

Production and Antioxidative Activity of Alcoholic Beverages Made From Newly Isolated Vietnamese *Men* Yeast

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Abstract

Fermentation yeast was newly isolated from a traditional Vietnamese microbial starter for brewing alcoholic beverages, called *men*. The isolated yeast was identified as a strain of the *Saccharomyces cerevisiae* and designated as *S. cerevisiae* Y3. The alcoholic beverage made with 3 yeast strains, Y3, NP01, and K7 from uncooked and cooked nonglutinous rice grains had an ethanol concentration of approximately 11.6 to 14.5% (v/v). Resulting alcoholic beverages made with Y3, NP01, and K7 yeasts had antioxidative activity. The DPPH radical scavenging activity of the alcoholic beverages made with 3 yeast strains is equivalent to approximately 500 to 600 μ M Trolox. The DPPH radical scavenging activity of the alcoholic beverage made with Y3 yeast was higher than that of the alcoholic beverage made with NP01 and K7 yeasts. The inhibitory activity of lipid peroxidation of the alcoholic beverages made with Y3 and NP01 yeasts was higher than that of the alcoholic beverages made with K7 yeast.

Key words: *men*, fermentation yeast, antioxidative activity, uncooked fermentation.

1. Introduction

We have been researching fermented foods worldwide and their microbial resources. We previously reported on the characteristics of a traditional Thai alcoholic beverage called *ou*, which was drunk through tubes, and its fermentation yeast [1]. We also reported on the characteristics of alcoholic beverages drunk through tubes in Uganda and Bahrain [2, 3].

Food materials containing antioxidative compounds are known to contribute to prevention of diseases such as arteriosclerosis and many studies were done [4, 5].

In this study, we tried to isolate and identify the yeast strain from a Vietnamese microbial starter called *men* and to utilize the isolated yeast for ethanol fermentation. The characteristics of the alcoholic beverage made with the isolated yeast were determined to develop the functional alcoholic beverage, which has antioxidative activity [6, 7]. For a comparative study, *S. cerevisiae* NP01, stocked in our laboratory, and the industrial sake brewing yeast *S. cerevisiae* K7 were used as well.

We are now trying hard to research the undeveloped microbial resources of tropical area under the project of Japan Society for Promotion of Science (JSPS)–National Research Council of Thailand (NRCT). Comparative studies of newly isolated yeast of tropical area and industrial brewing yeast are also carried

out in order to utilize the isolated yeast to modern brewing industry.

2. Experimental

2.1 Yeast strain

We attempted to isolate a fermentation yeast strain from commercial *men*, Nam Hoa, used in the local microdistillery in the Cai Lay District in Vietnam, using plates of an agar-solidified YPD medium (yeast extract, 10 g; peptone, 20 g; glucose, 20 g; tap water, 1,000 ml) containing 50 μ g of chloramphenicol/ml. The isolated strain of yeast was maintained on YPD agar slopes. A taxonomical study concerning rDNA analysis of the isolated yeast strain was carried out by Toyobo Biologics, Inc. (Tsuruga, Japan).

For a comparative study, *S. cerevisiae* NP01 isolated in Thailand and stocked in our laboratory [1] and the industrial sake yeast *S. cerevisiae* K7 purchased from the Brewing Society of Japan (Tokyo, Japan) were used.

2.2 Rice grains

Commercial nonglutinous rice grains (*Oryza sativa* var. *Japonica* cv. *Hinohikari*) were ground to particles of 2–3 mm in diameter with an electric grinder and were used for ethanol fermentation.

2.3 Saccharifying agent

A glucoamylase preparation, Sumizyme, kindly donated by Shin Nihon Kagaku Kogyo Co., Ltd. (Anjo, Japan), was used as the saccharifying agent.

2.4 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).

2.5 Ethanol fermentation procedure

Traditional ethanol fermentation with cooking was performed according to the procedure below. Thirty grams of uncooked unpolished nonglutinous 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 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.

Ethanol fermentation without cooking was done as follows. Thirty grams of uncooked unpolished nonglutinous rice 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. 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 CO₂ gas was measured every 24 h.

2.6 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.

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 [8, 9]. The amount of total phenolic compounds, expressed as gallic acid, was determined according to the Folin-Ciocalteu method [10, 11].

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).

2.7 Determination of antioxidative activity

The DPPH radical scavenging activity as the Trolox equivalent was measured on the basis of the method of Yamaguchi *et al.* [12]. The lipid peroxidation inhibitory activity as the BHT equivalent was determined using β-carotene [11].

3. Results and Discussion

Efforts were made to research traditional Vietnamese alcoholic beverages in November 2011. Research was done in Go Den District, Cai Lay District, Tra Vinh District, and O Mon District in the Mekong Delta [13]. In Vietnam, alcoholic beverages have traditionally been made using a special microbial starter, the native name of which is *men*.

A strain of yeast designated Y3 was isolated from the Vietnamese commercial *men* Nam Hoa. Y3 yeast was found to be fermentation yeast with globose or subglobose cells. Pseudohyphae were not observed during cultivation. D-Glucose, D-galactose, mannose, maltose, and sucrose were fermented, while lactose was not.

rDNA analysis of Y3 yeast was carried out by Toyobo Biologics, Inc., and was identified as a strain of *Saccharomyces cerevisiae*. According to these characteristics, the isolated yeast was identified as a strain of *S. cerevisiae*.

The fermentation curves of the mashes made from the 3 yeast strains, *S. cerevisiae* Y3, NP01, and K7 are shown in Fig. 1. A larger amount of CO₂ was generated from the mash with Y3 and NP01 yeasts than that with K7 yeast on day 1. Fermentation was completed after 5 days.

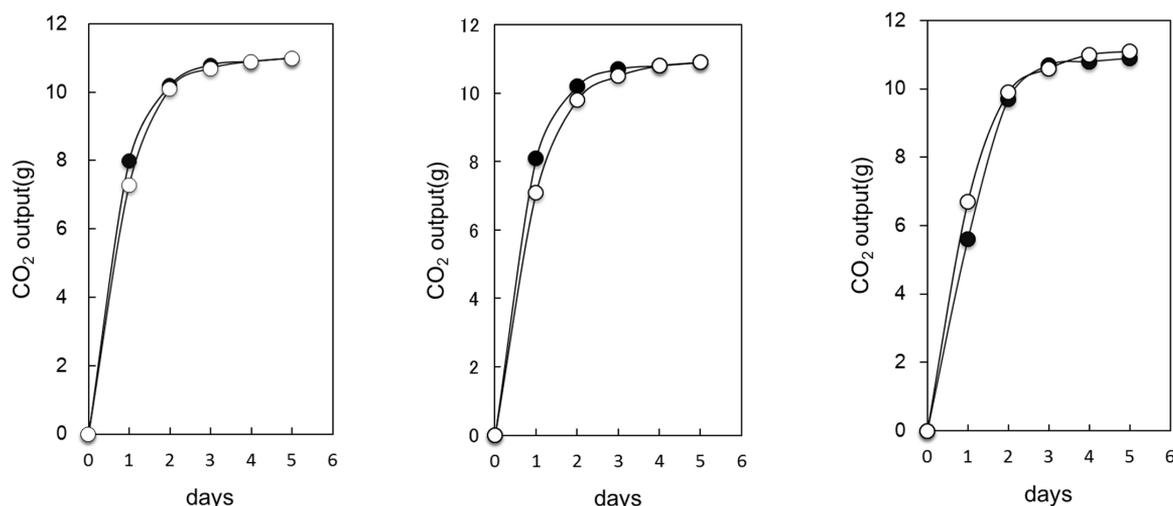


Figure 1 : Time courses of fermentation of mashes using 3 yeast strains and nonglutinous rice grains. Symbols: ●, fermentation with cooking; ○, fermentation without cooking. Values are the mean of 3 replicates. A, Y3 yeast; B, NP01 yeast; C, K7 yeast.

Y3 yeast is applicable to conventional ethanol fermentation with cooking and ethanol fermentation without cooking as well. In the system of ethanol fermentation without cooking, material rice grain was directly applied to ethanol fermentation, then we can save energy for cooking or steaming.

The characteristics of alcoholic beverage made from the 3 yeasts are shown in Table 1. The ethanol concentration of the alcoholic beverages was approximately 11.6 to 14.5% (v/v). We use gas trap containing concentrated H_2SO_4 to release CO_2

only and capture aqueous vapor and volatile compounds such as higher alcohols and esters. Then, sometime, depends on the fermentation condition, too much aqueous vapor and volatile compounds were captured by gas trap and the volume of resulting filtrate decrease and ethanol concentration increase. We are not determined but there is a possibility, instead of ethanol other metabolite such as organic acid might be produced. We observed sometime amount of released CO_2 and amount of ethanol are not precisely parallel.

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

	Y3		NP01		K7	
	uncooked	cooked	uncooked	cooked	uncooked	cooked
Polished rice (g)	30	30	30	30	30	30
Sumizyme (g)	0.2	0.2	0.2	0.2	0.2	0.2
Deionized water (ml)	0	50	0	50	0	50
Yeast suspension (ml)*	100	50	100	50	100	50
pH	4.2	4.2	3.9	4.2	3.9	4.0
Acidity (ml)	2.1	2.0	2.3	1.8	2.6	2.4
Ethanol concentration (% , v/v)	13.2	11.6	12.6	14.1	14.2	14.5
Total phenolic compound content ($\mu g/ml$)	1438	1047	1172	836	1256	893
Reducing sugar content ($\mu g/ml$)	664	699	635	619	653	711

*Cell number of the initial mash was adjusted to be 3.0×10^7 cells/ ml.

Acidity of the resulting alcoholic beverages was 1.8 to 2.6 and pH of them was 3.0 to 4.2. Acidity and pH of resulting alcoholic beverages were almost similar to Japanese sake.

Reducing sugar of the resulting alcoholic beverages were around 600 to 700 $\mu g/ml$. This means ethanol fermentation proceeded properly and there are not so many residual sugar.

The total amount of phenolic compounds of alcoholic beverage made from uncooked rice grains was higher than that of the beverage made from cooked rice grains. In the case of fermentation without cooking, rice grain was not heated or steamed; the phenolic compounds might be not decomposed by heating. Alcoholic beverages made with Y3 yeast contained relatively higher amount of phenolic compounds. Y3 yeast might produce much phenolic compounds including protein and aromatic amino acids.

The antioxidative activity of the alcoholic beverage was determined. The DPPH radical scavenging activity of various alcoholic beverages is equivalent to approximately 500 to 600 μM Trolox (Fig. 2). The DPPH radical scavenging activity of the alcoholic beverage made with Y3 yeast was higher than that of the alcoholic beverage made with NP01 and K7 yeasts.

The inhibitory activity of lipid peroxidation of the alcoholic beverages is shown in Fig. 3. The inhibitory activity of lipid peroxidation of the alcoholic beverage made with Y3 and NP01 yeasts was higher than that of the beverages made with K7 yeast.

Y3 and NP01 yeasts were screened from the warm region. Then it was considered that fermentation advanced quickly in a warm environment of 25 °C. It has been suggested that compounds related to DPPH radical scavenging activity and the inhibitory activity of lipid peroxidation are different. We would like to determine the compounds related to DPPH radical scavenging activity and the inhibitory activity of lipid peroxidation.

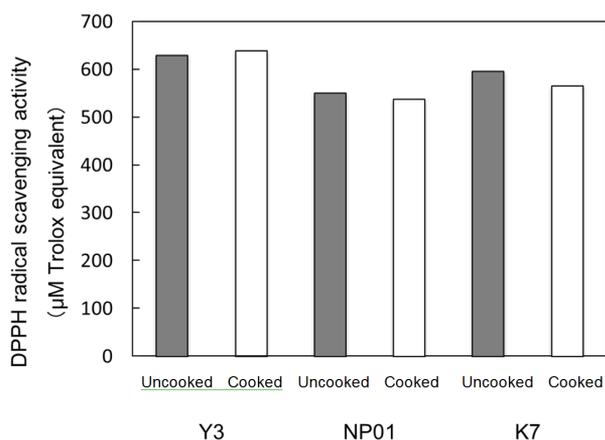


Figure 2 : DPPH radical scavenging activity of alcoholic beverages made from 3 yeast strains and nonglutinous rice grains.

Closed bars, alcoholic beverage made by fermentation without cooking; open bars, fermentation with cooking.

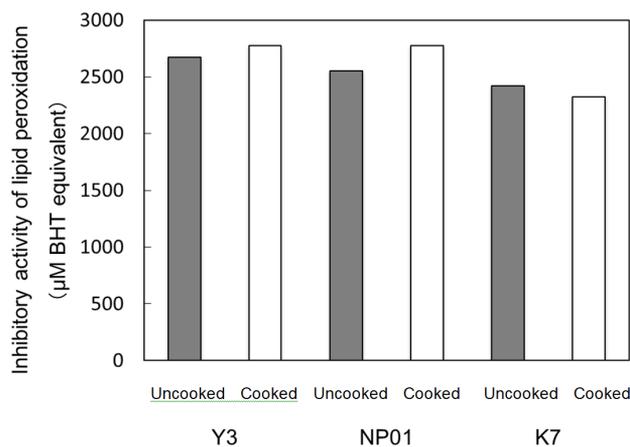


Figure 3 : Inhibitory activity of lipid peroxidation of alcoholic beverages made from 3 yeast strains and nonglutinous rice grains.

Closed bars, alcoholic beverage made by fermentation without cooking; open bars, fermentation with cooking.

4. Conclusion

Newly isolated yeast strain *S. cerevisiae* Y3 was applicable for ethanol fermentation. Comparative study with *S. cerevisiae* NP01 isolated in Thailand and *S. cerevisiae* K7 industrial sake yeast used in Japan was done. Y3 yeast had similar fermentation ability compared with NP01 yeast isolated in Thailand and K7 industrial sake yeast used in Japan. Y3 yeast was applicable for traditional ethanol fermentation with cooking and ethanol fermentation without cooking.

The alcoholic beverages made from various yeasts and nonglutinous rice grains showed antioxidative activity. The DPPH radical scavenging activity of the alcoholic beverage made with Y3 yeast was higher than that of the alcoholic beverage made with NP01 and K7 yeasts. We would like to determine the compounds contained in the alcoholic beverages which have DPPH radical scavenging activity and the inhibitory activity of lipid peroxidation.

We would like to improve the taste and aroma of the alcoholic beverage and produce a fine alcoholic beverage that also has physiological advantages. We also apply the tropical microbial resources to modern brewing industry.

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