Effects of distillation process on antioxidant activity of Japanese traditional spirits rice-*shochu*

Noriaki Saigusa* and Yuji Teramoto

Department of Applied Microbial Technology, Faculty of Biotechnology and Life Science,
Sojo University, 4-22-1 Ikeda, Nishi-ku Kumamoto 860-0082, Japan

Abstract

In this study we investigated the effects of the distillation process on the antioxidant activity of Japanese traditional spirits "rice-*shochu*", particularly focusing on DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity and lipid peroxidation inhibitory activity. Using polished rice, white-*koji (Aspergillus kawachii)* and S-2 yeast *(Saccharomyces cerevisiae)*, we prepared rice-*shochu* using both vacuum- and atmospheric-distillation processes. The lipid peroxidation inhibitory activity of vacuum-distilled *shochu* was 8.2 (± 0.4) μM BHT eq., whereas that of atmospheric-distilled *shochu* was 12.3 (±0.7) μM BHT eq., representing a significant difference ($p<0.01$). However, DPPH radical scavenging activity of vacuum-distilled *shochu* was 3.9 (± 1.2) μM Trolox eq., whereas that of atmospheric-distilled *shochu* was 3.4 (±0.1) μM Trolox eq., representing no significant difference ($p<0.01$). Furthermore, we analyzed the absorption spectra of both types of *shochu* and observed a common absorption maximum at 260 nm. Notably, the absorption values of the atmospheric-distilled *shochu* were higher than that of vacuum-distilled *shochu*. In addition, HPLC analysis of potential components with absorption maximum at 260 nm identified two peaks in the atmospheric-distilled *shochu* that were not detected in the vacuum-distilled *shochu*.

Keywords: rice-*shochu*, antioxidant activity, lipid peroxidation inhibitory activity, DPPH radical scavenging activity, vacuum-distillation, atmospheric-distillation.

1. Introduction

Shochu is Japanese traditional spirits produced with rice-*koji* as saccharifying agents. However, reports on the antioxidant activity of *shochu* are limited compared to that of brewed beverages. This is likely due to the fact that the antioxidant activity of *shochu* is markedly lower compared to that of brewed beverages, which contain antioxidants extracted directly from raw materials. Concerning the functional properties of *shochu*, only an anti-thrombotic effect has been reported [1, 2]. However, numerous studies have described the beneficial properties of *shochu* stillage. For example, Nakamura *et al*. [3] investigated the functional properties of rice *shochu* stillage fermented by *Lactobacillus brevis* IFO-12005 and found high levels of γ-aminobutyric acid, which lowers hypertension, autonomic neuropathy, and liver function. Furthermore, Hokazono *et al*. [4] examined concentrations of γ-aminobutyric acid in barley-*shochu* mash and its distillation residue of different *koji* ratio. And they reported that the concentration of γ-aminobutyric acid was depending on the *koji* ratio in both atmospheric- and vacuum-distillation residues. On the other hands, it was reported that vinegar made from the distillation residue of sweet potato *shochu* had high anti-radical activity in human leukocyte cancer cells. And an active agent in the vinegar was identified as caffeic acid ethyl ester [5]. The aim of the present study was to examine the effect of the distillation process on the antioxidant activity of rice-*shochu*.

2. Experimental

2.1. Chemicals

β-Carotene was purchased from Sigma Chemical Co. (St. Louis, MO). DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Nacalai Tesque (Kyoto, Japan). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma-Aldrich Inc. (St. Louis, Mo, USA). BHT (2,6-di-*tert*-butyl-*p*-cresol) was purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan). All other chemicals were of reagent grade.

2.2. Yeast strain

Saccharomyces cerevisiae S-2 purchased from the Brewing Society of Japan (Tokyo, Japan) was used.

2.3. Saccharifying agent

Tane-*koji* i.e. starter of *koji* mold *(Aspergillus kawachii)*, for making rice-*koji* was purchased from Kawachi Genichiro Shoten Co., Ltd. (Kagoshima, Japan).

*Corresponding author.
E-mail address : noriaki@bio.sojo-u.ac.jp

2.4. Rice-*koji* making

Rice-*koji* was prepared according to the method of Saigusa *et al.* [6]. Briefly, polished rice (200 g) was soaked in water at 15 °C for 20 min and the water was then drained over a 2-h period. The rice was then steamed for 40 min in a pot-type steamer, removed, and allowed to stand until the temperature decreased to 40 °C. Next, 0.2 g of tane-*koji (Aspergillus kawachii)* spores was inoculated onto the steamed rice, which was then mixed to uniformly disperse the spores. Except for the steaming process, the cultivation procedure was performed under sterile conditions. After inoculation, the steamed rice was transferred to a petri dish of 152 mm in diameter, and compacted with a spoon. A piece of filter paper was inserted inside the cover, and the plate was incubated at 30°C for 48 h. During the incubation period, the rice-*koji* was mixed every 12 h.

2.5. Production of rice-*shochu*

Rice-*shochu* was made according to the method described in The Production Techniques of *Shochu* [7]. Briefly, a mash was prepared with water (130 ml), rice-*koji* (110 g) and S-2 yeast (final concentration: 1.0×108 cells/ml) and was then fermented for 4 days at 25 °C. A total of 400 ml water and 220 g steamed rice was added to first mash, and the secondary mash was allowed to ferment for a further 6 days at 25 °C. The decrease in weight of the Erlenmeyer flask and its contents as a result of the evolution of CO_2 gas was measured every 24 h. The fermented secondary mash was then distilled using atmospheric-distillation process. In this method, the fermented mash was distilled under normal pressure (temperature was 90-95 °C) using steam to heat the mash. Steam was produced by heating with mantle heater (TAIKA Electric Co., Ltd., Osaka, Japan) (Fig.1). Alternatively, an vacuum-distilled *shochu* making was performed under reduced pressure (50 °C, 70 mm Hg) using an aspirator (ULVAC KIKO Inc., Miyazaki, Japan) (Fig. 2).

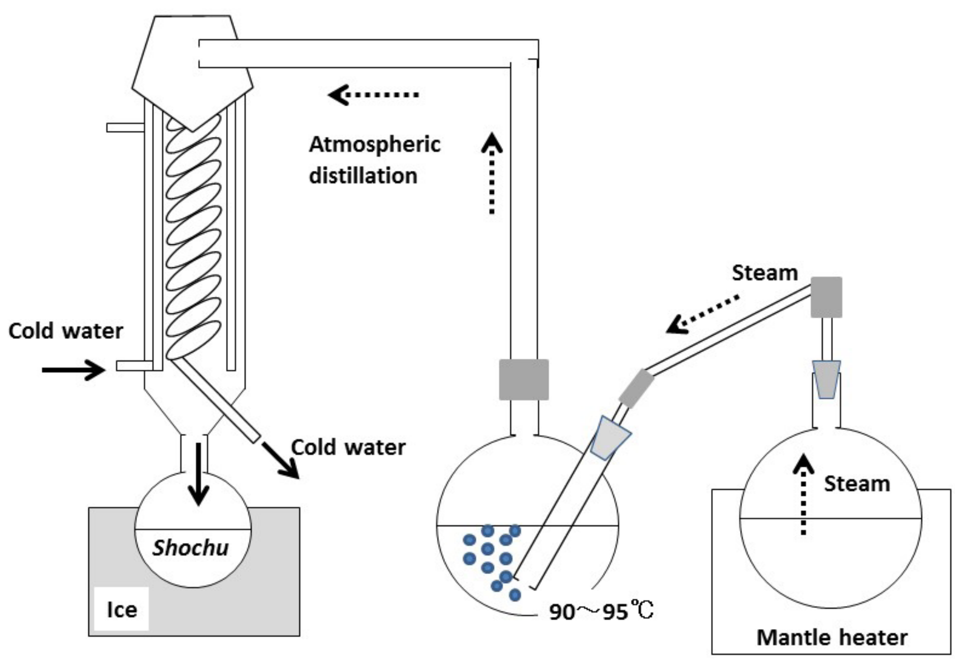

Fig.1 : Schematic diagram of atmospheric distillation apparatus

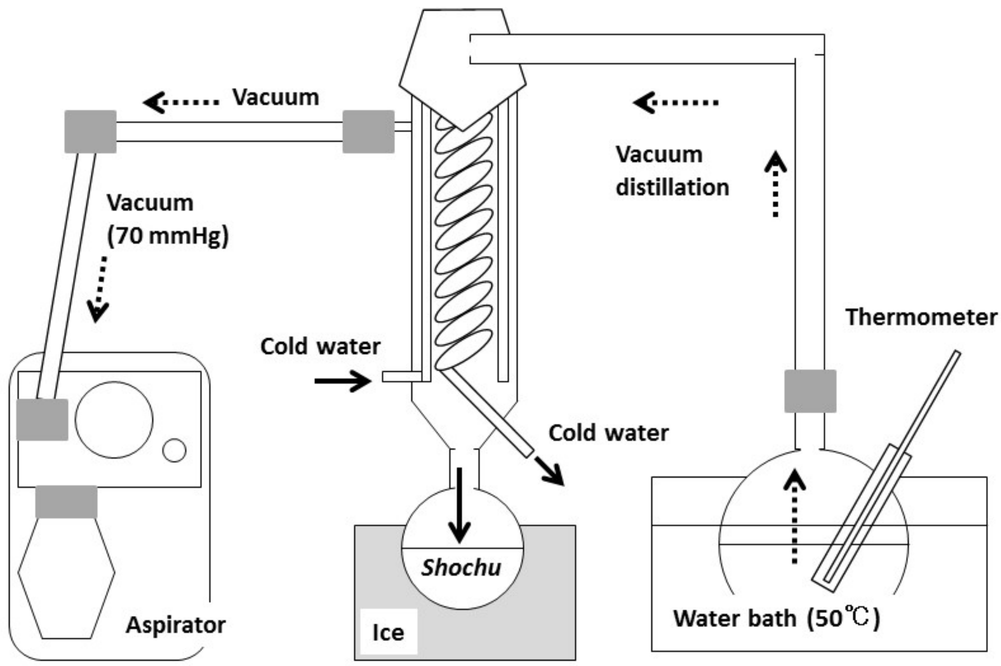

Fig.2 : Schematic diagram of vacuum distillation apparatus

2.6. Analysis of components in shochu

The concentrations of ethanol and aromatic components were analyzed using gas chromatography (Shimadzu Co., Ltd. Kyoto, Japan) by a direct injection method. For components concentration analysis, ethanol concentration of *shochu* was unified 25%(v/v). Gas chromatography conditions were identical to those used in the method described by method of Ohba *et al.* [8].

2.7. Determination of antioxidant activity

The DPPH radical scavenging activity as the Trolox equivalent was measured on the basis of the method of Yamaguchi *et al.* [9]. The lipid peroxidation inhibitory activity as the BHT equivalent was determined using β-carotene [10]. For the analysis, the ethanol concentration of *shochu* was adjusted to 40% (v/v).

2.8. Absorption spectra analysis

To determine the absorption spectra of the prepared *shochu*, samples were applied to a model U-3010 spectrophotometer (Hitachi, Tokyo, Japan). Prior to the analysis, the ethanol concentration of the *shochu* samples was adjusted to 40% (v/v).

2.9. HPLC analysis

HPLC analysis of components present in *shochu* samples were performed according to the method of David *et al.* [11] using a liquid chromatography (Shimadzu Co., Ltd. Kyoto, Japan) equipped with a CTO-10AC column oven and SPD-10AV UV-VIS detector. Analytical HPLC was performed using an Inertsil ODS-3 (4.6 i.d. × 250 mm, GL Sciences, Inc., Tokyo, Japan) column with solvents A (H_3PO_4 : AcOH : H_2O = 0.2 : 2.0 : 1000) and B (H_3PO_4 : AcOH : MeCN = 0.2 : 2.0 : 1000) under gradient conditions at a flow rate of 1 ml min^{-1}. Detection was performed at 260 nm and the column temperature was set at 25 ºC. Prior to analysis, the *shochu* samples were adjusted to 40%(v/v) ethanol and then filtered through a disposable membrane filter unit DISMIC-13HP (0.2 μm, ADVANTEC, Tokyo, Japan).

3. Results and Discussion

3.1. Characteristics of *shochu*

As shown in Fig. 3, after alcoholic fermentation was completed, rice-*shochu* was produced by atmospheric- and/or vacuum-distillation processes. The distillation percentage of atmospheric-distilled *shochu* was 83.2% (±2.4%) and that of vacuum-distilled *shochu* was 77.7% (±2.2%). The ethanol concentration of the atmospheric-distilled *shochu* was 44.6(±3.7)%(v/v) and that of vacuum-distilled *shochu* was 46.2 (±0.4)%(v/v). The concentration of aromatic compounds was analyzed by GC (Table 1). According to The Production Techniques of *shochu* [7], isoamyl alcohol, isobutyl alcohol, *n*-propyl alcohol, ethyl acetate and acetaldehyde in atmospheric-distilled *shochu* was 540, 272, 176, 100 and 23 ppm, respectively. On the other hands, vacuum-distilled *shochu* was 512, 253, 165, 60 and 10 ppm, respectively.

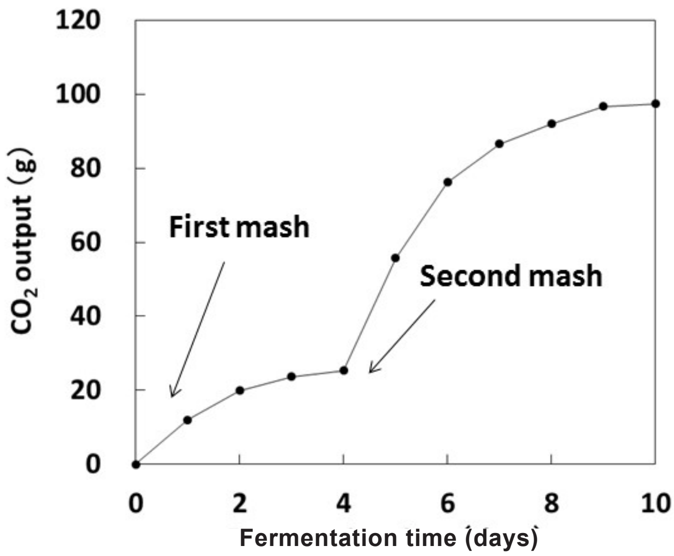

Fig.3 : Time course of fermentation.

TABLE 1 : Results of aromatic component analysis for atmospheric- and vacuum-distilled *shochu*.

Aromatic component	Atmospheric distillation	Vacuum distillation
Isoamyl alcohol (ppm)	703 (± 30)	777 (±51)
Isobutyl alcohol (ppm)	302 (± 18)	323 (±12)
n-Propyl alcohol (ppm)	176 (±18)	153 (±28)
Ethyl acetate (ppm)	150 (±14)	157 (±12)
Isoamyl acetate (ppm)	13 (±1)	13 (±0)
Acetaldehyde (ppm)	50 (±0)	66 (±1)

Each value is the mean ± SD of three replicated.

3.2. Antioxidant activity of *shochu*

The DPPH radical scavenging activity of vacuum-distilled *shochu* was 3.9 (±1.2) μM Trolox eq. and that of atmospheric-distilled *shochu* was 3.4 (±0.1). μM Trolox eq. No significant difference was detected between the both distillation methods. However, the lipid peroxidation inhibitory capacity of the vacuum-distilled *shochu* was 8.2 (±0.4) μM BHT eq., whereas that of the atmospheric-distilled *shochu* was 12.3 (±0.7) μM BHT eq., which represented a significant difference ($p < 0.01$) (Fig. 4).

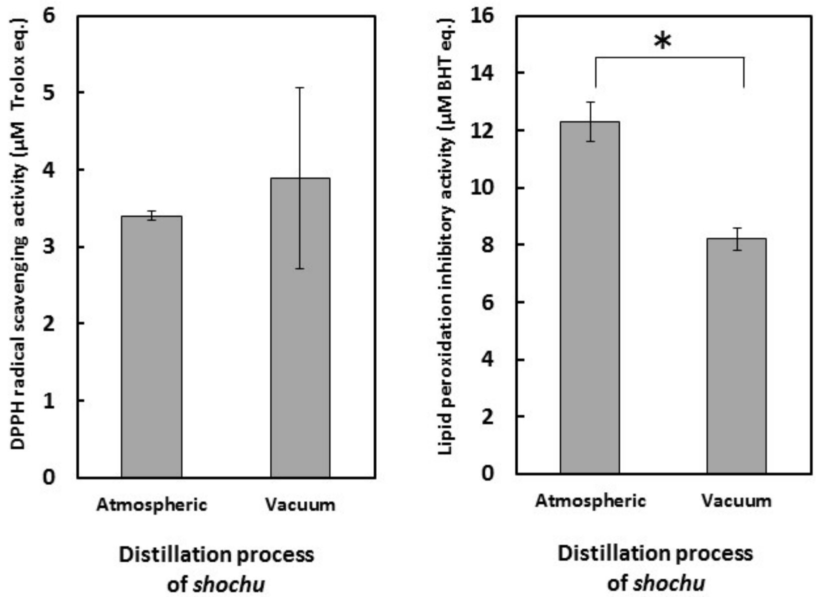

Fig. 4 : Antioxidant activity of *shochu* produced with different distillation process. DPPH radical scavenging activity was left side, and lipid peroxidation inhibitory activity was right side. Each value is the mean ± SD of three replicated. *p<0.01.

Koseki *et al*. [12] reported phenolic compounds (ferulic acid, vanillin, vanillic acid) found in Japanese rice spirits made in Ryukyu islands called *awamori*. Therefore, we guessed that the difference of the lipid peroxidation inhibitory activity was related to phenolic compounds. So, we examined the absorption spectra of *shochu* prepared using the two different distillation processes and observed a common peak at 260 nm (Fig. 5).

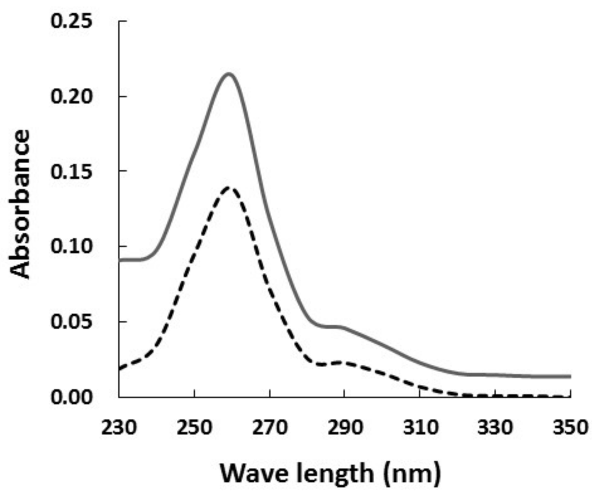

Fig.5 : Absorption spectra of *shochu* produced using different distillation process. Atmospheric-distillation process was solid line, and vacuum-distillation process was dashed line. Ethanol concentration of the *shochu* samples was adjusted to 40%(v/v).

However, the absorption values of the atmospheric-distilled *shochu* were higher than that of the vacuum-distilled *shochu*. So, we guessed that the atmospheric-distilled *shochu* contained large amounts of component indicating an absorption maximum at 260 nm than the vacuum-distilled *shochu*. Furthermore, according to the study by Davit *et al*. [11] who analyzed the antioxidant and phenolic compounds contained in distilled spirits, we separated compounds with absorption maximums around 260 nm in the *shochu* samples by HPLC. In particular, we investigated and compared the levels of 2-furoic acid (255 nm), ellagic acid (256.6 nm), vanillic acid (260.4 nm), and protocatechuic acid (260.4 nm) in atmospheric- and vacuum-distilled *shochu*. On comparison of the HPLC patterns, two peaks (B and C) that were not detected in the vacuum-distilled *shochu* were confirmed in the atmospheric-distilled *shochu* (Fig. 6).

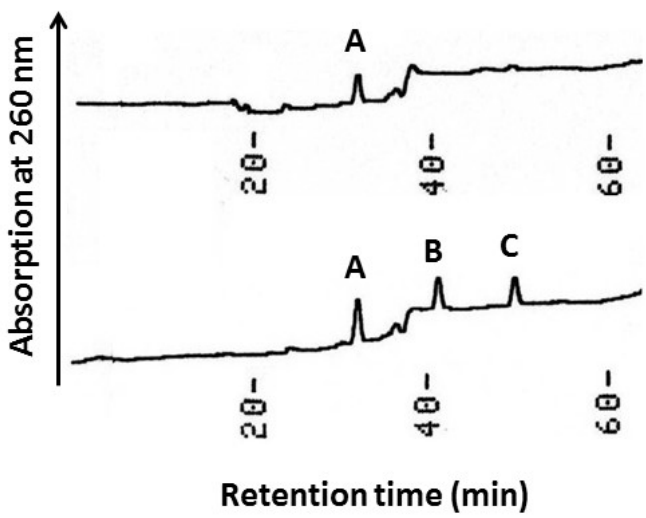

Fig.6 : HPLC chromatograms of *shochu* produced using different distillation process. Vacuum-distillation process was upper, and atmospheric-distillation process was lower. Ethanol concentration of the *shochu* sample was adjusted to 40%(v/v).

On the other hand, peak A of which retention time is the same in both the *shochu* samples has been confirmed. A comparison of the retention times of the three compounds suggested that peak C was consistent with protocatechuic acid. But, components corresponding to the peaks A and B could not be confirmed. Protocatechuic acid is produced by the action of microbial enzymes from ferulic acid derived from bran [13] and has demonstrated antioxidant activity. Koseki *et al*. [14] also detected phenolic compounds in *awamori* that were formed from ferulic acid, vanillin, and vanillic acid during storage. Based on these findings, while it is insufficient in this experimental data, it was considered that during the production of *shochu*, especially in the atmospheric-distillation process, protocatechuic acid and peak B may be generated. And these components may possibly affect the lipid peroxidation inhibitory activity of *shochu*. To date, the antioxidant activity of *shochu* has not received much attention. Our present findings suggest that *shochu* with added functionality can be produced by identifying and elucidating the formation mechanism of functional components contained in *shochu*. From the results, it was realized that the antioxidant activity of the atmospheric-distilled *shochu* was higher than that of vacuum-distilled *shochu*.

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