World Applied Sciences Journal 34 (1): 56-62, 2016
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2016.34.1.10258

Characteristics and Antioxidant Activity of Alcoholic Beverages Made from Various Polished Cereal Grain and Their Bran

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Abstract: Alcoholic beverages were properly made from various polished cereal grains and their bran. Wild rice bran shows black color, however, resulting alcoholic beverages have pale yellow color. Alcoholic beverage made from uncooked black rice bran shows brilliant red color just like grape wine. The ethanol concentration of various alcoholic beverages made from polished rice and bran was approximately 11.3 to 15.1% (v/v). The DPPH radical scavenging activities of the alcoholic beverages made from polished wild rice and polished black rice were relatively higher than that of alcoholic beverage made from polished white rice. The compounds related with DPPH radical scavenging activity of black rice were mostly located in the bran layer; however, in the case of wild rice, the compounds might be distributed over whole grain. The compounds related with DPPH radical scavenging activity and the compound related to inhibitory activity of lipid peroxidation are thought to be different.

Key words: Wild Rice · Black Rice · Antioxidant Activity · Uncooked Fermentation

INTRODUCTION

Wild rice (*Zizania aquatica*) has been used as food by Native Americans, however, it is not actually rice, genus Oryza. Wild rice contained black pigment in the bran layer and rich in nutrient such as vitamins and minerals become popular among consumer. However, reports concerning alcoholic beverage made from wild rice are very rare.

As progress is made in fermentation technology and the brewing industry, many kinds of alcoholic beverages are being produced year by year to catch the interest of consumers. In Japanese sake brewing, the polishing of rice grains is an important process. Rice bran, which contains proteins, lipids, vitamins and minerals, is usually removed from the rice grains by polishing before fermentation to produce an alcoholic beverage of high quality.

The characteristics of alcoholic beverages made from polished wild rice and its bran were determined to develop the functional alcoholic beverage, which has antioxidant activity [1, 2, 3, 4]. Further we determined the localization of antioxidant compounds in the wild rice grain.

For a comparative study, alcoholic beverages were also made from polished black rice (*Oryza sativa* var. *Japonica* cv. *Shiun*) [5], black rice bran and polished white rice (*Oryza sativa* var. *Japonica* cv. *Hinohikari*).

In this study, we tried to made novel alcoholic beverage from polished wild rice and its bran.

MATERIALS AND METHODS

Yeast Strain: Industrial sake yeast *Saccharomyces cerevisiae* K7 purchased from the Brewing Society of Japan (Tokyo, Japan) were used for ethanol fermentation. K7 yeast is maintained on the plates of an agar-solidified YPD medium (yeast extract, 10 g; peptone, 20 g; glucose, 20 g; tap water, 1, 000 ml).

Cereal Grains: Wild rice having black pigments in the bran layer was purchased from Suzusho Co. Ltd., Tokyo, Japan (Fig. 1). Black rice having red pigments in the bran layer was purchased from Kajiwara Beikoku Co. Ltd., Kyoto, Japan.

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Fig. 1: Picture of polished white rice, black rice and wild rice

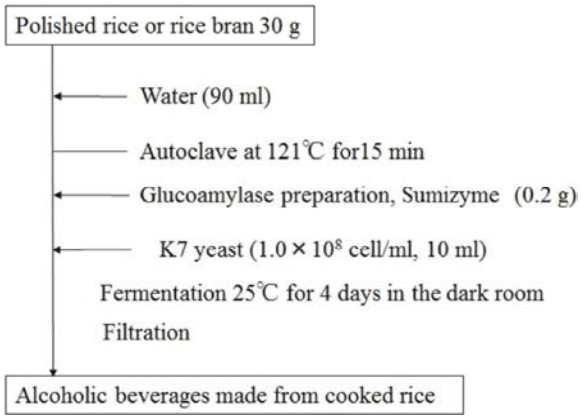

Fig. 2: Procedure for ethanol fermentation with cooking

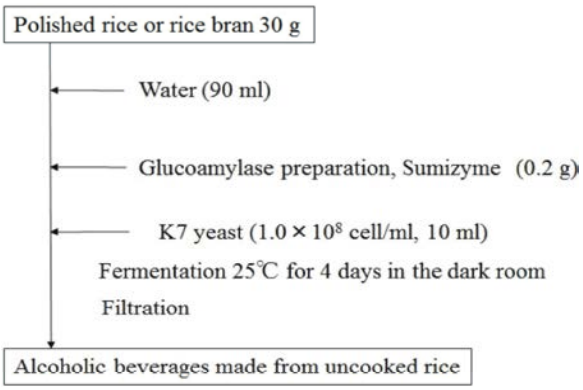

Fig. 3: Procedure for ethanol fermentation without cooking

These cereals were ground to particles of 2 to 3 mm in diameter with an electric grinder and fractionated into polished rice and rice bran. The ratio of whole rice grain: polished rice: rice bran was 100: 70: 30. Polished rice and rice bran was used as material for ethanol fermentation.

Commercial polished white rice (*Oryza sativa* var. *Japonica* cv. *Hinohikari*) was ground to particles of 2 to 3 mm in diameter and used as material for ethanol fermentation.

Saccharifying Agent: A glucoamylase preparation, Sumizyme, kindly donated by Shin Nihon Kagaku Kogyo Co., Ltd. (Anjo, Japan), was used as the saccharifying agent. Sumizyme produced by *Rhizopus* sp. is known to have raw starch digestibility and digest cooked rice and uncooked rice as well. Sumizyme is applicable to uncooked fermentation procedure [5].

Ethanol Fermentation Procedure: Conventional fermentation with cooking was performed according to the procedure below (Fig. 2). Thirty grams of polished rice or rice bran and 50 ml of deionized water were dispensed into a 300-ml Erlenmeyer flask and autoclaved at 121 for 15 min. After cooling, the cooked material was mixed with 0.2 g of Sumizyme as the saccharifying agent, 40 ml of deionized water and 10 ml of a yeast suspension, which readily brought the population of yeast in the initial mash to 3.0×10^7 cells/ml. Ethanol fermentation was conducted at 25 in the dark.

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.

Ethanol fermentation without cooking was done as follows (Fig. 3). Thirty grams of uncooked polished rice or uncooked rice bran, 90 ml of deionized water, 0.2 g of Sumizyme and 10 ml of a yeast suspension were dispensed into a 300-ml Erlenmeyer flask. The population of yeast in the initial mash was adjusted to 3.0×10^7 cells/ml and fermentation was conducted in the same manner as for ethanol fermentation with cooking. In this procedure, there is no cooking or steaming process.

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.

General Analytical Methods: Fermented mash made from polished rice or rice bran were centrifuged at 3, 000 rpm for 15 min and filtered through No. 101 filter paper (Advantec Toyo Co., Ltd., Tokyo, Japan) and the resulting alcoholic beverage was analyzed.

Acidity was measured by titrating 10 ml of alcoholic beverage with 0.1 N NaOH. Reducing sugar as glucose was determined according to the methods of Somogyi and Nelson [6, 7]. The amount of total phenolic compounds, expressed as gallic acid, was determined according to the Folin-Ciocalteu method [2, 8].

The ethanol concentration of alcoholic beverage was determined with a gas chromatograph (model GC-14A; Shimadzu Co., Kyoto, Japan) equipped with a 3.1-m PEG-HT column (Gasukuro Kogyo, Inc., Tokyo, Japan).

The anthocyanin content of alcoholic beverage, as the cyanidine 3-glucoside equivalent (μg/ml), was measured according to the method of Boyles and Wrolstad [9].

Chemicals: 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).

Determination of Antioxidant Activity: The DPPH radical scavenging activity as the Trolox equivalent was measured on the basis of the method of [10]. The lipid peroxidation inhibitory activity as the BHT equivalent was determined using β-carotene [2].

RESULTS AND DISCUSSION

The fermentation curves of the mashes contained polished wild rice, polished black rice and polished white rice are shown in Figure 4. The fermentation curves of the mashes contained wild rice bran and black rice bran are shown in Figure 5. Fermentation was properly preceded in each run.

Picture of resulting alcoholic beverages are shown in Figures 6 and 7. Wild rice bran shows black color, however, resulting alcoholic beverages have pale yellow color. Alcoholic beverage made from uncooked black rice bran shows brilliant red color just like grape wine. While the color of alcoholic beverage made from cooked black rice bran is faded. During cooking process, a part of anthocyanin pigment might be denatured [5].

The characteristics of alcoholic beverage made from various materials are shown in Table 1. The ethanol concentration of various alcoholic beverages was approximately 11.3 to 15.1% (v/v). The total amount of phenolic compounds of alcoholic beverage made from uncooked materials was relatively higher than that of the beverage made from cooked materials except for that of alcoholic beverage made from black rice bran (Table 1 and Figure 8). Alcoholic beverages made from polished black rice and black rice bran contained anthocyanin. The alcoholic beverage made from uncooked black rice bran contained highest anthocyanin, 221.2 μg/ml as Cyanidin 3-glucoside equivalent.

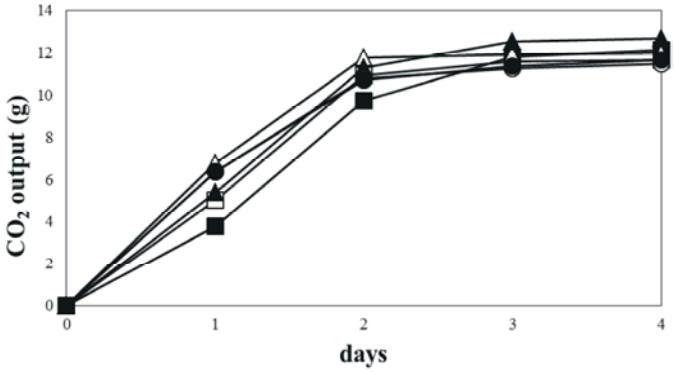

Fig. 4: Time courses of fermentation of the mashes made from polished wild rice, polished black rice and polished white rice using K7 yeast, Symbols: ○, cocked polished wild rice; ●, uncooked polished wild rice; Δ, cooked polished black rice; ▲, uncooked polished black rice; □, cooked polished white rice; ■, uncooked polished white rice. Values are the mean of triplicates

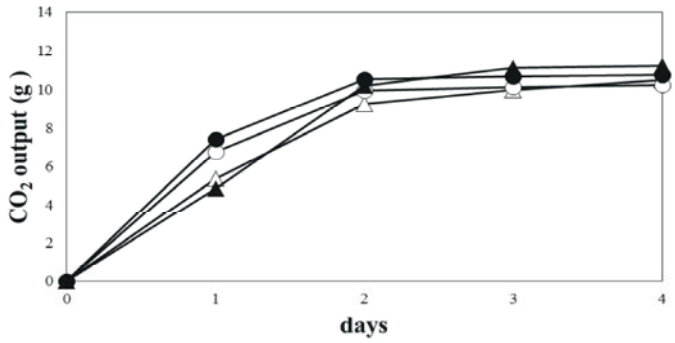

Fig. 5: Time courses of fermentation of the mashes made from wild rice bran and black rice bran using K7 yeast Symbols: ○, cocked wild rice bran; ●, uncooked wild rice bran; Δ, cooked black rice bran; ▲, uncooked black rice bran. Values are the mean of triplicates

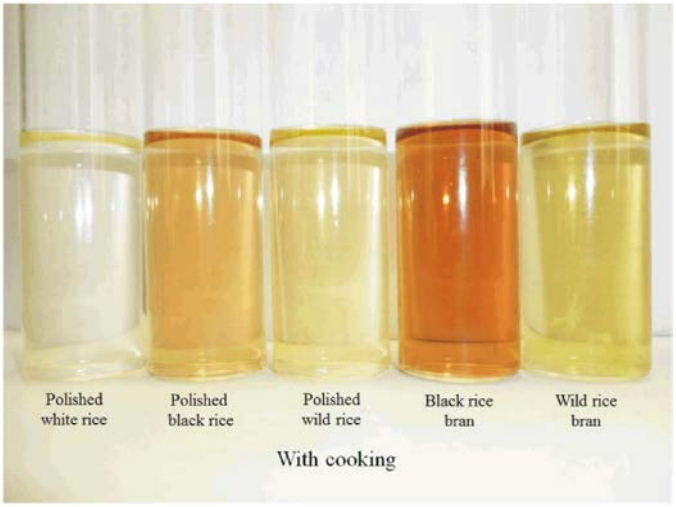

Fig. 6: Pictures of alcoholic beverage made from various cooked materials using K7 yeast. From left, alcoholic beverage made from polished white rice, polished black rice, polished wild rice, black rice bran, wild rice bran

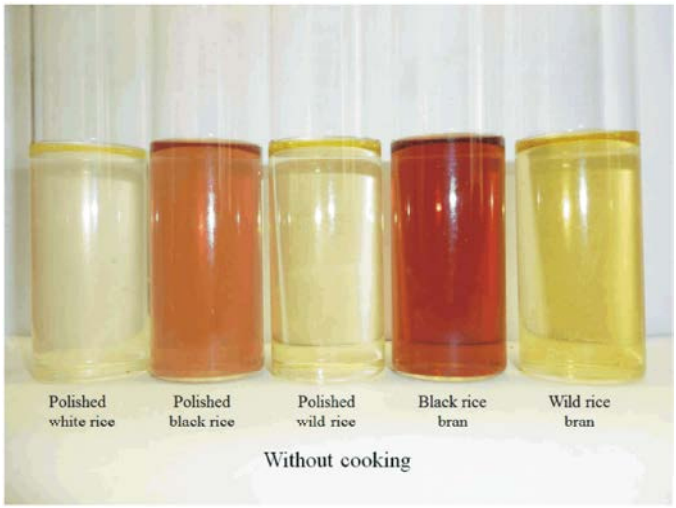

Fig. 7: Pictures of alcoholic beverage made from various uncooked materials using K7 yeast. From left, alcoholic beverage made from polished white rice, polished black rice, polished wild rice, black rice bran, wild rice bran.

Table 1: Characteristics of alcoholic beverages made from various materials

	Polished white rice		Polished black rice		Polished wild rice		Black rice bran		Wild rice bran	
	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked
Initial pH	6.5	6.4	6.2	6.1	6.1	6.2	6.2	6.4	6.2	6.3
Final pH	4.4	4.3	4.8	4.8	4.9	5.0	5.1	5.4	5.1	5.2
CO_2 output (g)	11.7	12.2	12.0	12.7	11.6	12.0	10.6	9.2	10.4	10.9
Filtrate (ml)	89	86	84	72	43	63	68	68	38	58
Acidity (ml)	1.6	1.7	2.2	2.2	2.4	2.6	2.2	2.8	2.4	2.7
Ethanol concentration (%, v/v)	13.8	14.4	14.0	15.1	13.1	14.1	12.3	11.3	13.7	13.0
Reducing sugar content (μg/ml, glucose eq.)	437	608	767	807	1003	1054	956	826	1315	1321
Total phenolic compounds (μg/ml, GA eq.)	379	524	500	612	577	809	687	669	743	992
Anthocyanin content (μg/ml, Cy 3-glc eq.)	ND	ND	33.5	91.5	ND	ND	80.7	221.2	ND	ND

Values are the means of three replicates. ND, Not detected

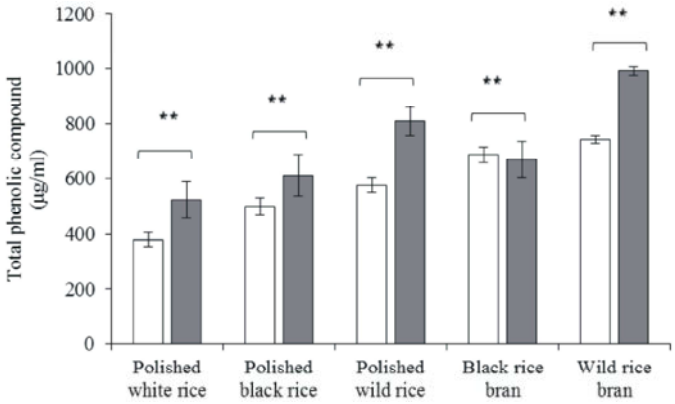

Fig. 8: Total phenolic compound of alcoholic beverages made from various materials using K7 yeast
Open bar shows alcoholic beverage made by fermentation with cooking and closed bar shows alcoholic beverage made by fermentation without cooking. Each value is the mean ± S.D. (n=3). Significant difference indicates $^{**}p < 0.01$

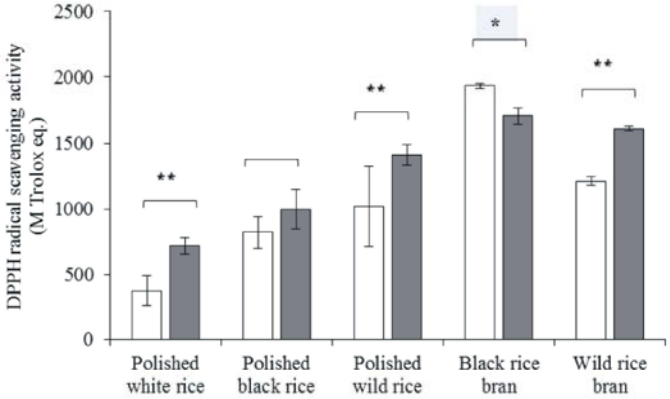

Fig. 9: DPPH radical scavenging activity of alcoholic beverages made from various materials using K7 yeast
Open bar shows alcoholic beverage made by fermentation with cooking and closed bar shows alcoholic beverage made by fermentation without cooking. Each value is the mean ± S.D. (n=3). Significant difference indicates $^{**}p < 0.01$ and $^{*}p < 0.05$.

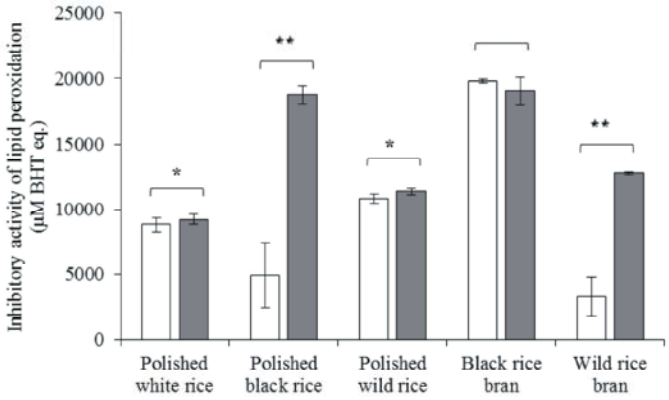

Fig. 10: Inhibitory activity of lipid peroxidation of alcoholic beverages made from various materials using K7 yeast
Open bar shows alcoholic beverage made by fermentation with cooking and closed bar shows alcoholic beverage made by fermentation without cooking. Each value is the mean ± S.D. (n=3). Significant difference indicates $^{**}p < 0.01$ and $^{*}p < 0.05$.

The antioxidant activity of various alcoholic beverages was determined. The DPPH radical scavenging activities of alcoholic beverages made from polished wild rice and polished black rice were relatively higher than that of alcoholic beverage made from polished white rice (Figure 9).

The alcoholic beverages made from black rice bran showed much higher DPPH radical scavenging activity than that of the alcoholic beverage made from polished black rice. It has been suggested that the compounds related with DPPH radical scavenging activity of black rice were mostly located in the bran layer [11, 12]. In the case of wild rice, the compounds might be distributed over whole grain.

The inhibitory activity of lipid peroxidation of the alcoholic beverages is shown in Figure 10. The profile of inhibitory activity of lipid peroxidation of various alcoholic beverages is quite complicated and different from that of DPPH radical scavenging activity of various alcoholic beverages. It has been suggested that the compounds related with DPPH radical scavenging activity and the compound related with inhibitory activity of lipid peroxidation are different.

CONCLUSION

The alcoholic beverages were properly made from various cereal materials. The DPPH radical scavenging activity of alcoholic beverage made from polished wild rice, wild rice bran, polished black rice and black rice bran was higher than that of the alcoholic beverage made from polished white rice.

Localization of antioxidant compounds in wild rice and black rice thought to be different. It has been suggested that the compounds related with DPPH radical scavenging activity of black rice are mostly located in the bran layer. In the case of wild rice, the compounds relating DPPH radical scavenging activity might be distributed over whole grain.

We would like to determine the compounds related with DPPH radical scavenging activity and inhibitory activity of lipid peroxidation. In this study we also try to improve the quality of taste and aroma of alcoholic beverages made from wild rice and black rice to produce a fine alcoholic beverage that also has physiological advantages.

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