BIOCHEMICAL AND SENSORIAL CHARACTERISTICS OF *BREM BALI*

by

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ABSTRACT

This study was an attempt to verify the biochemical and sensorial characteristics of *brem bali*, an alcoholic beverage produced traditionally in Bali island. The results revealed that the *brem bali* contained 8.6% (w/v) ethanol, 27.8% (w/v) glucose, 111.5 mM organic acids and 436.5 ppm of volatile compounds. Lactic acid was the main organic acid in *brem bali*. The presence of large amount of lactic acid resulted in its typical flavour, which has a nice combination of sweet, sour and alcoholic taste with fruity aroma dominated by the higher molecular weight alcohols such as *i*-amyl alcohol, *i*-butanol, *n*-propanol and other unidentified volatile compounds.

Keywords : Biochemical characteristics, sensorial characteristics, alcoholics, beverage

RINGKASAN

Penelitian dilakukan untuk menentukan karakteristik biokimiawi dan organoleptik pada produk brem bali, sebuah produk tradisional minuman beralkohol yang diproduksi di Pulau Bali. Hasil penelitian menunjukkan bahwa brem bali mengandung 8.6%(w/v) alkohol, 27.8% (w/v) glukosa, 111.5 mM asam-asam organik dan 436.5 ppm senyawa mudah menguap. Kandungan asam laktat yang tinggi menyebabkan rasa khas pada brem bali yang merupakan kombinasi dari rasa manis, rasa asam dan rasa alkohol serta beraroma buah-buahan yang sebagian besar didominasi oleh golongan alkohol yang mempunyai berat molekul lebih tinggi seperti *i*-amyl alcohol, *i*-butanol dan *n*-propanol serta senyawa-senyawa mudah menguap lainnya.

Kata kunci : Karakter biokimia, karakter sensorial, alkohol, minuman beralkohol

INTRODUCTION

Brem bali is a popular alcoholic beverage produced traditionally in Bali Island. The alcoholic beverage plays an important role in the local diet and in the ritual ceremonies of Balinese Hindu (Steinkraus, 1996). The traditional fermentation of *brem bali* has been practiced by the people in the island for centuries, however, only a little has been known about its biochemical characteristics particularly on the compounds responsible for the typical flavor of the product.

The traditional fermentation of *brem bali* is carried out at two fermentation steps (Fig. 1). The first step is fermentation of steamed rice after inoculation with *ragi tape* in solid-state fermentation. The second one is fermentation of liquid portion obtained by pressing *tape* mash and is usually carried out in a tank. The first fermentation step is locally called *penapean* (*Balinese* and *Indonesian)* or *tape* fermentation (for review see Ardana and Fleet, 1989; Ko, 1972).

In fermentation of *brem bali* (using *ragi tape* as a dry-starter), bacterial flora (*Bacillus* sp., and lactic acid bacteria), amylolytic yeasts and ethanol-producing yeasts proliferate soon after the growth of amylolytic fungi. Thus, the liquefaction of steamed rice and the alcoholic fermentation occur almost simultaneously. It is therefore difficult to control processes that ensure a stable quality of the *brem bali* and this is considered as a main problem in its fermentation.

The *brem bali* contains alcohol, 8.7 - 12.7% (v/v); total sugars 4.0 - 5.6 % (w/v); reducing sugars, 3.6 - 5.0% (w/v); amino nitrogen, 0.15 - 0.16% (w/v); total soluble solids, 14.8 -19.1°Brix, and has a pH value of 4.1- 4.2 (Aryanta, 1980). Though these data imply the main taste components of *brem bali*, the compounds that responsible for the unique aroma are still unclear. Therefore, this work was attempted to verify the compounds accountable for the typical flavor (taste and aroma) of *brem bali*. Such data are fundamental information to clearly understand the process and to improve the technology for a more commercial production of this alcoholic beverage.

MATERIALS AND METHODS

Brem bali samples

Two samples of *brem bali* (three bottles per samples) were purchased at two different supermarkets at Denpasar, Bali, Indonesia. The samples were commercially sold in amber bottle containing 200 ml of *brem bali*. After arrival at Sapporo, Japan the samples were stored at 5°C prior to analysis. One bottle from each sample was used for chemical analysis and the others were used for the sensorial characterization. The data presented for the chemical analysis are the means of two time replications; on the other hand, sensorial characteristics were obtained from 13 panelists.

Analytical Methods

Organic acids were determined using HPLC (Shimadzu) with Shim-pack SCR-102H column (8 mm ID X 300 mm L) fitted with SCR-102H (6 mm IDX50 mm L) guard column in CTO-10A Vp column oven which was held at 45°C. The HPLC was equipped with SDD-10AD Vp auto sampler and SDD-6A conductivity detector. The mobile phase was 5 mmol/l *p*-toluenesulfonic acid in combination with a buffer containing 5 m mol/l *p*-toluenesulfonic acids, 100 mol/l EDTA and 20 mmol/l Bis-Tris. Both mobile phases were run at a flow rate of 0.8 ml/min. Samples were diluted up to the final organic acid concentration of about 15 mmol/l using milliQ water. About 500 l aliquot was added with 100 l of 25 m mol/l crotonic acid in 100 mmol/l NaOH (internal standard) and was centrifuged at 15,000 rpm for 10 min at 4°C. The upper layer (500 l) was added with 500 l chloroform following centrifugation at 15,000 rpm for 10 min at 4°C. The upper layer (200 l) was filtered through 0.2 m cellulose acetate filter (dismic 13CP, Advantec Toyo, Japan) then frozen at –80°C for 20 min. After thawing, samples were centrifuged again at 15,000 rpm for 10 min at 4°C, and then a 100 l portion of the upper layer was transferred into the HPLC vial. About 10 l sample was injected to the HPLC and the data were analyzed and calculated by comparing area of sample peak with standard peak on Shimadzu Class-VP V5.02.

Glucose concentration was determined using mutarotase-glucoseoxidase method (Glu-cose AR II Kit, Wako, Japan) as instructed by manufacturer. Ethanol concentration was determined using enzymatic method (ethanol kit, BOEH-RINGER MANNHEIM). The pH value was measured after a 1:1 sample dilution (HM-20E, TOA pH meter). Volatile compounds were analyzed on a headspace gas chromatography (Shimadzu HSS 2B) with the temperature of syringe held at 60°C for 20 min. An amount of 1 ml of headspace gas was injected into a capillary column (TC-Wax, df 0.5 m; 0.32 mm ID X 30 m) then the compounds were detected using flame ionization detector. The column temperature was programmed to initiate at 40°C (5 min) then gradually increased at 3°C per min and final temperature was held at 210°C for 5 min using helium gas as carrier. The volatile compounds were recorded and analyzed in Shimadzu R4A Chromatopac. The amount of each compound was calculated by comparing the peak area with the peak of authentic sample.

Sensory analysis

The structured scaling method by Poste *et al* (1991) was employed to verify the fruity aroma, flavor richness, sweetness and sourness of *brem bali*. The sensorial characteristics of *brem bali* were scaled from 1 to 6 where 6 is for extreme, 5 for very, 4 for moderate, 3 for slightly, 2 for trace and 1 for no sensory response (Fig. 2). Thirteen students and staffs (8 males and 5 females) of the Laboratory of Applied Microbiology, Hokkaido University, who were selected in the basis of their motivation and availability, participated as judges. The test was initiated by a short explanation about *brem bali* to introduce the product to the panelists.

RESULTS AND DISCUSSION

In Table 1 it is shown that the ethanol content in *brem bali* was quite low (about 8.6% w/v) compared to other rice wine such as *Japanase sake* and aromatic red rice wine, which contain about 17% (w/v) and 10% (w/v) ethanol, respectively. On the other hand, *brem bali* contained very high residual glucose (about 27.8% (w/v)), while the red rice wine produced in Kumamoto, Japan only contained about 0.8 – 1.6% glucose (Ueda *et al.,* 1990). The lower ethanol content and higher residual glucose in *brem bali* is thought to be affected by the type of yeast used in fermentation. In Kumamoto, the red rice wine was produced using the commercial compressed baker's yeast (Ueda *et al,* 1990), and recently the quality was improved using the *Kyokai no.9* yeast, a strain that often used in *Japanese sake* fermentation (Ueda *et al,* 1991a). On the other hand, *brem bali* is produced using *ragi tape* as a dry starter, which contains *Endomycopsis fibuligera* (synonym: *Saccharomycopsis fibuligera* Lindner) and *Pichia anomala* as the principal yeast (Kuriyama *et al,* 1997). *Saccharomyces cerevisiae* has been reported also to be present during the *tape ketan* fermentation (Ardana and Fleet, 1989). Aside from the amylolytic activity of *S. fibuligera* (Kato *et al,* 1976; Sukara *et al,* 1998), this yeast is also capable of producing ethanol therefore used in rice wine fermentation (Nga *et al*, 1995). *S. fibuligera* is the principal yeast during the liquefaction step in *brem bali* fermentation. Our observation in the laboratory studies of *brem bali* fermentation also showed that *S.fibuligera* cocultured with *Rhizopus oryzae* could produce about 5-6% (w/v) of ethanol from steamed rice after 3 days fermentation (unpublished data). Taking into account that *brem bali* contained about 8.6% (w/v) ethanol, it could be speculated that other alcohol producing yeasts such as *S. cerevisiae* may grow during the fermentation. However, since the filtrate obtained by pressing the *tape* mash contained high amount of glucose, *S. cerevisiae* may not able to grow properly and is inhibited during fermentation. The phenomenon is partially supported by our observation that most of *S. cerevisiae* strains isolated from *brem bali* showed a slow growth in *tape* juice containing 32% (w/v) glucose and only a small portion of glucose was fermented resulting in high residual glucose (20-26% (w/v)) in the broth (unpublished data). Therefore, high glucose content in the *brem bali* is due to the inability of the yeasts to convert glucose into ethanol and consequently, the taste of *brem bali* is very sweet.

The acidity of *brem bali* was about 5.4 ml (ml 0.02 N NaOH/ml) equaivalent to pH 3.8 and 111.5 mM organic acids (Table 1). These observations imply that *brem bali* has an acidic taste. In alcoholic beverages, the types of organic acids are more significant to the flavor of the product. The famous example is malolactic fermentation in wine by which the L-malic acid is transformed into L-lactic acid resulting in deacidification and flavour improvement of wine (Lonvaud-Funel, 1999). The results showed that lactic acid, acetic acid and succinic acid were the predominant organic acid found in the *brem bali*, whereas fumaric acid, tartaric acid and citric acid were present in less than 1 mM. The lactic acid was found to be beneficial in developing a specific sour taste of *brem bali.* Succinic acid, which is the most important organic acid in *Japanese sake*, is known exhibit to about two folds of the lactic acid concentration (Rose and Harrison, 1970). This acid is the main secondary metabolite in alcoholic fermentation of *sake* yeasts. In *brem bali*, however, the concentration of succinic acid was about one tenth to one twentieth of the lactic acid.

The higher molecular weight alcohols such as *i*-amyl alcohol, *n*-propanol and *i*-butanol were the predominant volatile compounds of *brem bali*, present in the amounts of 160.4 ppm, 68.0 ppm and 135.2 ppm, respectively, whereas *i*-amyl acetate, the most important ester for the aroma of alcoholic beverages, was found in trace amount only and its concentration was below the detection limit (Table 1 and Fig. 2). Higher molecular weight alcohols may influence certain sensorial characteristic of the alcoholic beverages. In the rice wine production using aromatic red rice, the concentration of *i*-butyl and *i*-amyl alcohols were reported to be about 250 ppm and 260 ppm, respectively, which attributed to the high sensorial quality of this wine (Ueki *et al*, 1991, Ueda *et al*, 1991a; 1991b). The *i*-

butanol and *i*-amyl alcohol were the main higher alcohols produced during the fermentation of *Indonesian tape ketan* (Cronk *et al*, 1979), however, *n*-propanol was not detected event after the fermentation was carried out until 196 h. Aside from the high amount of *n*-propanol in *brem bali*, there were also some unidentified compounds detected which where thought also to contribute to the aroma of *brem bali* (Fig.2).

Sensory analysis of the *brem bali* showed that the product has the following characteristics: slightly to moderately fruity aroma (score 3.4), moderately to very rich flavor (score 4.4), slightly to moderately sour taste (score 3.9) and moderately to very sweet taste (score 4.8) (Tab.2). These sensory data imply that the *brem bali* is a sour and heavy alcoholic beverage.

The *brem bali* has a combination of sweet, sour and alcoholic taste. The biochemical properties of *brem bali* greatly affect the flavor of the product. The presence of lactic acid as the major organic acid might be correlated with the involvement of lactic acid bacteria during the fermentation. The lactic-sour taste is a typical flavor of *brem bali*, making it different from *Japanese sake* and the aromatic red rice wine produced in Kumamoto, Japan. The higher molecular weight alcohols such as *i*-amyl alcohol, *n*-propanol and *i*-butanol were responsible for the principal aroma of this alcoholic beverage. In order to improve the quality of *brem bali*, the use of pure starter culture such as ethanol producing yeast, lactic acid bacteria and amylolytic micro-organisms are greatly recommended.

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Table 1. Biochemical characteristics of *brem bali*

Compounds		Compounds	
Glucose (%w/v)	27.8	pH	3.8
Ethanol (%w/v)	8.6	Titratable acidity*	5.4
Volatile compounds (ppm)	436.5	Organic acids (mM)	111.5
η-propanol	68.0	lactic acid (mM)	90.5
i-butanol	135.2	succinic acid	2.0
i-amyl alcohol	160.4	acetic acid	15.9
i –amyl acetate	trace	tartaric acid	0.8
un	58.9	citric acid	0.1
un	14.0	fumaric acid	1.1

Un : unidentified compound
*Titratable acidity was expressed in ml of 0.02 NaOH/ml
Data are the means of two time replications

Table 2. Sensorial characteristics of *brem bali*

Fruity aroma	Flavor richness	Sourness	Sweetness
3.4 ± 0.1	4.4 ± 0.1	3.9 ± 0.2	4.8 ± 0.1

The test was carried out at 27°C
The data are average ± standard error (SE) out of 13 panelists

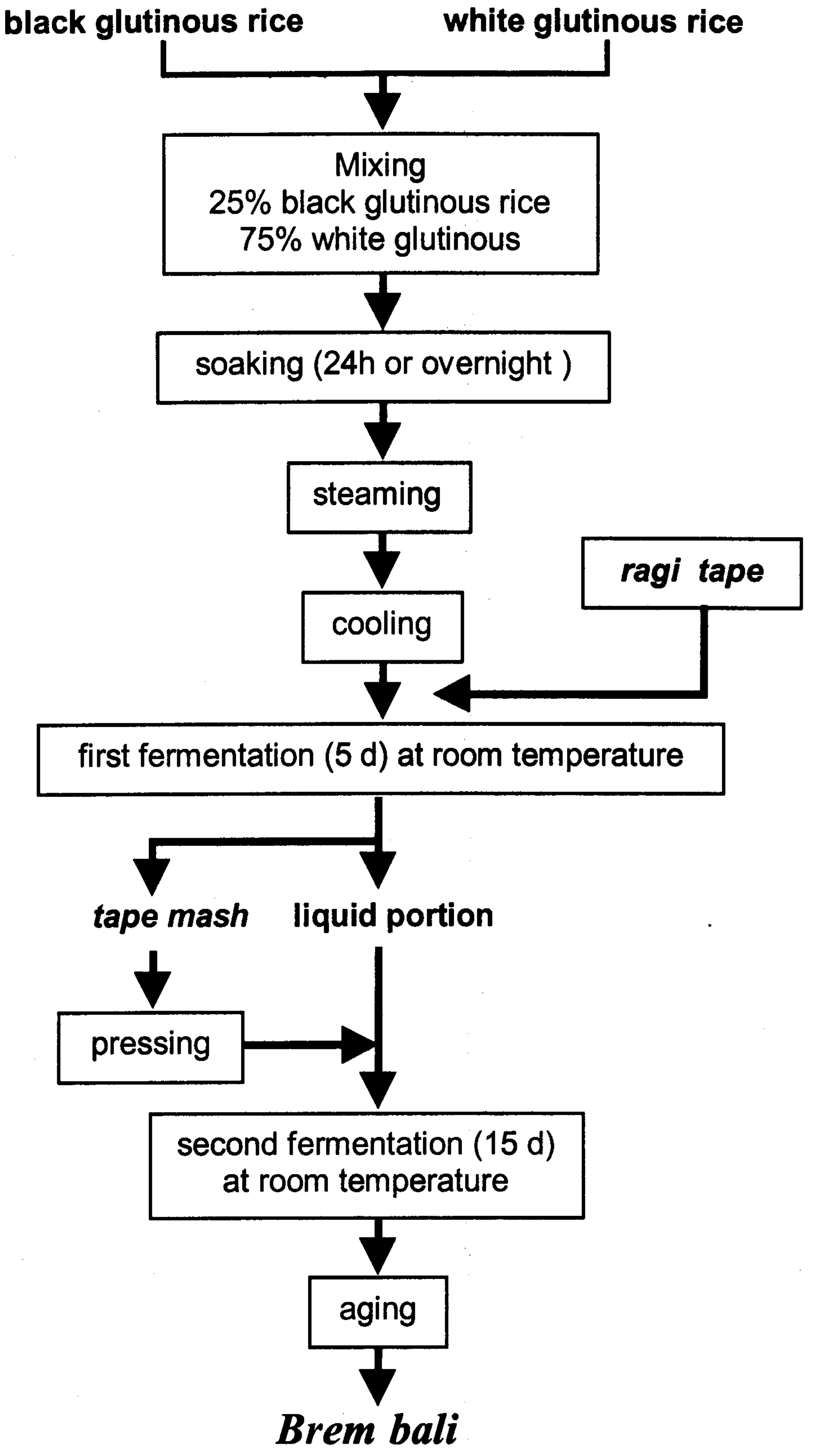

Figure 1. General procedure of the traditional fermentation of *brem bali*.

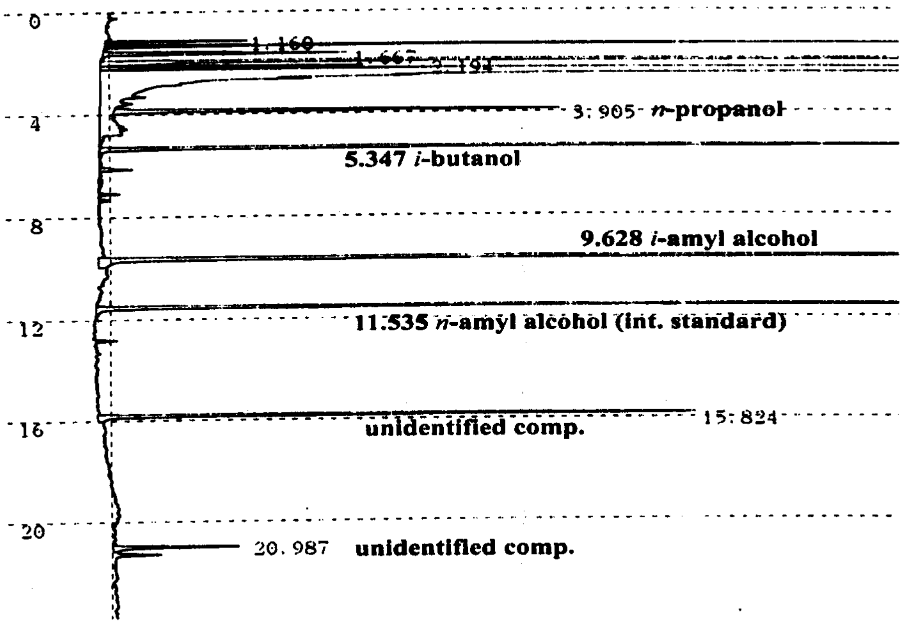

Figure 2. The volatile compounds of *brem bali* determined using headspace gas chromatography.

QUESTIONAIRE FOR STRUCTURED SCALE

PRODUCT : *Brem bali* (Balinese rice wine)
NAME : DATE :

Evaluated the fruity aroma, flavor richness, sourness and sweetness of the *brem bali*. Indicate the value of each item based on the scale below

	Characteristics			
Scale	Fruity aroma	Flavor richness	Sweetness	Sourness
6	Extremely fruity	Extremely rich	Extremely sweet	Extremely sour
5	Very fruity	Very rich	Very sweet	Very sour
4	Moderately fruity	Moderately rich	Moderately sweet	Moderately sour
3	Slightly fruity	Slightly rich	Slightly sweet	Slightly sour
2	Trace of fruitiness	Trace of richness	Trace of sweetness	Trace of sourness
1	Not fruity	Not rich	Not sweet	Not sour

Comments :

Figure 3. The questionaire used to determine the sensorial characteristics of *brem bali*.