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Full Length Research Paper

Characteristics of fermentation yeast isolated from traditional Ethiopian honey wine, *ogol*

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Indigenous honey wine, known locally as *ogol*, was collected in a village of the Majangir ethnic group in Southwest Ethiopia, and the procedure for *ogol* fermentation was investigated. A fermentation yeast was first isolated from *ogol* and identified as being a strain of the genus *Saccharomyces cerevisiae*. Honey wine made with *S. cerevisiae* ET99 contains 16.5% (v/v) ethanol; the acidity and pH were 6.0 and 3.8, respectively. Volatile ester and higher alcohol were detected by gas chromatography. A relatively higher amount of propan-1- ol (43 mg/l) was found in the honey wine than in those made with wine yeast W4 and sake yeast K7. The aroma characteristics of honey wine made with yeast ET99 were acceptable, as determined by organoleptic tests, and were found to be applicable to ethanol fermentation.

Key Words: Honey wine, ogol, indigenous alcoholic beverage, fermentation, *Saccharomyces*, Ethiopia.

INTRODUCTION

Honey wine, an indigenous fermented beverage, was invented thousands of years ago (Steinkraus, 1983). Many kinds of honey wine, such as mead, metheglin, hydromel, aguamiel, and medovukha, have been made in various parts of Europe (Campbell-Platt, 1989). In Africa, honey wine was also brewed traditionally in many areas.

Honey wine generally containing 6 to 17% (v/v) ethanol is well known all over the world, and many ethnological reports have been published (Steinkraus, 1983). However, scientific studies of honey wine are very rare.

In the present study, the Ethiopian honey wine, *ogol,* was investigated, and an attempt was made to characterize the fermentation yeast isolated from *ogol.* Honey wines were also made with the yeast strain isolated from *ogol* as well as the yeasts from industrial wine and sake, and the characteristics of these honey wines were compared.

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MATERIALS AND METHODS

Samples of traditional honey wine, *ogol*

A sample of *ogol* was collected in a local village of the Majangir ethnic group, Kumi Village, Godare Wereda, Gambella Region in Southwest Ethiopia. The precise method for making *ogol* was determined during fieldwork in Southwest Ethiopia.

Honey and black rice grain

Mandarin orange honey was purchased from Sugi Yohoen, Kumamoto. Black rice *Oryza sativa* var. *Indica* cv. *Shiun* was purchased from Kajiwara Beikoku, Kyoto.

Industrial microorganisms and dried rice koji

The industrial wine yeast *S. cerevisiae* W4 and sake yeast *S. cerevisiae* K7 were purchased from the Brewing Society of Japan, Tokyo. Dried rice *koji*, a Japanese microbial starter made from cooked rice grain and spores of *Aspergillus kawachii* that can produce a saccharifying enzyme, such as glucoamylase, was purchased from Kawachi Gen-ichiro Shoten, Kagoshima.

Figure 1. The bark of *mange* (*Blighia unijungata* Bak) used for *ogol* brewing.

Isolation and taxonomical study of fermentation yeast from *ogol*

Fermentation yeast was isolated on plates of an agar-solidified YPD medium (yeast extract, 10 g; peptone, 20 g; glucose, 20 g; tap water, 1,000 ml) containing 50 g chloramphenicol/ml. The isolated strain of yeast was maintained on YPD slopes. A taxonomic study of the yeast strain was carried out by NCIMB Japan, Co., Ltd., Shimizu.

Ethanol fermentation using yeast isolated from *ogol*

An isolated strain of yeast was cultivated statically in 1,000 ml of a sterilized YPD medium in a 3-l Erlenmeyer flask at 30°C for 3 days. The number of cells in the culture broth was calculated with a haemocytometer. Yeast cells were collected by centrifugation at 3,000 x g and washed twice with a 0.9% solution of NaCl.

Twenty-five grams of mandarin orange honey, 5 g of dried rice *koji* as a source of fungal glucoamylase and nutrition for yeast, 5 g of ground black rice as natural colorant and nutrition for yeast, and fresh yeast cells suspended in 75 ml of tap water were added. The initial number of yeast cells in the mash was adjusted to 3 x 10^7/ml. The Erlenmeyer flask, equipped with a gas trap, was weighed every day, and the amount of CO_2 evolved was calculated. For comparison, industrial wine yeast W4 and industrial sake yeast K7 were also used for ethanol fermentation.

General analyses

Acidity was measured by titrating 10 ml of sample with 0.1 N NaOH and indicated as the volume of 0.1 N NaOH (ml) needed for neutralization. Reducing sugars were quantitated as described previously (Teramoto et al., 1998). Levels of ethanol and other aromatic components were analyzed by gas chromatography (Teramoto et al., 1998).

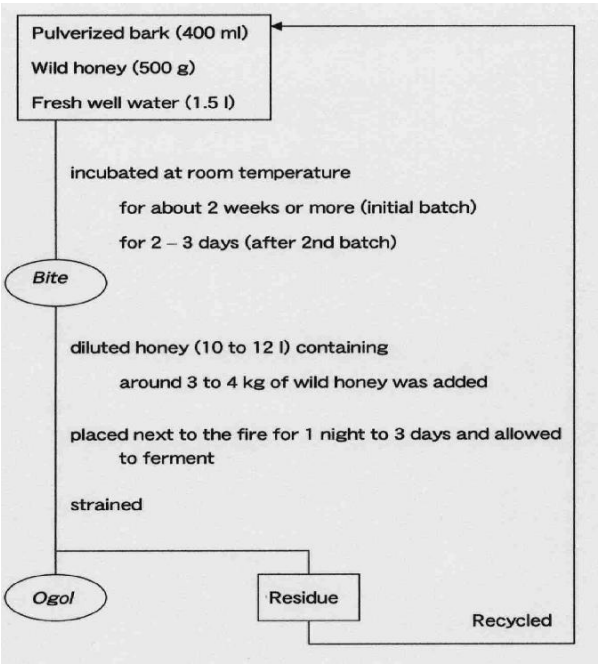

Figure 2. Procedure for *ogol* brewing.

RESULTS AND DISCUSSION

Procedure for *ogol* brewing

Four hundred ml of pulverized bark of the native tree, *mange* (*Blighia unijungata* Bak), 500 g of wild honey,

and 1.5 l of fresh well water were mixed and incubated at room temperature in the tropical area for about 2 weeks or more to propagate the yeast and prepare a seed culture called *bite* (Figures 1and 2). *Mange* was one of the plants of the honey source as well. The bark might provide some nutrition for yeast growth and chemical compounds that prevent microbial contamination and function as a kind of supporting material for the fermentation yeast.

Around 3 to 4 kg of wild honey containing beeswax and bodies of honeybees was diluted with well water to 10 to 12 l. Diluted wild honey was inoculated with *bite* as described above in a 15 l earthen pot. The pot was placed next to a dying fire to maintain the mixture at the proper fermentation temperature. Wax and other residues floating on the surface kept the fermentation more anaerobic, and the impurities in the wild honey served as yeast nutrients (Steinkraus, 1983).

After 1 night to 3 days, the fermented mash was ready to be drunk (Figure 3). It was strained to prepare the *ogol*. The residue composed of the bark and viable yeast cells was recycled to prepare *bite* for the next batch of *ogol* brewing. When recycled bark containing yeast cells was used, it took only 2 to 3 days to prepare the *bite* for the next batch.

Figure 3. Vigorously fermenting mash of *ogol,* the traditional honey wine of Southwest Ethiopia.

Isolation and taxonomical study of the yeast isolated from ogol

A strain of yeast designated ET 99 was isolated from *ogol.* ET 99 was found to be a fermentation yeast with globose or subglobose cells. Pseudohyphae were not observed during cultivation.

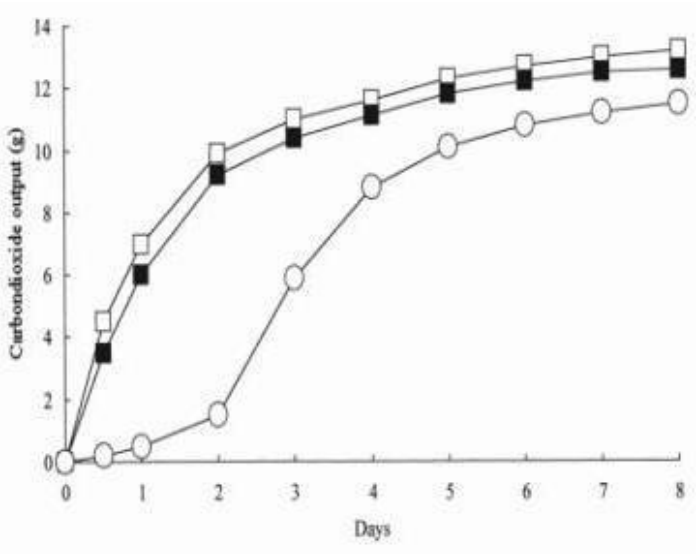

Figure 4. Fermentation curves for honey wine mashes with *S. cerevisiae* ET 99, wine yeast *S.. cerevisiae* W4, and sake yeast *S.. cerevisiae* K7 at 30^{O}C. Symbols: , mash with ET 99; , wine yeast W4; , sake yeast K7.

Table 1. Characteristics of honey wine made with *S. cerevisiae* ET99, wine yeast *S. cerevisiae* W4, and sake yeast *S. cerevisiae* K7 at 30^{O}C.

Parameters	Honey wine made with		
	ET99	W4	K7
Ethanol (%,v/v)	16.5	17.5	17.5
2-Methylpropanol (mg/l)	89	99	74
3-Methylbutanol (mg/l)	154	150	260
Propan-1-ol(mg/l)	43	28	24
Ethyl acetate (mg/l)	30	63	49
Isoamyl acetate(mg/l)	0.4	2.6	0.7
CO_2 output (g)	11.5	13.2	12.6
Acidity (ml)	6.0	6.0	6.4
Initial pH	4.5	4.5	4.5
Final pH	3.8	3.9	3.8
Reducing sugar (mg/ml)	9.7	2.8	2.4

D-Glucose, maltose, D-galactose, and sucrose were fermented, but lactose was not fermented. D-glucose, D-galactose, maltose, and sucrose were assimilated, but glycerol, 2-keto-D-gluconate, L-arabinose, D-xylose, adonitol, xylitol, inositol, D-sorbitol, methyl- -D-glucoside, N-acetyl-D-glucosamine, D-cellobiose, lactose, D-trehalose, D-melezitose, and D-raffinose were not. According to these and other characteristics, the strain was identified as belonging to the genus *Saccharomyces* and was considered to closely resemble *S. cerevisiae* Meyen *ex* E. C. Hansen (Barnett et al., 2000).

Another yeast strain was also isolated from *ogol* and identified as *Pichia membranifaciens* E. C. Hansen (data not shown), but this yeast strain did not show any fermentation ability. *P. membranifaciens* is well known as a pellicle-forming yeast and is often isolated from various alcoholic beverages, foods, and food spoilage.

In the villages of Southwest Ethiopia, fermentation of honey was properly done using traditional procedures without modern methods, such as sterilization and cleaning, but wild yeast, which did not have fermentation ability, was found in *ogol*.

Ethanol fermentation using yeast isolated from *ogol*

The fermentation curves of the mash that contained yeast strain W4 and strain K7 were similar (Figure 4). After 8 days, the amount of CO_2 generated from the mash using W4 and K7 was 13.2 and 12.6, respectively. In comparison with the mash containing W4 and K7, the mash containing ET99 yeast did not show vigorous fermentation. However, in 8 days, the fermentation of the mash progressed, and, finally, 11.5 g of CO_2 was generated.

The analytical data for the resulting honey wine are shown in Table 1. The final concentrations of ethanol in honey wine made with ET99, W4, and K7 were 16.5, 17.5, and 17.5 % (v/v), respectively. The acidity and final pH of these honey wines were 6.0 to 6.4 and 3.8 to 3.9.

The gas chromatographic analysis showed that the levels of the aromatic components in the honey wines made with ET99, W4, and K7 were relatively similar. However, the amount of 2-methylpropanol, ethyl acetate, and isoamyl acetate was higher in honey wine made with W4 yeast. The amount of propan-1-ol and acetaldehyde was higher in honey wine made with ET99 yeast. The amount of 3-methylbutanol was higher in honey wine made with K7 yeast.

The aromatic characteristics of honey wine made with yeast ET99 were acceptable, as demonstrated by an organoleptic test, and found to be applicable to ethanol fermentation.

In conclusion, fermentation yeast was first isolated from Ethiopian traditional honey wine *ogol* and characterized. *S. cerevisiae* ET99 was isolated from *ogol* and found to be applicable to ethanol fermentation. It has been suggested that a novel alcoholic beverage could be brewed with the ET99 yeast used in the brewing of traditional Ethiopian honey wine.

Efforts are currently underway to research indigenous alcoholic beverages brewed in tropical and subtropical areas and to isolate useful microbial resources (Teramoto et al., 2001, 2002; Teramoto, 2003) so that the methods and microorganisms used in their production can be studied and possibly applied to modern brewing.

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