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ETHANOL FERMENTATION WITH *SHOCHU* DISTILLERY WASTE AND AROMATIC RED RICE BRAN

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Shochu distillery waste which had been exhausted by a vacuum-distillation procedure at low temperature (35–40°C) was used for secondary ethanol fermentation with the bran of aromatic red rice (_Oryza sativa_ var. _Indica, Tapol_). The filtrate of the fermented mash made from _kome shochu_ distillery waste and aromatic red rice bran had a characteristic wine-like red color, contained about 12% ethanol (v/v), and possessed a fine aroma that was fortified with the aromas of higher alcohols and volatile esters during secondary ethanol fermentation. A novel red alcoholic beverage was produced from the industrial by-product of _shochu_-making, _kome shochu_ distillery waste and a by-product of _Sekihan_-cooking, aromatic red rice bran. The filtrate of the fermented mash had a characteristic absorbance at 530 nm. As the amounts of aromatic red rice bran in the mash were increased, the absorbance at 530 nm increased. Thus, a novel system utilizing _shochu_ distillery waste, which is conventionally treated as wastewater, and aromatic red rice bran was established economically using a simple vacuum-distillation and secondary ethanol-fermentation procedure without the need for any complicated or expensive processes.

Key Words: *Distillery waste, aromatic red rice, ethanol fermentation, rice bran, shochu*

Introduction

As food industries and bioindustries develop, treatment of industrial by-products and industrial wastes are causing increasingly serious problems.

Details have previously been reported both batchwise[18] and semicontinuous[12] ethanol fermentation with *shochu* distillery waste. A novel vacuum-distillation procedure has been applied at 35–40°C to *shochu* distillation to utilize the viable yeast and various enzymatic activities contained in the distillery waste as part of an attempt to reuse the distillery waste for secondary ethanol fermentation with carbon sources for production of useful products, such as alcoholic beverages, foods, and so on. *Shochu* distillery waste has been used in a semicontinuous ethanol fermentation to establish a suitable system for the utilization of distillery waste.

The application of aromatic red rice (*Oryza sativa* var. *Indica, Tapol*) to the brewing of rice wine[17] has been reported. Aromatic red rice wine produced by uncooked ethanol fermentation of aromatic red rice had a characteristic wine-like colour and aroma. The red colour originated from the anthocyan pigment contained in the bran layer of the red rice[20].

In this work *shochu* distillery waste and aromatic red rice bran[20] have been used in an attempt to produce a novel type of alcoholic beverage, with the idea of developing a systematic recycling system for the food industry using biotechnological methods.

Materials and Methods

Shochu Distillery Waste

Shochu distillery waste generated at Tohi Jozo Co. Ltd., Kumamoto, was used for the experiments. Distillation of *kome* (rice) and *mugi* (barley) *shochu moromi* (fermented mash) was performed at 35–40°C in vacuo.

Aromatic Red Rice Bran

The bran of aromatic red rice (*Oryza sativa* var. *Indica, Tapol*) was kindly donated by Kumamoto Agricultural Research Institute.

Procedure for Batchwise Secondary Ethanol Fermentation with Shochu Distillery Waste and Aromatic Red Rice Bran

Shochu distillery waste (200 ml), various amounts of aromatic red rice bran and glucose (40 g) were dispensed into a 300-ml Erlenmeyer flask with a gas trap. The composition of the initial mash is shown in Table 1.

Fermentation without cooking was performed at 30°C. The decrease in weight of the Erlenmeyer flask and its contents, equivalent to the amount of CO_2 gas evolved, was measured every 24 h[14].

Measurement of Ethanol

Ethanol was measured as described previously[18] using a gas chromatograph (model GC-14A; Shimadzu Co., Kyoto) equipped with a 3.1-m PEG-HT column (Gaskuro Kogyo Inc., Tokyo).

Measurement of Enzymatic Activity

To determine glucoamylase activity[17], 1 ml of a 2% solution of soluble starch and 0.2 ml of 0.2 M acetate buffer (pH 3.5) were mixed and incubated at 40°C for 5 min. Then, 0.1 ml of a suitably diluted solution of glucoamylase was added to the mixture, and it was again incubated at 40°C.

TABLE I. Composition of the initial mash prepared with *kome shochu* distillery waste and aromatic red rice bran

Run		1	2	3	4	5
Kome shochu distillery waste	(ml)	200	200	200	200	200
Glucose	(g)	40	40	40	40	40
Aromatic red rice bran	(g)	0	1	5	10	20

After a 20 min incubation, 0.1 ml of NaOH was added to stop the enzymatic reaction. Five ml of 0.2 M Tris-maleic acid buffer were added to the mixture which was then boiled (100°C) for 5 min. After cooling, the pH of the mixture was adjusted to 7.0 with 1 N HCl. The amount of glucose contained in the reaction mixture was measured with "Glucose C test Wako" (Wako Pure Chemical Co. Ltd., Osaka). One unit of glucoamylase activity was defined as the amount of enzyme that released 1 mg of glucose/ml/60 min at 40°C.

Proteolytic activity was determined with casein (Hammersten, Merck Co. Ltd., Germany) as substrate by the method of Tsuru *et al.*[2,11].

β-Glucosidase activity was measured by the methods of Mega *et al.*[6] and Paus and Christensen[9] with *p*-nitrophenyl-β-D-glucopyranoside (Seikagaku Kogyo Co. Ltd., Tokyo) as substrate.

General Analytical Methods

Reducing sugars in the hydrolysate were quantitated by the micro-Bertrand method[1]. Aroma analysis of the filtrate of fermented mash was performed by gas chromatography[18]. Organoleptic tests were also performed, using more than 10 persons in our laboratory, to evaluate the various filtrates of the fermented mash[17].

Spectrophotometric analysis of various filtrate of the fermented mash was performed with a spectrophotometer (model DU-7; Beckman, U.S.A.).

Results

Batchwise Secondary Ethanol Fermentation with Shochu *Distillery Waste and Aromatic Red Rice Bran*

Batchwise secondary ethanol fermentation was effectively achieved with a mash composed of *kome shochu* distillery waste, various amounts of red rice bran, and glucose (Figure 1). Since the distillery waste contained a large number of viable yeast cells, ethanol fermentation advanced spontaneously for the first 2 d and ethanol fermentation of the mash was completed in 3–4 d.

Because of the viable yeast present in the distillery waste and the acidity of the distillery waste, ethanol fermentation was achieved without any contamination.

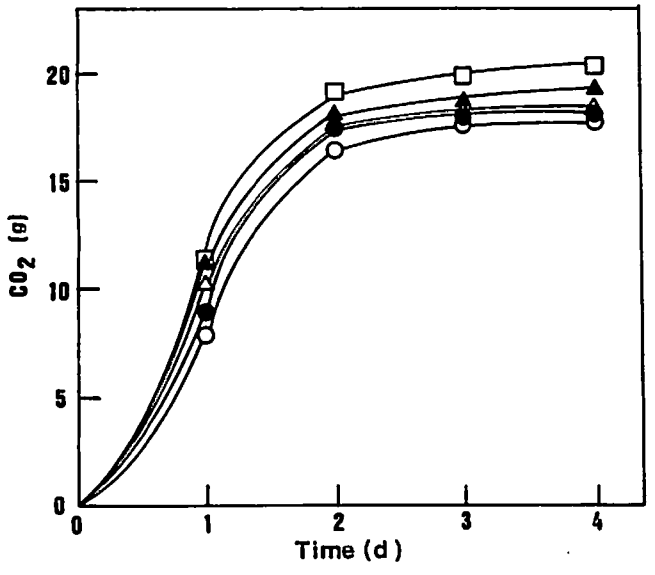

FIG. 1. Time courses of the fermentation of mashes prepared with *kome shochu* distillery waste and aromatic red rice bran. Symbols: ○, 40 g of glucose was used as the carbon source; ●, 1 g of aromatic red rice bran and 40 g of glucose; △, 5 g of aromatic red rice bran and 40 g of glucose; ▲, 10 g of aromatic red rice bran and 40 g of glucose; □, 20 g of aromatic red rice bran and 40 g of glucose. Fermentation was performed at 30°C.

Characteristics of the Filtrate of the Fermented Mash Generated from Aromatic Red Rice Bran and Distillery Waste

Table II shows that the final pH and the acidity of the various filtrates of secondarily fermented *kome shochu* distillery waste were around 4.2 and 10.2, respectively. About 12% (v/v) ethanol was produced. The aroma of the distilley waste was fortified with ethanol, higher alcohols and esters, and was dramatically improved during secondary ethanol fermentation as judged both by gas-chromatographic analysis (Table III) and organoleptic testing. Compared with the distillery waste, large amounts of isobutyl alcohol, isoamyl alcohol and ethyl acetate were found in the filtrate of the secondarily fermented *kome shochu* distillery waste. The quality in term of aroma of the secondarily fermented *kome shochu* distillery waste was much higher than that of distillery waste.

The alcoholic beverage prepared from the filtrate of secondarily fermented *kome shochu* distillery waste had a characteristic wine-like red colour and a wine-like aroma according to the results of organoleptic tests. Characteristic absorbance at 530 nm was observed upon spectrophotometric analysis (Figure 2). As the amounts of aromatic red rice bran in the mash were increased, the absorbance at 530 nm increased. The filtrate of run 5, made from 200 ml of *kome shochu* distillery waste, 20 g of aromatic red rice bran and 40 g of glucose had a conspicuous peak at 530 nm. Figure 3 shows pictures of the various alcoholic beverages made from *kome shochu* distillery waste and the bran of aromatic red rice. The alcoholic beverage from run 1 was almost colourless, but the alcoholic beverage from run 5 had a characteristic wine-like ruby colour.

Batchwise secondary ethanol fermentation was also performed satisfactorily with *mugi shochu* distillery waste, aromatic red rice bran and glucose (data not shown). The quality of the filtrate from *mugi shochu* distillery waste and aromatic red rice bran was inferior to that of the filtrate generated from *kome shochu* distillery waste and aromatic red rice bran.

Table IV shows the various enzymatic activities present in the *kome shochu* distillery waste and in secondarily fermented *kome shochu* distillery waste (run 5). About 25% of the original glucoamylase activity and 50% of the original acid protease activity remained in the secondarily fermented *kome shochu* distillery waste. β-Glucosidase activities in the filtrate of the fermented broth varied from 0.11–0.17 U/ml. The residual cake generated from the secondarily fermented distillery waste, containing ethanol, red pigment and aromatic substances, had fine characteristics in terms of its aroma, and it could serve the same purpose as conventional *sake-kasu*.

Discussion

The quality of the filtrate from secondarily fermented *kome shochu* distillery waste that had been incubated with aromatic red rice bran was much better than that of unfermented distillery waste. The aroma of the distillery waste was fortified with those of ethanol, higher alcohols, and esters, and most of the unacceptable odour of the distillery waste was masked or eliminated during ethanol fermentation.

Many reports have been published on treatment of *shochu*[3,4], beer[10] and spirit[8] wastes. Valuable products, such as saccharifying enzyme, can be produced from *shochu* distillery wastewater[7].

Attempts were made to develop a system for utilization of by-products from the food industry, *e.g. shochu distillery* waste[12,18], aromatic red rice bran[20] and soybean cooked syrup[5]. We have also tried to develop novel alcoholic beverages and novel fermented foods, namely, aromatic red rice

TABLE II. Characteristics of various fermented mashes

Run	1	2	3	4	5	Distillery waste (*kome shochu*)
Final pH	4.1	4.1	4.2	4.2	4.3	4.0
Acidity (ml)	10.5	10.2	10.1	10.0	10.2	9.9
Reducing sugar in filtrate (mg/ml)	1.1	0.6	0.3	0.2	0.2	ND[a]
Total sugar in filtrate (mg/ml)	5.5	4.0	2.5	3.5	4.6	ND
Total sugar in residue (g)	23.2	24.2	24.1	30.3	35.0	1.9

[a] ND, Not detected.

TABLE III. Analysis of the aromas of various fermented mashes

Run		1	2	3	4	5	Distillery waste (*kome shochu*)
Ethyl alcohol	(%, v/v)	11.4	11.4	11.6	11.6	12.5	—
Isobutyl alcohol	(ppm)	183	240	166	179	133	11
Isoamyl alcohol	(ppm)	305	379	317	327	255	40
n-Propyl alcohol	(ppm)	23	30	28	31	22	2
Acetaldehyde	(ppm)	485	601	554	357	220	0.4
Ethyl acetate	(ppm)	212	268	276	337	288	10
Ethyl lactate	(ppm)	0.5	0.6	0.8	1.1	ND[a]	ND
Isoamyl acetate	(ppm)	9.2	10.2	9.0	11.4	10.3	1.6

[a] ND, Not detected.

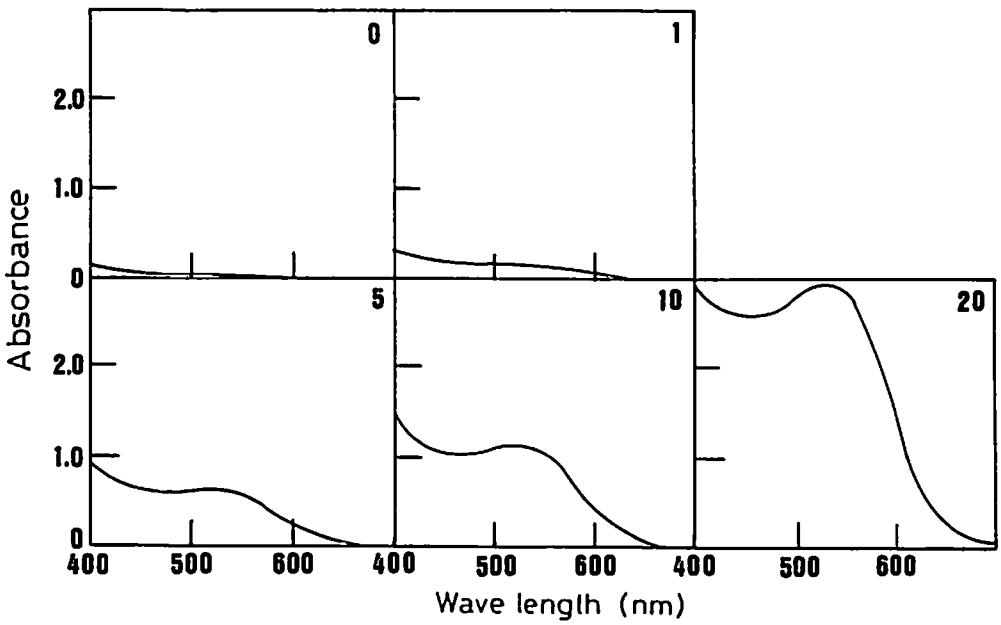

FIG. 2. Spectrophotometric analysis of various filtrates generated from *kome shochu* distillery waste and aromatic red rice bran.
Absorption curve of filtrate generated from the mash used 40 g of glucose as the carbon source (0);
1 g of aromatic red rice bran and 40 g of glucose (1);
5 g of aromatic red rice bran and 40 g of glucose (5);
10 g of aromatic red rice bran and 40 g of glucose (10);
20 g of aromatic red rice bran and 40 g of glucose (20).

wine made from aromatic red rice[15,16,17], sprouting rice wine made from germinating rice seed[13,19] and soybean cooked-syrup cheese made from soybean cooked syrup[5].

In the present work attempts were made to utilize *shochu* distillery waste and aromatic red rice bran directly for secondary ethanol fermentation to produce a red-coloured alcoholic beverage. We developed a novel system for utilizing *shochu* distillery waste and aromatic red rice bran without the need for any complicated processes. In this system, these by-products of the food industry were directly applied to ethanol fermentation.

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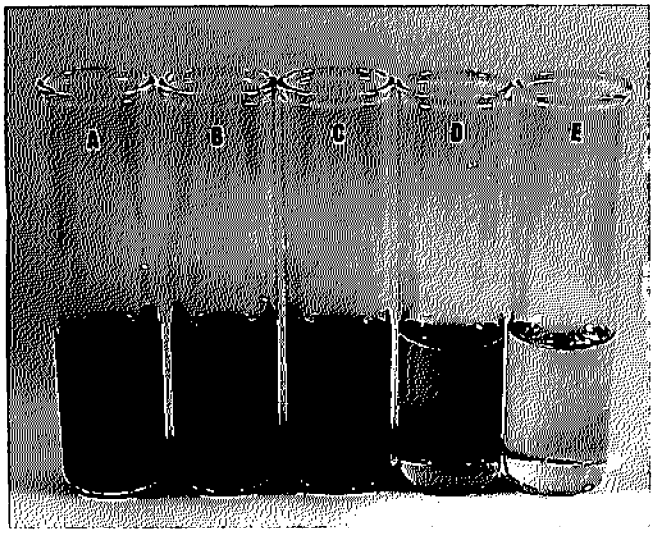

FIG. 3. Pictures of various filtrates generated from *kome shochu* distillery waste and aromatic red rice bran.
Filtrate generated from the mash used 20 g of aromatic red rice bran and 40 g of glucose (A);
10 g of aromatic red rice bran and 40 g of glucose (B);
5 g of aromatic red rice bran and 40 g of glucose (C);
1 g of aromatic red rice bran and 40 g of glucose (D);
40 g of glucose (E).

TABLE IV. Enzymatic activities of various fermented mashes

Run	1	2	3	4	5	Distillery waste (*kome shochu*)
Glucoamylase (U/ml)	30.9	30.8	30.0	26.6	22.0	88.6
Acid protease (U/ml)	2380	2360	2444	1621	1848	3500
β-Glucosidase (U/ml)	0.17	0.17	0.17	0.14	0.11	0.11

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