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Production and antioxidant activity of alcoholic beverages made from various colored rice and wild rice

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In order to develop a new type of alcoholic beverage with antioxidant activity, the authors carried out this research. In this study, alcoholic beverages were made from unpolished black rice, unpolished red rice, unpolished green rice, polished white rice and unpolished wild rice using conventional cooked fermentation and economical uncooked fermentation system. The resulting alcoholic beverages made from cooked and uncooked rice grains had an ethanol concentration of approximately 11.0 to 13.8% (v/v). Alcoholic beverages made from various grains had antioxidant activity. Especially, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the alcoholic beverages made from black rice, red rice and wild rice were relatively higher as compared to other alcoholic beverages. On the other hand, the inhibitory activity of lipid peroxidation of the alcoholic beverages made from wild rice using cooked and uncooked fermentation system was higher than that of the other alcoholic beverages. From these results, the possibility of new types of alcoholic beverages with antioxidant activity was suggested.

Key words: Antioxidant activity, fermentation, colored rice, wild rice.

INTRODUCTION

As progress is made in fermentation technology and the brewing industry, many kinds of alcoholic beverages are being produced each year to catch the interest of consumers. In Japanese sake brewing, the cooking 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. Black rice (*Oryza sativa* var. *Japocica* cv. *Shiun*) contains anthocyanin pigments, such as cyanidin and peonidin glucosides, in the bran layer. Anthocyanin having antioxidant activity is known to have physiological functions and contributes to the prevention of diseases such as arterial sclerosis, and is a type of functional food. Many reports were published concerning antioxidant activity of various foods (Chen et al., 1998; Hamasaka et al., 2004; Saigusa and Teramoto, 2014).

In this study, the authors tried to brew novel alcoholic beverages from various unpolished colored rice and wild rice. The characteristics of the alcoholic beverage made

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Figure 1. Picture of various rice and wild rice.

from various grains were determined to develop the functional alcoholic beverage, which has antioxidant activity (Yuwa-amornpitak et al., 2012). For a comparative study, alcoholic beverages are brewed from nonglutinous rice as well.

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).

Rice grains and wild rice

Black rice (*O. sativa* var. *Japonica* cv. *Shiun*), red rice (*O. sativa* var. *Japonica* cv. *Engimai*), green rice (*O. sativa* var. *Japonica* cv. *Midorinoka*) purchased from Kajiwara Beikoku Co. Ltd. (Kyoto Japan) and wild rice (*Zyzania aquatica*) purchased from Suzusho Ltd. (Tokyo, Japan) were used for ethanol fermentation (Figure 1). Wild rice is belonging to genus *Zyzania* and actually, it is not rice.

For comparative study, commercial polished nonglutinous white rice grains (*O. sativa* var. *Japonica* cv. *Hinohikari)* were used as material for ethanol fermentation. These cereals were ground to particles of 2 to 3 mm in diameter with an electric grinder and were used 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 is known to have raw starch digestibility and digest cooked rice and uncooked rice as well. Sumizyme is applicable to uncooked fermentation procedure (Teramoto et al., 1994).

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) 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).

Ethanol fermentation procedure

Conventional fermentation with cooking was performed according to the procedure below. Thirty grams of grains and 50 ml of deionized water were dispensed into a 300-ml Erlenmeyer flask and autoclaved at 121°C for 15 min. Various grains such as unpolished black rice, unpolished red rice, unpolished green rice, unpolished wild rice and polished white rice were used as material for ethanol fermentation. After cooling, the cooked rice 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°C in the dark (Figure 2). Ethanol fermentation without cooking was done as follows. Thirty grams of raw grains, 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 (Figure 3). 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 nonglutinous rice grains was 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.

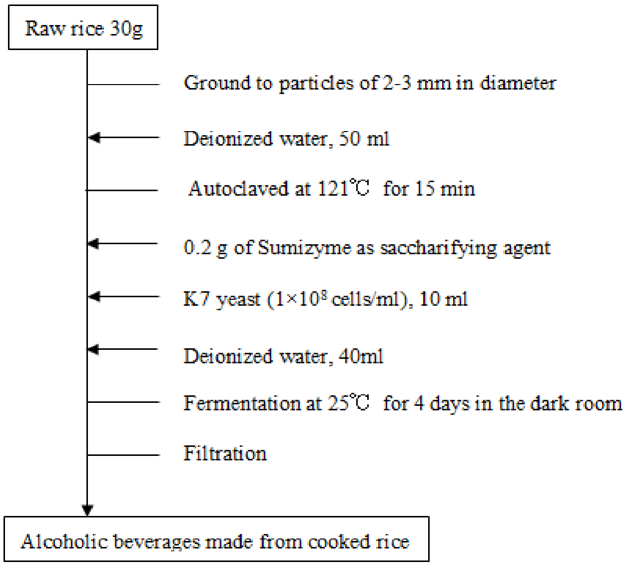

Figure 2. Procedure for ethanol fermentation with cooking.

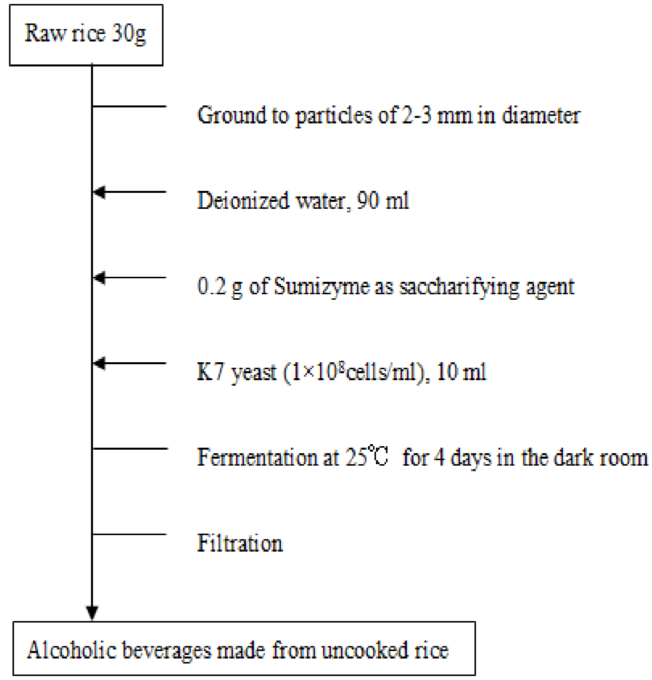

Figure 3. Procedure for ethanol fermentation without cooking.

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 (Somogyi, 1952; Nelson, 1944). The amount of total phenolic compounds, expressed as gallic acid, was determined according to the Folin-Ciocalteu method (Singleton et al., 1999; Hamasaka et al., 2004).

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).

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. (1998). The lipid peroxidation inhibitory activity as the BHT equivalent was determined using β-carotene (Hamasaka et al., 2004).

RESULTS AND DISCUSSION

The fermentation curves of the mashes made from various grains such as unpolished black rice, unpolished red rice, unpolished green rice, unpolished wild rice, and polished white rice are shown in Figure 4. In the case of cooked fermentation, fermentation of the mash containing wild rice proceeded quickly in one day; while, fermentation of the mash containing black rice proceeded slowly in one day. Final CO_2 output of each mashes are ~11.2 to 12.2 (Table 1). In the case of uncooked fermentation, fermentation of the mash containing wild rice proceeded quickly until two day. Wild rice grain is relatively easy to be digested comparing with other rice grains belonging to *O. sativa.*

Picture of resulting alcoholic beverages are shown in Figure 5. Alcoholic beverage made from uncooked black rice shows brilliant red color just like grape wine. While the color of alcoholic beverage made from cooked black rice is faded. During cooking process, a part of anthocyanin pigment might be denatured. Red rice, green rice, and wild rice shows pinky color, green color, and black color, however, resulting alcoholic beverages have pale yellow or pale brown color.

The characteristics of alcoholic beverage made from various grains are shown in Table 1. The ethanol concentration of the alcoholic beverages was approximately 11.0 to 13.8% (v/v). The total amount of phenolic compounds of alcoholic beverage made from uncooked grains were higher than that of the beverage made from cooked grains (Table 1 and Figure 6). Alcoholic beverages made from black rice contained anthocyanin. From the results of Table 1, it was confirmed that only alcoholic beverage made from black rice contains anthocyanin. Min-Kyoung Kim et al. (2008) reported that anthocyanin have been identified in hydrochloric acid - methanol extract of black rice and wild rice, respectively. And both main anthocyanin was a cyaniding-3-glucoside. Though it is a small amount, it may be appeared that there is a possibility that contain anthocyanin also in the alcoholic beverage made from wild rice, too.

The antioxidant activity of various alcoholic beverages was determined. The DPPH radical scavenging activities of alcoholic beverages made from colored rice and wild rice are relatively higher than that of alcoholic beverage made from polished white rice (Figure 7). A bran fraction of black rice or red rice was taken by changing the rice milling rate, to examine the DPPH radical scavenging activities. As a results, black rice had high activity in bran

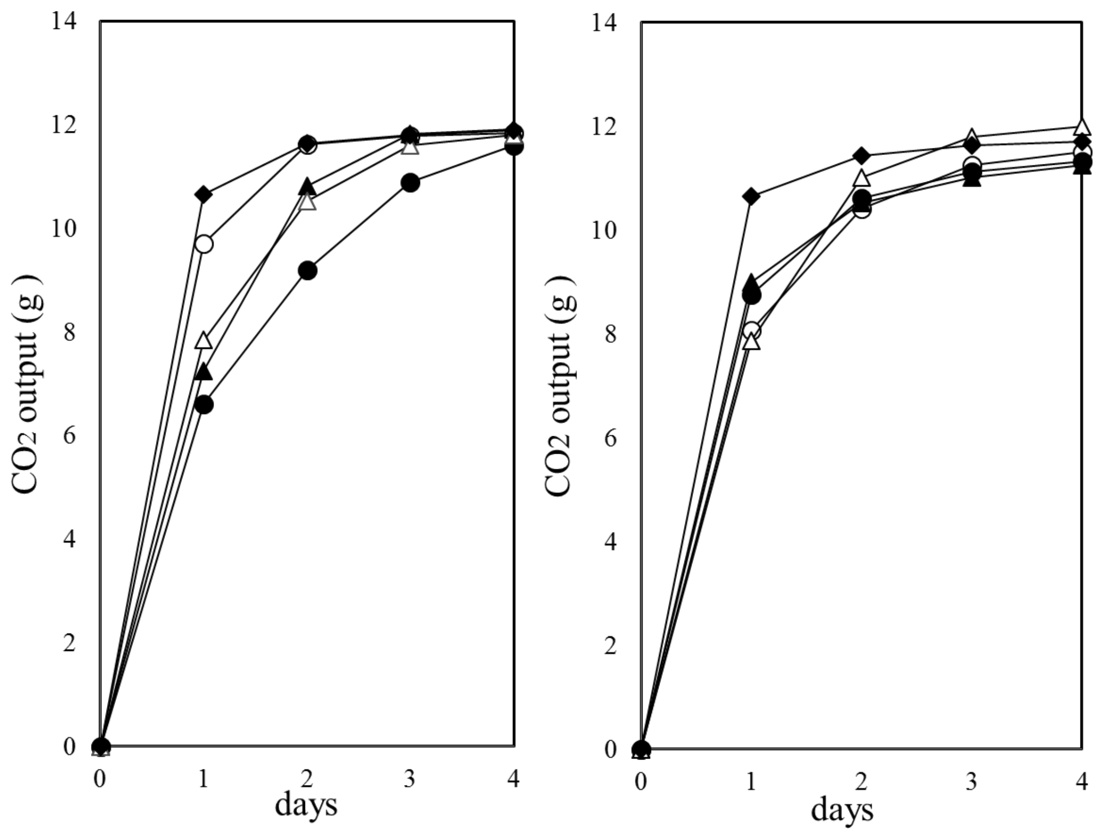

Figure 4. Time courses of fermentation of the mashes made from various grains with cooking [left] and without cooking [right] using K7 yeast. ○, polished white rice; ●, black rice; △, red rice; ▲,green rice; ◆, wild rice. Values are the mean of triplicates.

Table 1. Composition of initial mash and analysis of resulting alcoholic beverages.

Rice	**Polished white rice**		**Black rice**		**Red rice**		**Green rice**		**Wild rice**	
Fermentation	With cooking	Without cooking	With cooking	Without cooking	With cooking	Without cooking	With cooking	Without cooking	With cooking	Without cooking
Initial pH	4.7	4.8	4.9	4.9	4.9	4.9	5.0	5.0	5.0	5.1
Final pH	4.5	4.5	4.6	4.9	4.7	4.8	4.6	4.9	4.9	5.0
CO_2 output (g)	11.8	11.5	11.8	11.3	11.9	12.2	11.9	11.2	11.9	11.8
Filtrate (ml)	76	83	67	75	66	76	73	73	48	63
Acidity (ml)	2.2	2.4	3.3	2.9	3.6	3.3	3.2	2.8	2.8	2.5
Ethanol concentration (%, w/v)	12.1	12.6	12.1	12.6	13.8	11.0	12.5	11.4	13.0	13.3
Reducing sugar content (μg/mL)	540	590	660	780	770	630	670	820	800	720
Total phenolic compound (μg/mL)	380	560	470	580	500	640	400	570	600	680
Anthocyanin content (μg/mL)	ND	ND	61.8	94.6	ND	ND	ND	ND	ND	ND

Values are the mean of triplicates. ND, not detected.

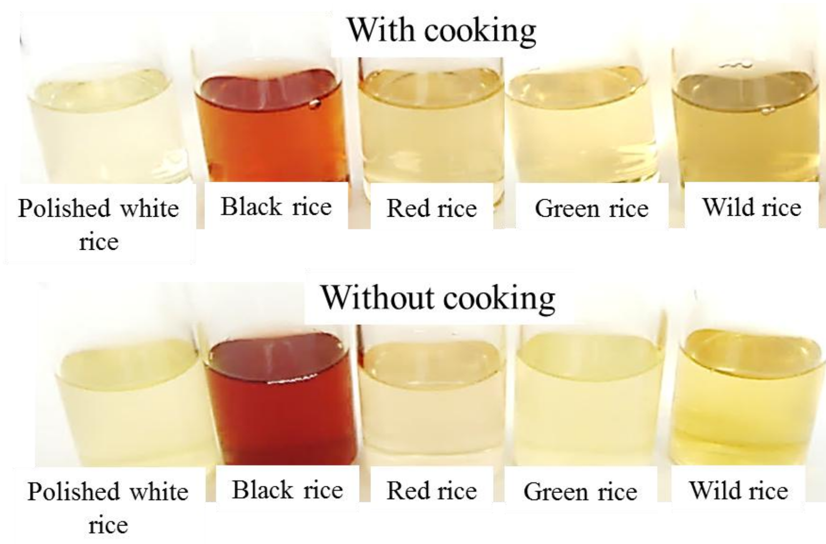

Figure 5. Pictures of alcoholic beverage made from various grains using K7 yeast.

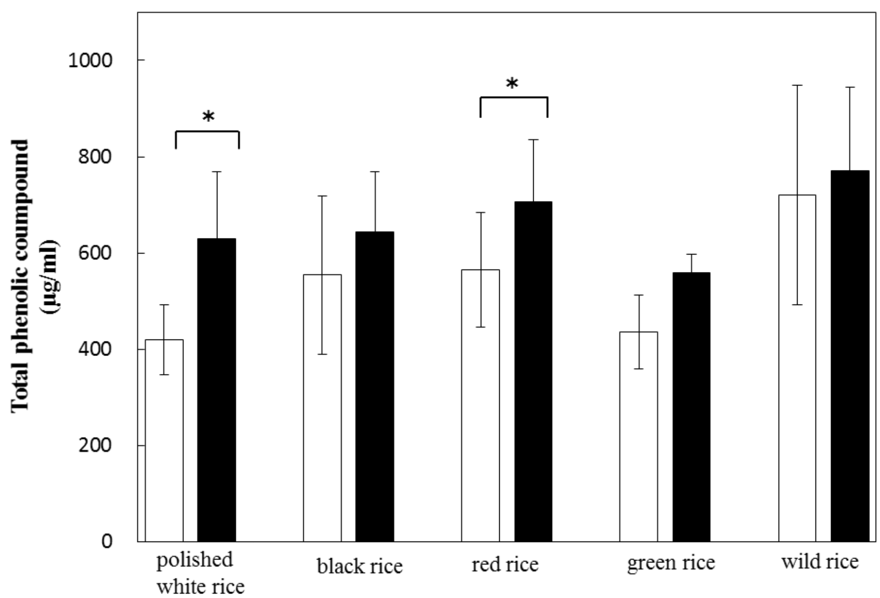

Figure 6. Total phenolic compound of alcoholic beverages made from various grains using K7 yeast. Open bar shows alcoholic beverage made by fermentation with cooking and closed bar shows alcoholic beverage made by fermentation without cooking.

obtained from 90-100 and 80-90%, and red rice had high activity in bran obtained from 90-100% (Fujita et al., 2010). In both of black rice and red rice, there is a high anti-oxidant substance on the outside of unpolished rice, and it was thought that these components have been extracted, in this time.

The inhibitory activity of lipid peroxidation of the alcoholic beverages is shown in Figure 8. The inhibitory activity of lipid peroxidation of the alcoholic beverage made from wild rice was much higher than that of other alcoholic beverage (Figure 8). It has been reported that the inhibitory activity of lipid peroxidation of 21 colored rice and 1 colorless rice water-ethanol mixed extract.

As a result, almost colored rice was higher antioxidant activity (Seok et al., 2006). However, from our results, other than the wild rice, there was no difference in the

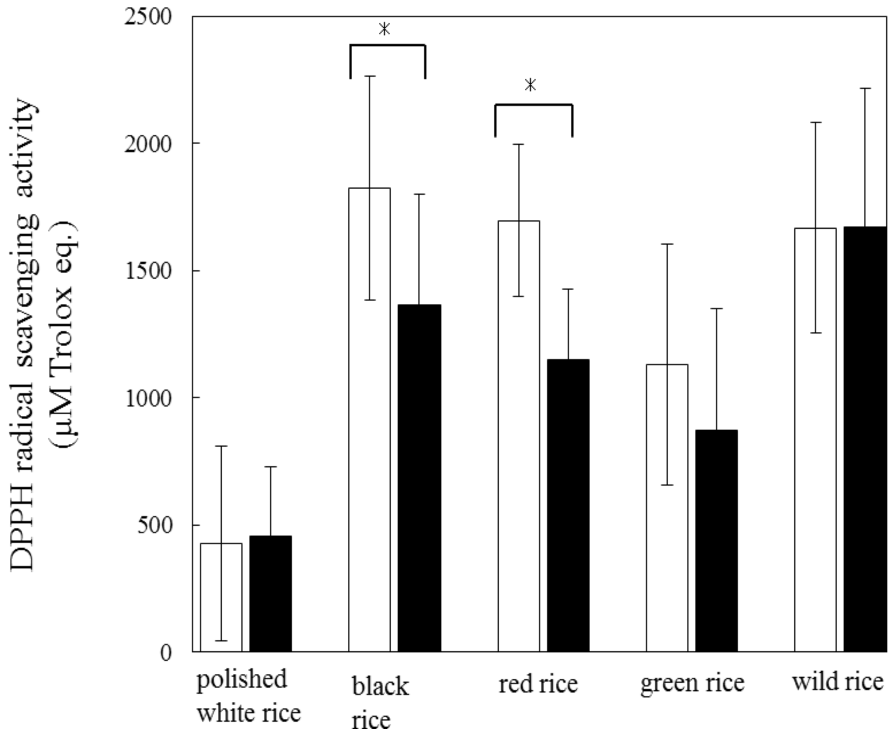

Figure 7. DPPH radical scavenging activity of alcoholic beverages made from various grains using K7 yeast. Open bar shows alcoholic beverage made by fermentation with cooking and closed bar shows alcoholic beverage made by fermentation without cooking.

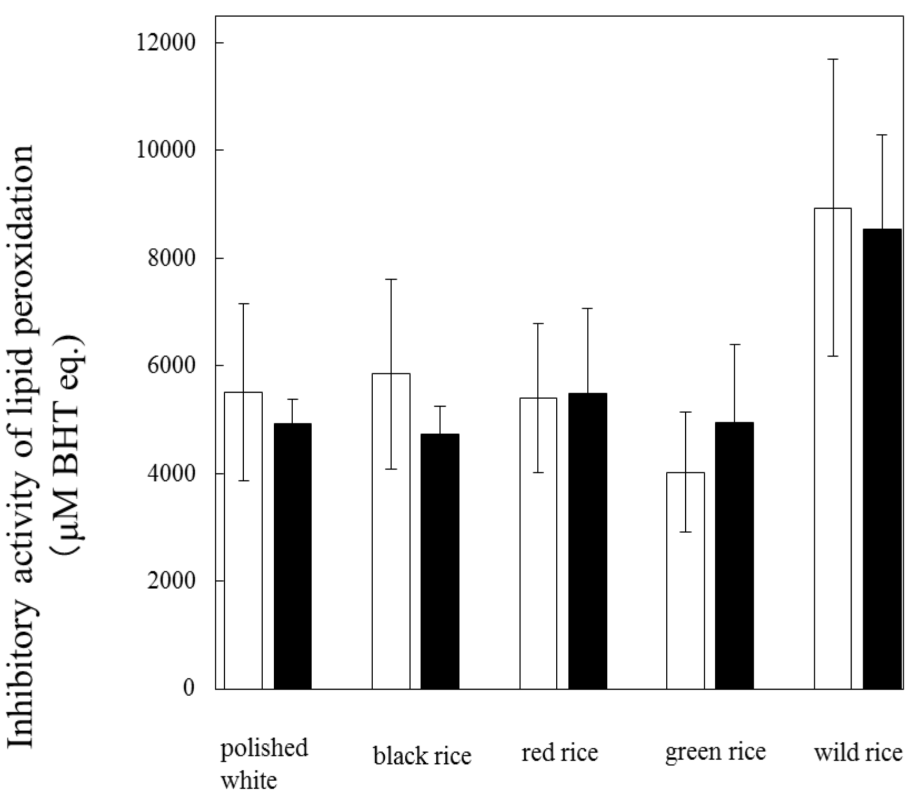

Figure 8. Inhibitory activity of lipid peroxidation of alcoholic beverage made from various grains using K7 yeast. Open bar shows alcoholic beverage made by fermentation with cooking and closed bar shows alcoholic beverage made by fermentation without cooking.

colored rice and polished white rice. In order to extract the component having the inhibitory activity of lipid peroxidation, it was thought to be necessary to increase the ethanol concentration.

It has been suggested that the compounds related with DPPH radical scavenging activity and the compounds related with inhibitory activity of lipid peroxidation are different. Chen et al. (1998) reported that peptides with lipid peroxidation inhibitory activity did not have DPPH radical-scavenging activity. It is also possible that the antioxidant substances in alcoholic beverages made from colored rice need to be identified, as they may be different substances.

DPPH radical scavenging activity and inhibitory activity of lipid peroxidation of the alcoholic beverage made from cooked and uncooked wild rice were relatively higher. The compounds having antioxidant activity contained in wild rice might be resistant against cooking. We would like to determine the compounds related with DPPH radical scavenging activity and the inhibitory activity of lipid peroxidation.

In this study, the colored rice pigment other than black rice could not be applied to alcoholic beverages. In the future, it is necessary to study the extraction technology of colored rice pigment in the alcoholic fermentation period. However, the alcoholic beverages made from black rice, red rice and wild rice showed high DPPH radical scavenging activity compared with polished white rice. In particular, alcoholic beverage made from wild rice showed high antioxidant activity regardless with or without cooking. In production of alcoholic beverages having functionality, usefulness of wild rice was suggested. From these results, the possibility of new types of alcoholic beverages with antioxidant activity was suggested.

Conflict of interest

The authors have not declared any conflict of interests.

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