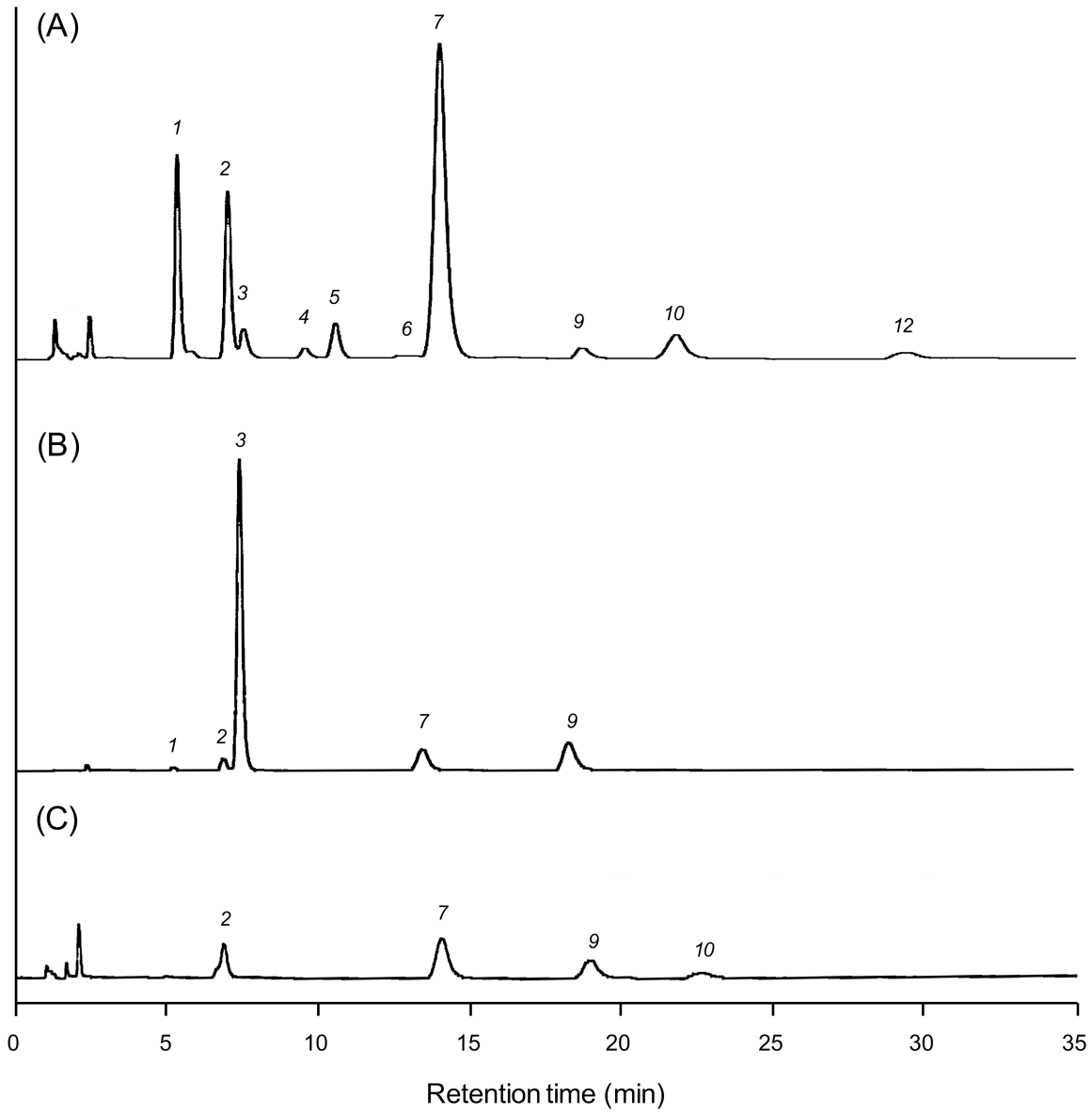


Figure S1. HPLC chromatogram of the AcOEt extract from (A) BRA2 made by Ayumasamune Co., (B) WRA2 made by Fukumitsuya Co. and (C) SCA2 made by Melodian Co.

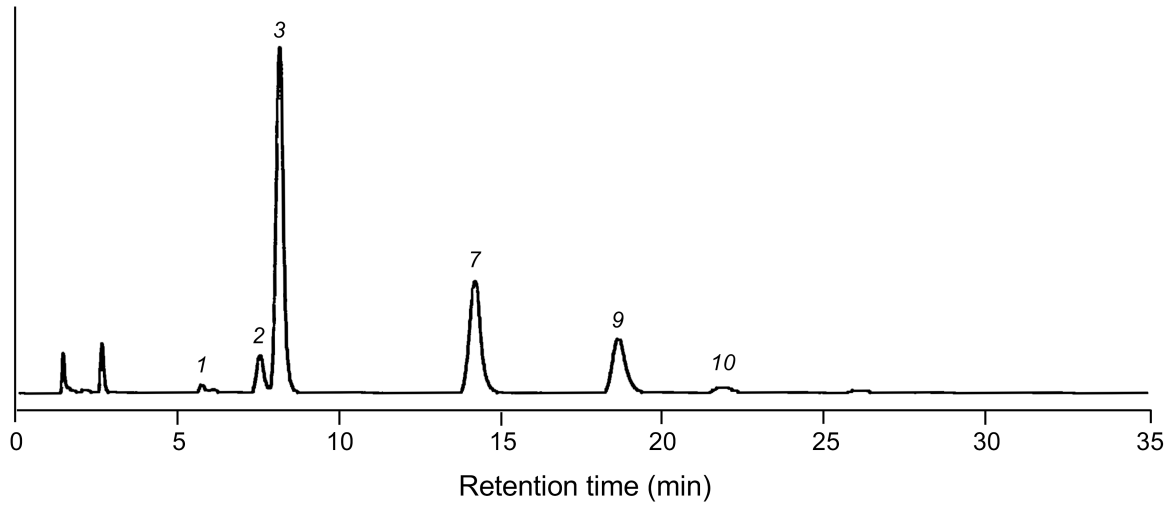


Figure S2. HPLC chromatogram of the extract from white rice koji provided by Misuzu Shouhin.

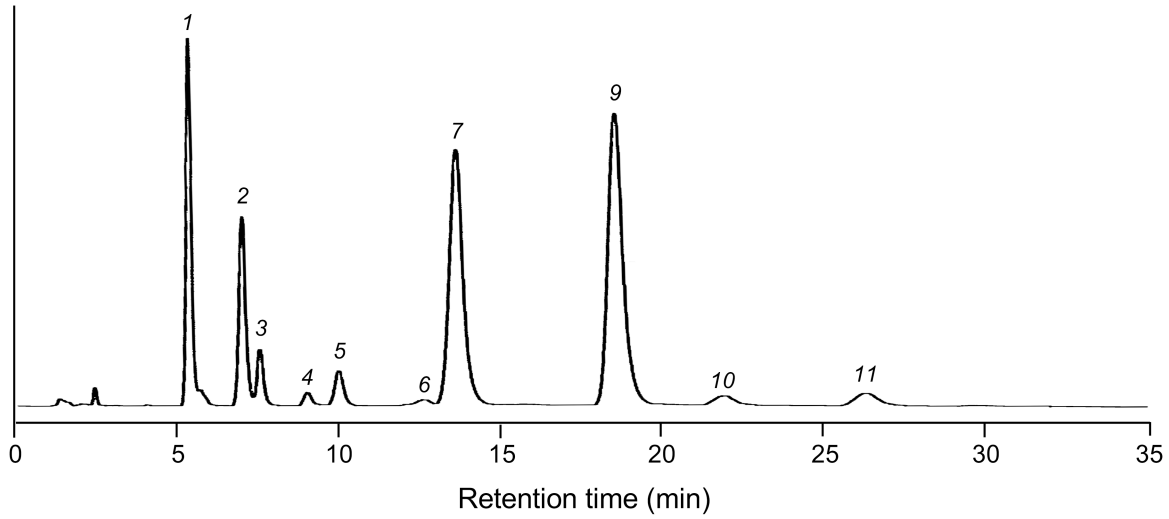


Figure S3. HPLC chromatogram of the extract from gelatinized brown rice powder.

3. Thermal degradation of α-tocopherol in water

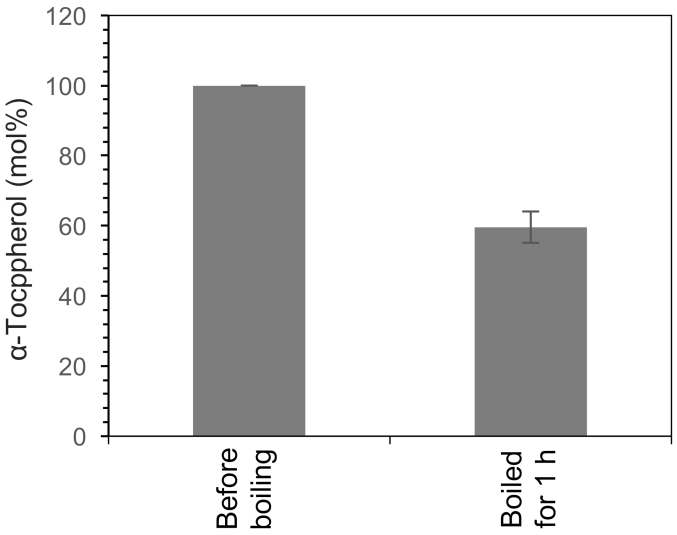


Figure S4. Decomposition of α-tocopherol in boiling water.

4. NP-HPLC chromatograms of the food samples during pre-saccharification process

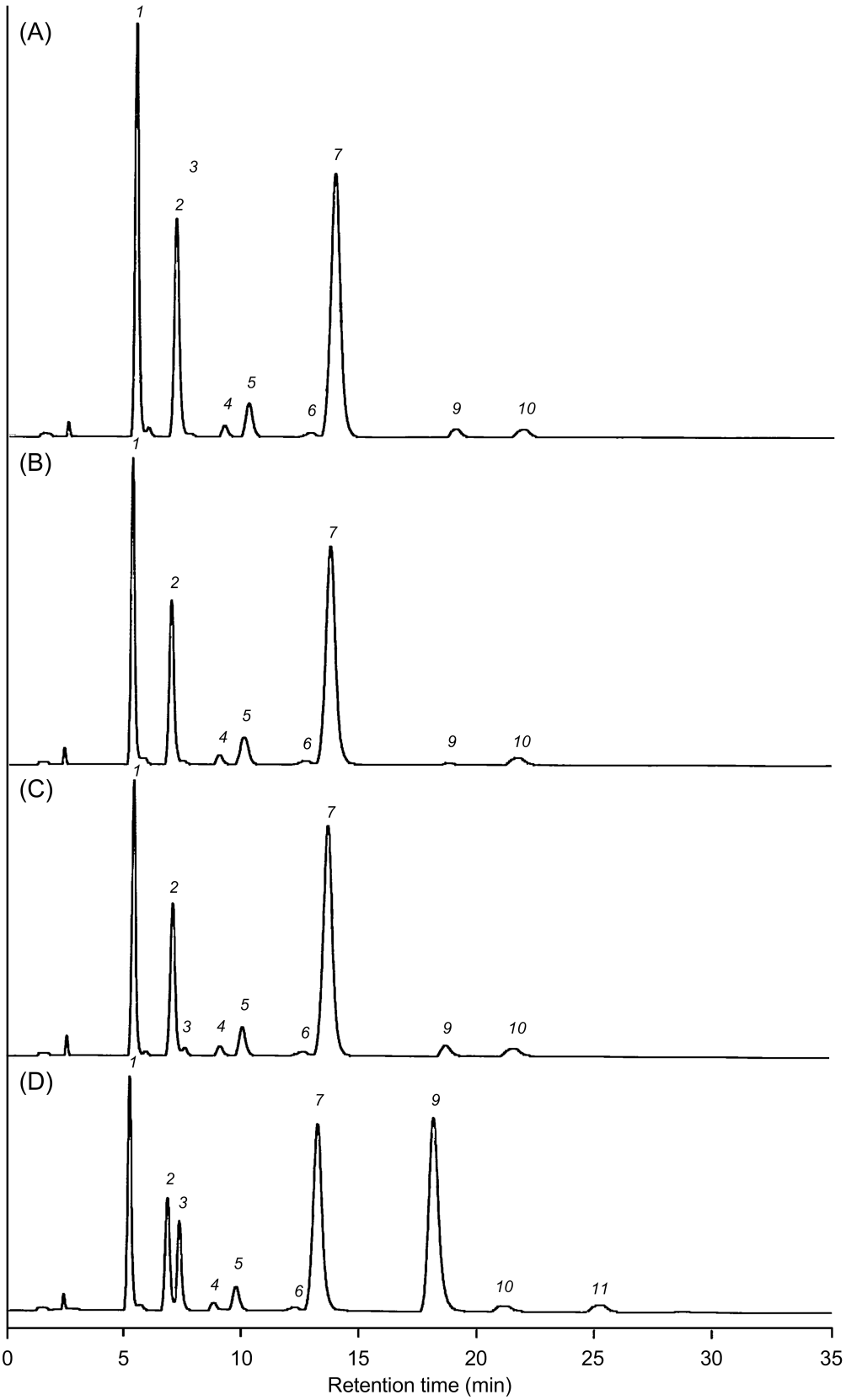


Figure S5. NP-HPLC chromatograms of the extracts from brown rice powder treated by multi-enzyme complex. (A) untreated control, (B) treated at 45 °C, (C)treated at 45 °C, and 70 °C, and (D) treated at 45 °C, 70 °C, and 95 °C.

5. NP-HPLC chromatograms of the food samples during saccharification process

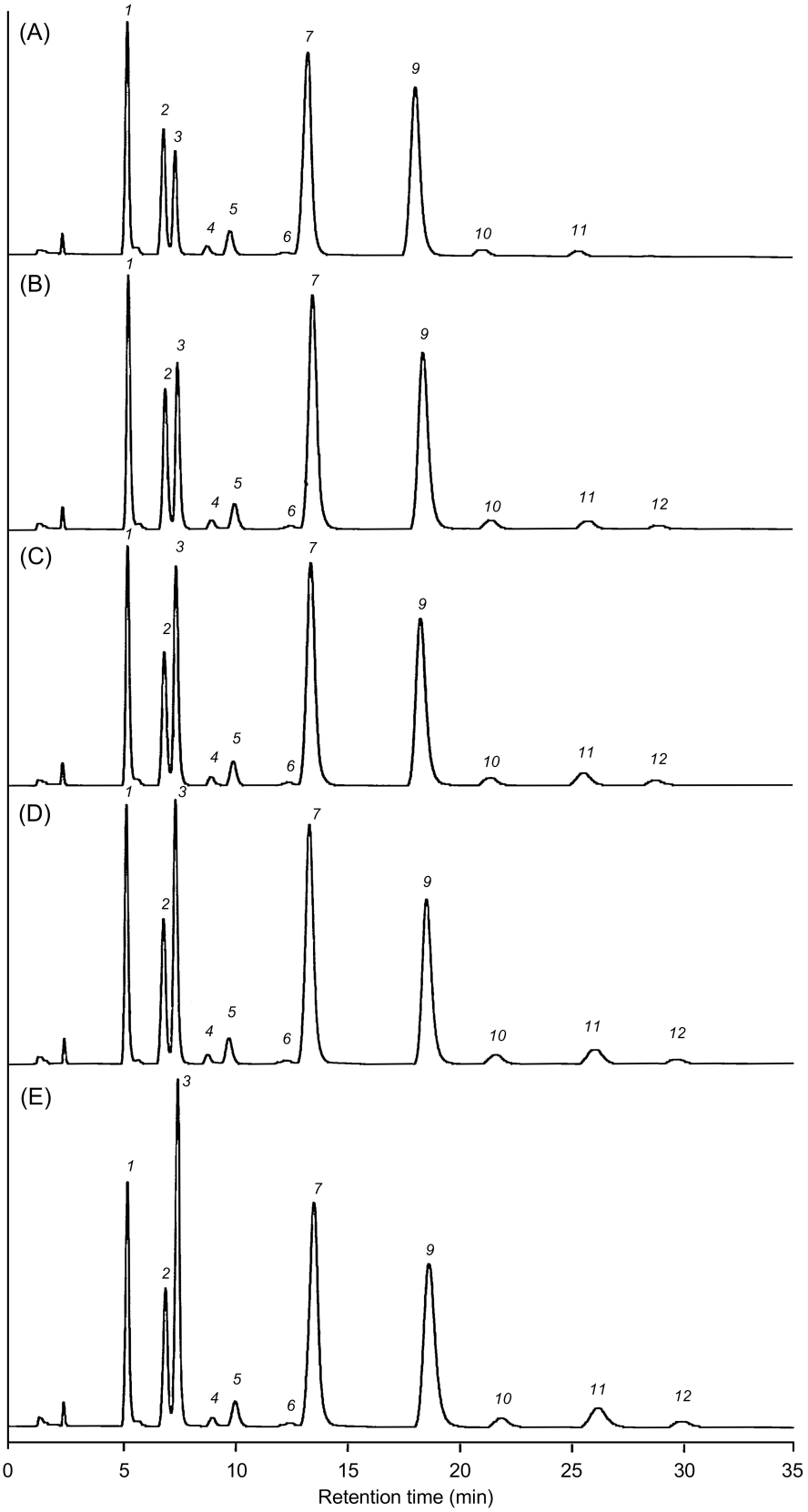


Figure S6. HPLC chromatograms of the extracts from saccharified brown rice with brown rice koji for (A) 0 h, (B) 6 h, (C) 12 h, (D) 18 h, and (E) 24 h.

6. Brix change during saccharification process

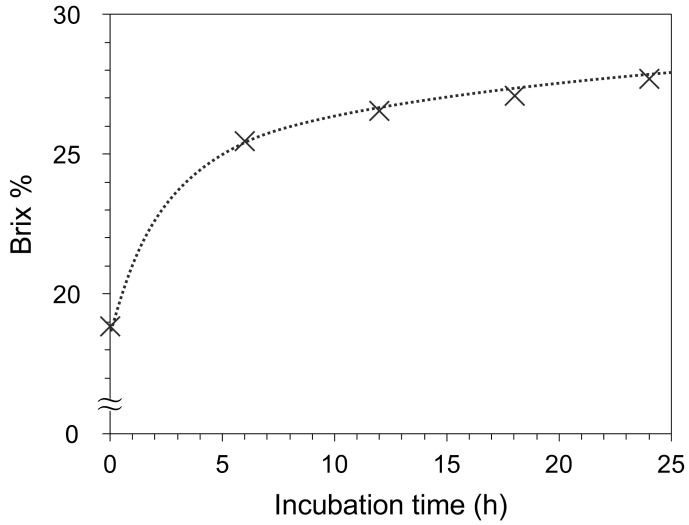


Figure S7. Brix change during saccharification process at 57 °C.

7. Assignment of Peak 3

Brown rice koji (1.2 kg) and water (1.6 L) were mixed and incubated at 57 °C for 24h. After shaking, obtained mush was extracted with hexane (1.2 L) twice. The combined extract was concentrated *in vacuo*. Yellow residue was dissolved in MeOH (5 mL) and stored at 4 °C for 12 h in order to remove wax. White precipitate was removed off by a centrifuge (800 G, 2 min) and this process was repeated until no precipitate formed. Hexane was evaporated off. Obtained yellow oil was dissolved in MeOH and subjected to preparative RP-HPLC (Inert sustain 14 x 150 mm, GL science). UV-vis absorption and fluorescence spectra of the target fraction were obtained with V-550 and FP-750 (JASCO). The absorption maximum appeared at 266 nm with a shoulder peak at ca. 290 nm (Figure S8). Fluorescence spectrum with an excitation at 266 nm has fluorescent maximum at 335 nm (Figure S9). To the target fraction was added DMSO-d_6 (0.6 mL) in order to prevent volatilization of target compound, and this solution was concentrated *in vacuo*. Isolated compound was analyzed by NMR spectroscopy. NMR analysis was conducted at the Advanced Research Center of Kanazawa University. The spectroscopic characters are consistent with known spectra of 4-VG.

^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.17 (s, 1H), 7.10 (d, J = 1.8 Hz, 1H), 6.91 (dd, J = 8.2, 1.8 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1H), 6.66 (dd, J = 17.8, 10.8 Hz, 1H), 5.69 (dd, J = 17.8, 1.2 Hz, 1H), 5.12 (dd, J = 10.8, 1.2 Hz, 1H), 3.85 (s, 3H), ^{13}C-NMR (100 MHz, DMSO-d6) δ 147.7, 146.7, 136.7, 128.8, 119.6, 115.4, 110.4, 109.6, 55.6.

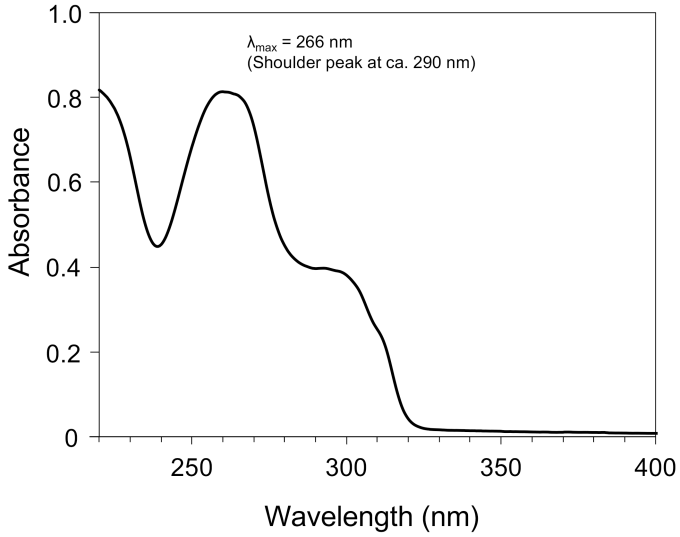


Figure S8. UV-vis absorption spectrum of 4-VG isolated from brown rice koji.

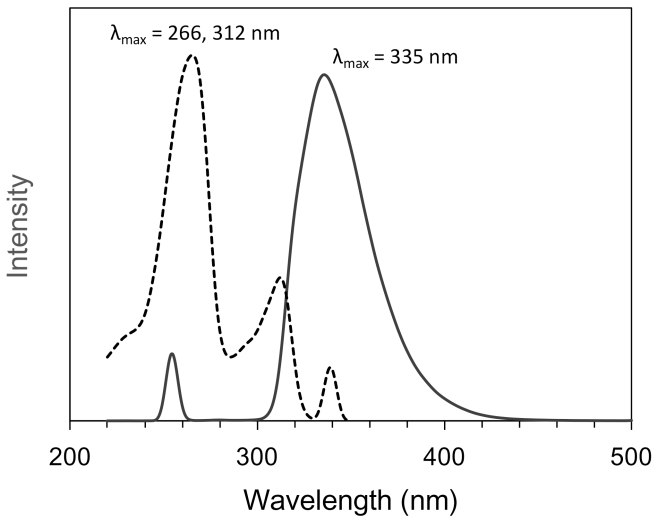


Figure S9. Excitation and emission spectra of 4-VG isolated from brown rice koji