African Journal of Biotechnology Vol. 10(52), pp. 10706-10711, 12 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1516
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Production and antioxidative activity of alcoholic beverages made from Thai *ou* yeast and black rice (*Oryza sativa* var. *Indica* cv. *Shiun*)

Teramoto, Y.[1]*, Koguchi, M.[1], Wongwicharn, A.[2] and Saigusa, N.[1]

[1]Department of Applied Microbial Technology, Faculty of Biotechnology and Life Science, Sojo University, Ikeda 4-22-1, Kumamoto 860-0082, Japan.
[2]Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi, 126 Prachautid Road, Bangmod, Ratburana, Bangkok 10140, Thailand.

Accepted 10 May, 2011

Fermentation yeast was isolated from a Thai traditional alcoholic beverage called Thai *ou*, which is drunk through bamboo tubes. The isolated yeast was identified as a strain of the genus *Saccharomyces cerevisiae*. The alcoholic beverage made with the isolated yeast designated as *S. cerevisiae* NP01 from black rice grains had an ethanol concentration of 12.4 to 13.1% (v/v) and a large amount of phenolic compounds. The resulting alcoholic beverages made from black rice grains were red in color, especially those made from uncooked black rice, which had a brilliant red hue similar to that of red or rosé wine. The amount of anthocyanin in the beverages made from uncooked black rice with NP01 and industrial wine yeast W-4 was 118 and 131 µg/ml, respectively. The anthocyanin content of beverages made from uncooked black rice was higher than that of the beverages made from the cooked black rice. The antioxidative activity of alcoholic beverages made from uncooked black rice was also higher than that of beverages made from cooked black rice. In the course of this study, the use of NP01 yeast produced black rice wine that was red in color and exhibited antioxidative activity.

Key words: Antioxidative activity, *ou*, black rice, anthocyanin, alcoholic beverage.

INTRODUCTION

In a previous paper, we described the characteristics of an alcoholic beverage made from black rice (*Oryza sativa* var. *Indica* cv. *Shiun*) (Koguchi et al., 2010). Black rice contains anthocyanin pigments, such as cyanidin and peonidin in the bran layer. Anthocyanins are widely present in fruits, vegetables and red wine.

Anthocyanins have been recognized as health-enhancing functional food ingredients due to their antioxidative activity (Jang and Xu et al., 2009; Kamiyama et al., 2009), anticancer activity (Spormann et al., 2008; Longo et al., 2008) and prevention of arterial sclerosis (Miyazaki et al., 2008).

We have been researching fermented foods worldwide and their microbial resources. We previously reported on the characteristics of a traditional Ethiopian honey wine, *ogol*, and its fermentation yeasts (Teramoto et al., 2005). We also reported on the characteristics of alcoholic beverages drunk through tubes in Thailand, Uganda, and Bahrain (Teramoto and Wongwicharn, 2002; Teramoto, 2003, 2007).

In this study, we utilized the isolated yeast strain *Saccharomyces cerevisiae* NP01 for ethanol fermentation and brewed a novel alcoholic beverage that was red in color and had physiological functions as well.

MATERIALS AND METHODS

Yeast strain

The precise method for making *ou* was determined through fieldwork conducted at Nakohn Phanom, a village located in northeast Thailand, in August 2001.

Fermentation yeast was isolated on plates of an agar-solidified YPD medium [yeast extract (10 g), peptone (20 g), glucose (20 g),

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

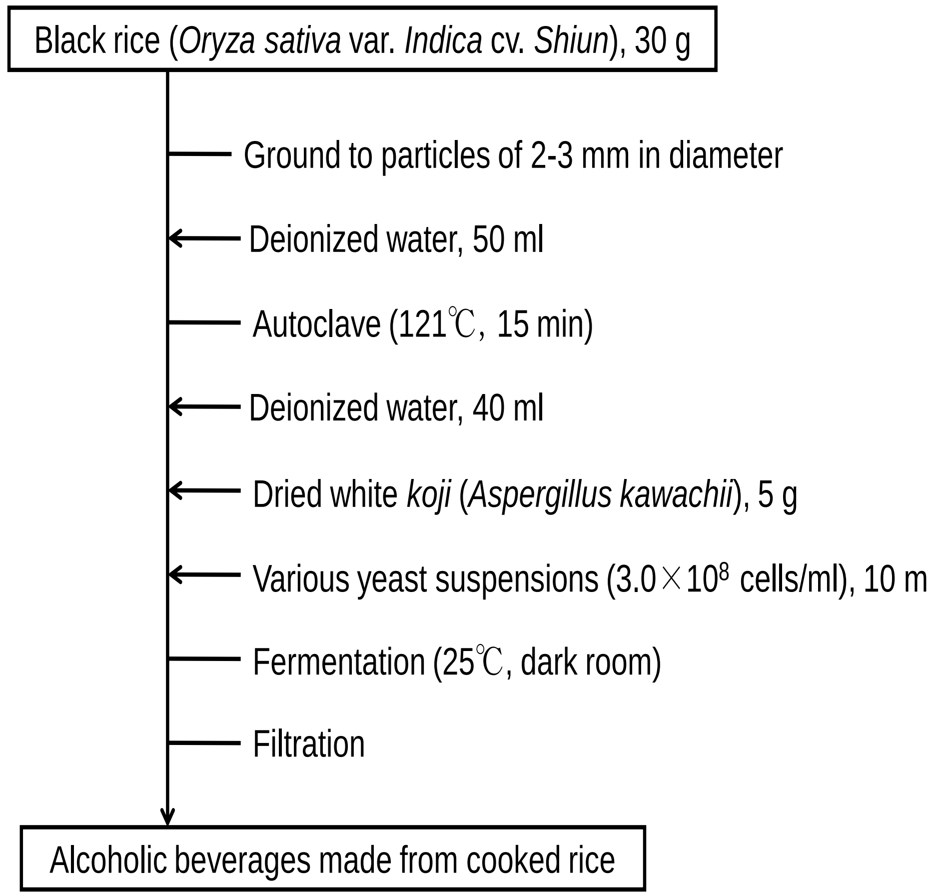

Figure 1. Procedure for black rice wine brewing with cooking.

tap water, (1000 ml)] containing 50 µg chloramphenicol/ml. The isolated strain of yeast was maintained on YPD slopes.

A taxonomical study of the yeast was carried out by NCIMB Japan, Co., Ltd. (Shimizu, Japan). For a comparative study, the industrial wine yeast *S. cerevisiae* W-4 was purchased from the Brewing Society of Japan (Tokyo, Japan) was used.

Rice grains

Black rice (*Oryza sativa* var. *Indica* cv. *Shiun*) was purchased from Kajiwara Beikoku Co., Ltd. (Kyoto, Japan). The black rice grains (Saigusa, 1994; Koguchi et al., 2010) were ground to particles of 2 to 3 mm in diameter with an electric grinder before ethanol fermentation.

Saccharifying agent

Dried white *koji* made from cooked rice grains and the fungal strain *Aspergillus kawachii* purchased from Kawachi Genichiro Shoten Co., Ltd. (Kagoshima, Japan) was used as the saccharifying agent.

Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Nacalai Tesque (Kyoto,Japan). Trolox (6-hydro xy-2,5,7,8-tetramethyl chroman-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

The procedure for ethanol fermentation with cooking is shown in Figure 1 (Koguchi et al., 2010). 30 g of unpolished black rice grains and 50 ml of deionized water were dispensed into a 300 ml Erlenmeyer flask and autoclaved at 121°C for 15 min. After cooling, the cooked rice was mixed with 5 g of dried white *koji* 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 x 10^7 cells/ml. Ethanol fermentation was conducted at 25°C in the dark.

The procedure for ethanol fermentation without cooking is shown in Figure 2. 30 g of unpolished raw black rice grains, 90 ml of deionized water, 5 g of dried white *koji*, 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 the ethanol fermentation with cooking.

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

After fermentation, the mash made from black rice grains was filtered through No.101 filter paper (Advantec Toyo Co., Ltd., Tokyo, Japan), and the resulting black rice wine was analyzed. Acidity was measured by titrating 10 ml of black rice wine with 0.1N NaOH.

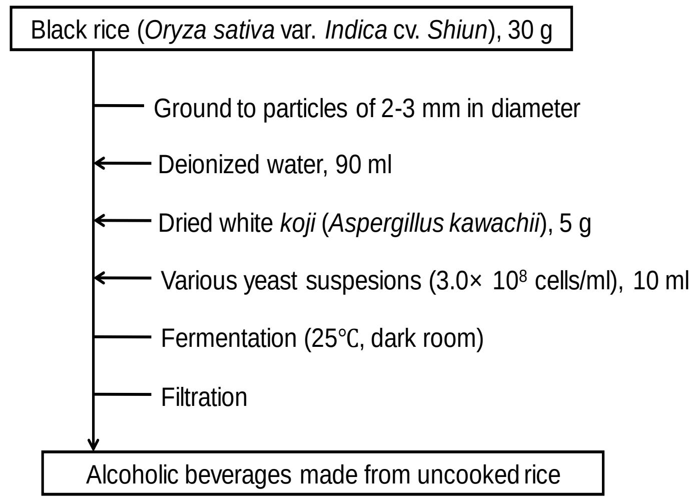

Figure 2. Procedure for black rice wine brewing without cooking.

Reducing sugar as glucose was determined according to the methods of Somogyi and Nelson (Somogyi, 1952; Nelson, 1944). Total phenol compounds, expressed as gallic acid, were determined according to the Folin-Ciocalteu method (Singleton et al., 1999; Hamasaka et al., 2004). The ethanol concentration of black rice wine was determined with a gas chromatographer (model GC-14A; Shimadzu Co., Kyoto, Japan) equipped with a 3.1 m PEG-HT column (Gasukuro Kogyo Inc., Tokyo, Japan).

Black rice wine was filtered through a membrane filter with a pore size of 0.45 μm and a diameter of 47 mm (Advantec Toyo Co., Ltd., Tokyo, Japan). To determine the color quality, the resulting filtrate was examined using a color meter (model ZE 2000; Nihon Denshoku Kogyo Co., Ltd., Tokyo, Japan). The color quality of black rice wine was examined using the Hunter value L, a, and b. The Hunter a value indicates the intensity of red, the Hunter b value, the intensity of yellow, and the Hunter L value, the degree of brightness. To determine the absorption spectra, the resulting filtrate was diluted 50-fold with 1 N HCl and examined with a spectrophotometer (model U-3010; HITACHI, Tokyo, Japan).

The anthocyanin content of black rice wine as the cyanidine 3-glucoside equivalent (μg/ml) was measured according to the method of Boyles et al. (1993).

Determination of anti-oxidative 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

A strain of yeast designated NP01 was isolated from the Thai traditional alcoholic beverage *ou*. NP01 yeast was found to be the fermentation yeast with globose or subglobose cells. Pseudohyphae were not observed during cultivation. D-Glucose, D-galactose, maltose, and sucrose were fermented, while lactose and trehalose were not. According to these and other characteristics, the isolated yeast was identified as a strain of the genus *S. cerevisiae.* The isolated yeast *S. cerevisiae* NP01 was available for ethanol fermentation.

The fermentation curves of the mashes made from the two yeasts and black rice grains are shown in Figure 3. The characteristics of alcoholic beverages, designated as black rice wine, made from the two yeasts and black rice grains, are shown in Table 1. The total amount of the CO_2 output was 11.4 to 12.0 g, and the ethanol concentration of the black rice wine was from 12.4 to 13.1% (v/v). The amount of total phenolic compounds of black rice wine made from the uncooked black rice grains was higher than that of the beverage made from cooked black rice grains. The anthocyanin content of black rice wine made from cooked and uncooked black rice grains with NP01 yeast was 52 and 118 μg/ml, respectively. The anthocyanin content of the black rice wine made from cooked and uncooked black rice grains with W-4 yeast was 57 and 131 μg/ml, respectively.

The black rice wine made from the two yeasts and the black rice grains was red in color; moreover, the one made from the uncooked black rice grains was brilliant red in color, just like red wine. The Hunter a values of the black rice wines made from the cooked and uncooked black rice grains with NP01 yeast were 2.65 and 6.89 (Table 2), respectively, and those of the black rice wines made from the cooked and uncooked black rice grains with W-4 yeast were 3.23 and 9.23, respectively. The Hunter, values of the beverages made with W-4 yeast were higher than those of the beverages made with NP01 yeast. The Hunter b values of the beverages made from the cooked and uncooked black rice grains with the two yeasts ranged from 4.22 to 4.72. The Hunter L value of the black rice wine made from the uncooked black rice grains was lower than that of beverages made from the cooked black rice grains.

The absorption spectra of the black rice wine made from NP01 yeast and black rice grains are shown in Figure 4.

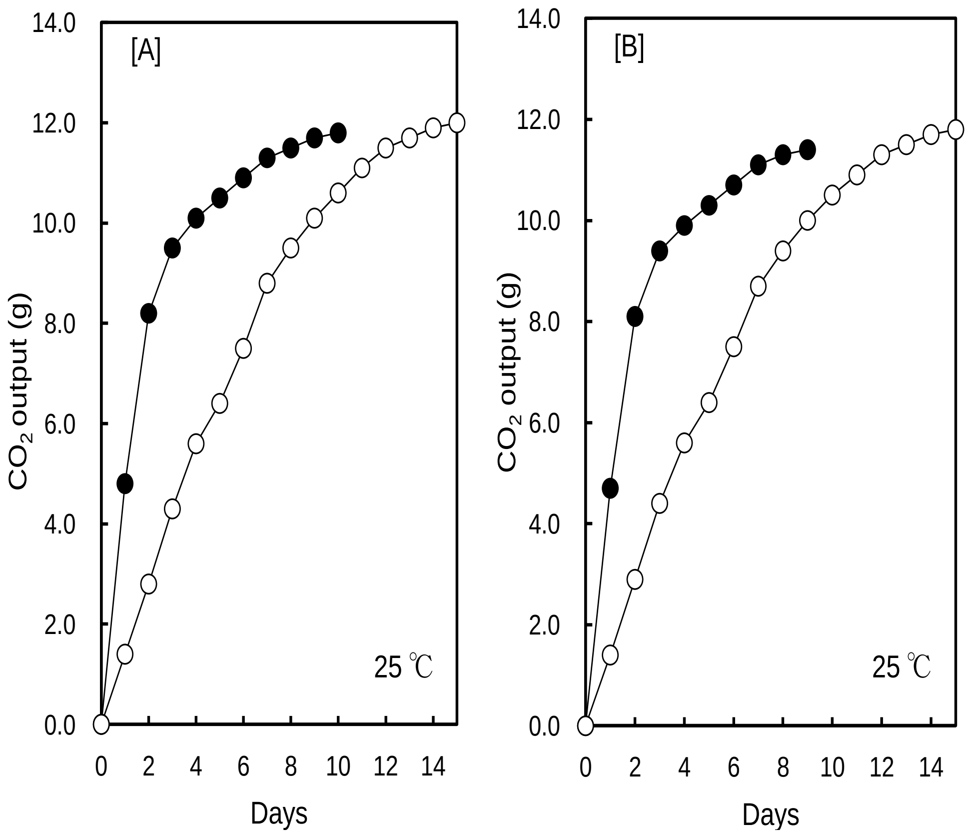

Figure 3. Time courses of fermentation of mashes using the two yeast strains and black rice grains. ●, fermentation with cooking; ○, fermentation without cooking. Values are the mean of three replicates. A, NP01 yeast; B, W-4 yeast.

Table 1. Characteristics of alcoholic beverages made from various yeasts and black rice grain.

Parameter	NP01 yeast		W-4 yeast	
	Cooked	Uncooked	Cooked	Uncooked
Initial pH	5.22 ± 0.04	4.58 ± 0.09	4.79 ± 0.09	4.58 ± 0.07
Final pH	3.92 ± 0.01	4.10 ± 0.02	3.72 ± 0.01	3.86 ± 0.01
CO_2 output (g)	11.8 ± 0.0	12.0 ± 0.1	11.4 ± 0.1	11.8 ± 0.1
Filtrate (ml)	79 ± 1	86 ± 1	78 ± 1	84 ± 1
Acidity (ml)	4.52 ± 0.09	5.62 ± 0.09	5.49 ± 0.22	6.67 ± 0.03
Ethanol concentration (%, v/v)	12.6 ± 0.2	12.8 ± 0.1	12.4 ± 0.3	13.1 ± 0.2
Reducing sugar content (mg/ml, glucose equivalent)	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.3 ± 0.0
Total phenolic compound content (μg/ml, GA equivalent)	851 ± 32	1157 ± 9	883 ± 51	1249 ± 72
Anthocyanin content (μg/ml, Cy 3-glc equivalent)	52 ± 5	118 ± 4	57 ± 5	131 ± 5

Values are the mean of three replicates ±SD. GA, gallic acid; Cy 3-glc, cyaniding 3-glucoside.

The absorption spectra of the black rice wine made from W-4 yeast and black rice grains are shown in Figure 4. The resulting rice wine made from the cooked and uncooked black rice grains showed absorbance at 520 nm. The absorbance of the rice wine made from the uncooked black rice grains was strong and relative to that of the rice wine made from cooked black rice grains. Part of the anthocyanin might have been decomposed during

Table 2. Hunter value of alcoholic beverages made from various yeasts and black rice grain.

Yeast	Black rice grain	Hunter value		
		L	a	b
NP01 yeast	Cooked	92.73 ± 0.14	2.65 ± 0.18	4.22 ± 0.10
	Uncooked	87.98 ± 0.33	6.89 ± 0.28	4.51 ± 0.11
W-4 yeast	Cooked	91.97 ± 0.16	3.32 ± 0.33	4.72 ± 0.08
	Uncooked	85.57 ± 0.67	9.23 ± 0.61	4.45 ± 0.19

Values are the mean of three replicates ±SD.

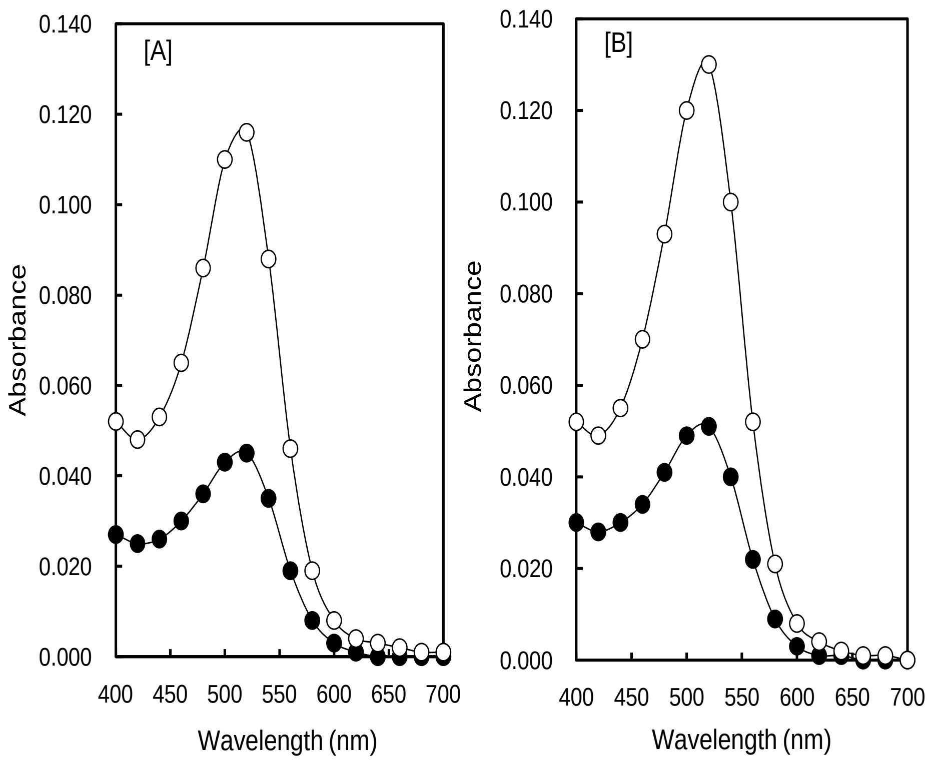

Figure 4. Absorption spectra of alcoholic beverages made from the two yeasts and black rice grains. ●, fermentation with cooking; ○, fermentation without cooking. Values are the mean of three replicates. A, NP01 yeast; B, W-4 yeast.

the cooking process because the rice wine made from the uncooked black rice grains showed typical peak of anthocyanin at 520 nm.

The antioxidative activity of the black rice wine was determined. The DPPH radical scavenging activity of the rice wine made from the uncooked black rice grains was higher than that of the rice wine made from the cooked black rice grains (Figure 5A). The lipid peroxidation of the alcoholic beverages was also determined. The inhibitory activity of the lipid peroxidation of the rice wine made from the uncooked black rice grains was higher than that of the rice wine made from the cooked black rice grains (Figure 5B). The antioxidative activity of various rice wines made from black rice was similar (Koguchi et al., 2010).

Black rice wine made from various yeasts and black rice grains showed antioxidative activity. We would like to improve the taste and aroma quality of the black rice wine to produce a fine alcoholic beverage that is red in color and has physiological advantages as well.

ACKNOWLEDGEMENTS

This work was partially funded by the Japan Society for Promotion of Science (JSPS) – National Research Council of Thailand (NRCT) Core University Program between Yamaguchi University and Kasetsart University from 1998 to 2007 and the JSPS-NRCT Asian Core

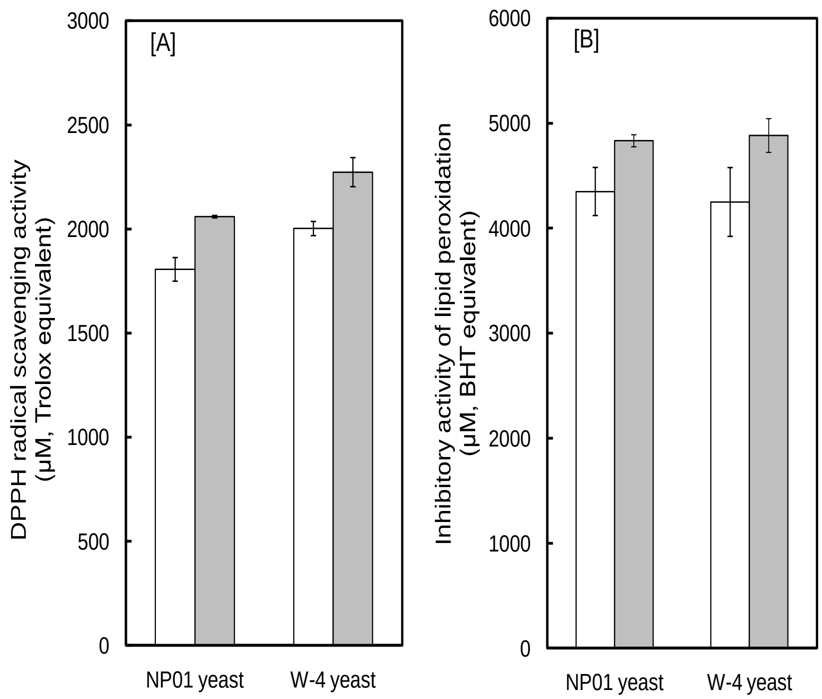

Figure 5. Antioxidative activity of alcoholic beverages made from the two yeasts and black rice grains. Open bars, fermentation with cooking; gray bars, fermentation without cooking. Values are the mean of three replicates ± SD. A, DPPH radical scavenging activity (μM Trolox equivalentB, Inhibitory activity of lipid peroxidation (μM BHT equivalent).

Program between Yamaguchi University and Khon Kaen University as of 2008.

REFERENCES

Hamasaka T, Kumazawa S, Fujimoto T, Nakayama, T (2004). Anti oxidant activity and constituents of propolis collected in various areas of Japan. Food Sci. Technol. Res. 10: 86-92.

Jang S, Xu Z (2009). Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. J. Agric. Food Chem. 57: 858-862.

Kamiyama M, Kishimoto Y, Tani M, Andoh K, Utsunomiya K, Kondo K (2009). Inhibition of low-density lipoprotein oxidation by Nagano purple grape (*Vitis vinifera×vitis labrusca*). J. Nutr. Sci. Vitaminol. 55: 471-478.

Koguchi M., Saigusa N, Teramoto Y (2010). Antioxidative activity of alcoholic beverages made from purple rice (*Oryza sativa* var. *Indica* cv. *Shiun*). Food Sci. Technol. Res. 16: 157-162.

Longo L, Platini F, Scardino A, Alabiso O, Vasapollo G., Tessitore L (2008). Autophagy inhibition enhances anthocyanin-induced apoptosis in hepatocellular carcinoma. Mol. Cancer Ther. 8: 2476-2485.

Miyazaki K, Makino K, Iwadate E, Deguchi Y, Ishikawa F (2008). Anthocyanins from purple sweet potato *Ipomoea batatas* cultivar ayamurasaki suppress the development of antherosclerotic lesions and both enhancements of oxidative stress and soluble vascular cell adhesion molecule-1 in apolipoprotein E-Deficient Mice. J. Agric.
Food Chem. 56: 11485-11492.

Nelson T (1944). A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375-380.

Saigusa N, Teramoto Y, Ueda S, Yoshizawa K (1994). Effects of β-glucosidase activity on the characteristics of aromatic red rice wine. J. Inst. Brew. 100:159-162.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152-178.

Somogyi M (1952). Notes on sugar determination. J. Biol. Chem. 195:19-23.

Spormann TM, Albert FW, Rath T, Dietrich H, Will F, Stockis JP, Eisenbrand G, Janzowski C (2008). Anthocyanin/polyphenolic-rich fruit juice reduces oxidative cell damage in an intervention study with patients on hemodialysis. Cancer Epidemiol Biomarkers Prev. 17: 3372-3380.

Teramoto Y (2003).Comparison of traditional Thai sake and Ugandan beer drunk with straws. The Brewer Intl. 3(12): 29-30.

Teramoto Y (2007). Drawing tubes of Bahrain, Uganda, and Thailand. The Brewer & Distiller Intl. 3(7): 33-34.

Teramoto Y, Wongwicharn A (2002). Characteristics of a traditional Thai alcoholic beverage drunk with a straw. The Brewer Intl. 2(7): 31-32.

Teramoto Y, Sato R, and Ueda S (2005). Characteristics of fermentationyeast isolated from traditional Ethiopian honey wine, *ogol*. African J. Biotechnol. 4: 160-163.

Yamaguchi T, Takamura H, Matoba T, Terao J (1998). HPLC method for evaluation of free radial-scavenging activity of foods by using 1,1-diphenyl-1-2-picrylhydrazyl. Biosci. Biotechnol. Biochem. 62: 1201-1204.