

EFFECTS OF β -GLUCOSIDASE ACTIVITY ON THE CHARACTERISTICS OF AROMATIC RED RICE WINE

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Received 24 August 1993

The pigment in the bran layer of aromatic red rice (*Oryza sativa* var. *Indica*, *Tapol*) is rather stable, has a characteristic red color just like that of grape wine and has a peak of absorbance at 530 nm at an acidic pH. A commercial saccharifying agent, glucoamylase AN-2, produced by *Aspergillus niger* was fractionated to separate glucoamylase and β -glucosidase activities by column chromatography on CM Sephadex C-50. The red rice wine made from uncooked, unpolished aromatic red rice using the fractionated, β -glucosidase-free preparation of glucoamylase had a characteristic red color. By contrast, red rice wine made with glucoamylase AN-2, which contained β -glucosidase activity, was inferior in color. The red pigment of aromatic red rice wine was decolorized and glucose originating from the red pigment was released by enzymatic digestion with the fractionated preparation of β -glucosidase. The partial decolorization of aromatic red rice wine was ascribed to the enzymatic action of β -glucosidase that was present in glucoamylase AN-2. Thus β -glucosidase activity has an undesirable effect on the brewing of aromatic red rice wine.

Key Words: Aromatic red rice (*Oryza sativa* var. *Indica*, *Tapol*), red rice wine brewing, ethanol fermentation without cooking, β -glucosidase, effects of β -glucosidase on rice wine brewing

INTRODUCTION

During the fermentation process, various enzymatic activities in the broth or mash play important roles. It has been previously reported that the quality of aromatic red rice wine made with a commercial saccharifying agent, glucoamylase AN-2, which is produced by *Aspergillus niger*, was inferior in color as compared with that of an aromatic red rice wine made with Sumizyme, a preparation of glucoamylase produced by *Rhizopus* sp.¹¹

There are many reports dealing with β -glucosidase. Ohta *et al.* reported that β -glucosidase produced by koji mold is closely correlated with aroma formation during the ethanol fermentation of *shochu moromi*². It was also reported that the β -glucosidase produced by lactic acid bacteria hydrolyzes the isoflavone glycoside in soybeans during preparation of soybean cooked-syrup cheese³.

In this study, a commercial preparation of glucoamylase was fractionated by column chromatography to isolate the β -glucosidase fraction and the mechanism for decolorization of red rice wine by β -glucosidase during the uncooked ethanol-fermentation process^{2,7,8,9} was investigated.

MATERIALS AND METHODS

Rice grains

Uncooked, unpolished grains of aromatic red rice (*Oryza sativa* var. *Indica*, *Tapol*), which contained a red pigment in the bran layer, were ground to particles of 2–3 mm in diameter and used as materials for the fermentation test.

Preparation of enzymes

Glucoamylase AN-2 produced by *Aspergillus niger* (Shinnihon Kagaku Kogyo Co. Ltd., Anjo) was used as the saccharifying agent for ethanol fermentation.

Column chromatography on CM Sephadex C-50

Glucoamylase AN-2 was fractionated on a column (50 mm i.d. \times 500 mm) of CM Sephadex C-50 that had been equilibrated with 0.05 M sodium acetate buffer, pH 4.0. Elution was performed with a linear gradient of 0 to 0.4 M NaCl in 0.05 M sodium acetate buffer, pH 4.0.

Ethanol fermentation with the fractionated preparation of glucoamylase

Thirty grams of uncooked, unpolished aromatic red rice, 3 g of compressed baker's yeast and 0.2 g of glucoamylase AN-2 (run 1) or 100 ml of the fractionated, β -glucosidase-free preparation of glucoamylase (1.5×10^4 U); (run 2) were dispensed into a 300-ml Erlenmeyer flask with a gas trap¹¹.

The pH of the initial mash was adjusted to 3.5 with 1 N HCl and 1 N NaOH. Ethanol fermentation was performed at 30°C. The decrease in weight of the entire Erlenmeyer flask and its contents, as a result of the evolution of CO₂ gas, was recorded at 24-h intervals.

Assay of enzyme activity

β -Glucosidase activity was measured by the method of Mega *et al.*⁴ and Paus and Christensen⁵. Glucoamylase activity was measured as previously described¹¹.

Extraction and spectrophotometric analysis of red pigment

Extraction of the fat fraction from the aromatic red rice bran was performed with diethyl ether (Nacalai Tesque, Inc., Kyoto) at 40–50°C for 8 h in a Soxhlet apparatus. One gram of defatted bran was extracted with 100 ml of 0.1 N HCl/95% ethanol at 25°C for 2 d. Five ml of McIlvaine buffer (pH 2.0–5.0) were added to 1.5 ml of the solution of extracted pigment and the mixture was analyzed with a Beckman model DU-7 spectrophotometer.

Decolorization test

Thirty grams of uncooked, unpolished aromatic red rice, 20 g of glucose, 3 g of compressed baker's yeast and 100 ml

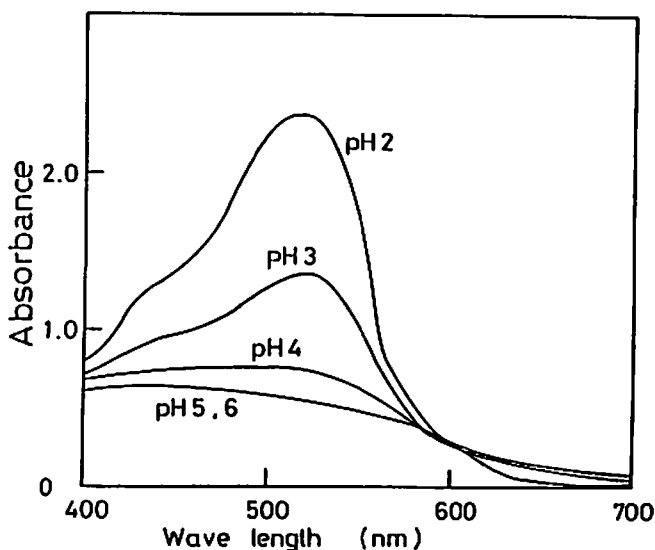


Fig. 1. Absorption curves of the red pigment contained in the bran layer of aromatic red rice.

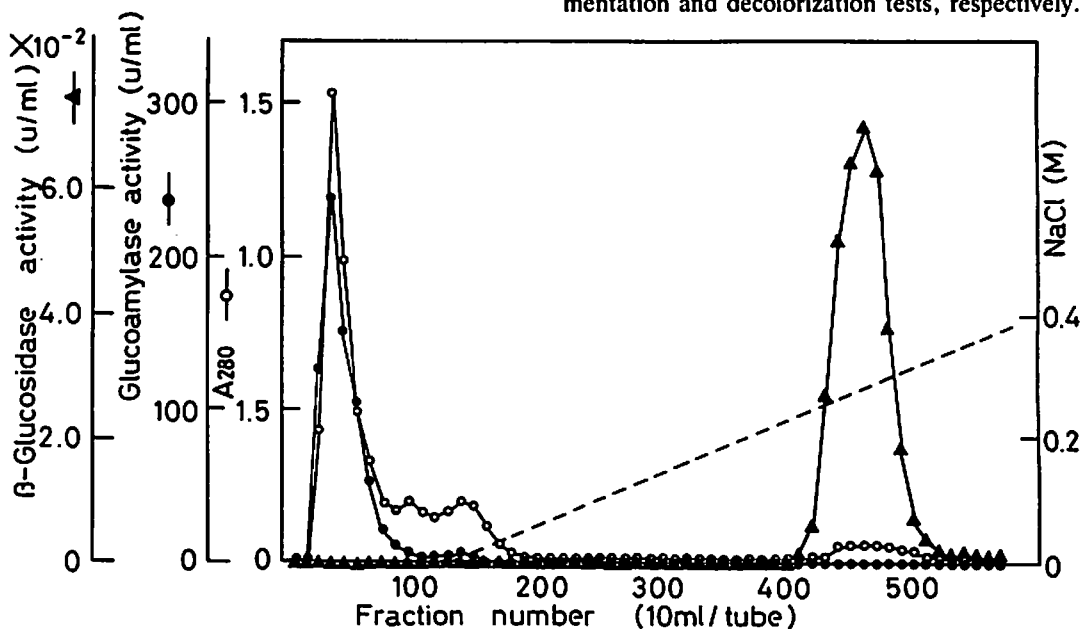


Fig. 2. Column chromatography on CM Sephadex C-50 of glucoamylase AN-2 produced by *Aspergillus niger*.

Symbols: ○, absorbance at 280 nm;
●, glucoamylase activity (U/ml);
▲, β-glucosidase activity (U/ml);
..., NaCl concentration.

Experimental details are described in the text.

of tap water were dispensed into a 300-ml Erlenmeyer flask with a gas trap. Ethanol fermentation was performed at 30°C. After filtration, 20 ml of the resultant red rice wine were mixed with 5 ml of various solutions of β-glucosidase, namely, the fractionated preparation of β-glucosidase (0.3 U/5 ml), a 0.1% solution of glucoamylase AN-2 (0.3 U/5 ml), a 1% solution of glucoamylase AN-2 (3.0 U/5 ml), or deionized water as the control.

The various reaction mixtures were incubated at 30°C. At suitable intervals, 4 ml of each reaction mixture were filtered through a 0.3-μm nitrocellulose filter (Advantec Toyo Co., Ltd., Tokyo). Absorbance at 530 nm (A_{530}) of the filtrate was measured in a Beckman model DU-7 spectrophotometer, and the amount of glucose in the filtrate was measured by a Glucose C Test Wako (Wako Pure Chemical Industries Ltd., Osaka).

Analysis of ethanol and aroma

Ethanol and volatile aromatic components of rice wines were analyzed on a Shimadzu model GC-14A gas chromatograph equipped with a 3.1 m PEG-HT column (Gasukuro Kogyo Inc., Tokyo), as previously described¹¹.

RESULTS

Spectrophotometric analysis of red pigment

Figure 1 shows the absorption curves of the red pigment contained in the bran layer of aromatic red rice (*Oryza sativa* var. *Indica*, *Tapol*). Characteristic absorbance of the red pigment at 530 nm was observed at an acidic pH. The peak at 530 nm of the red pigment was not observed at pH 5.6.

Column chromatography on CM Sephadex C-50

Glucoamylase activity was collected in the flow-through fraction from the column (Figure 2). β-glucosidase activity was adsorbed to the column of CM Sephadex C-50 that had been equilibrated with 0.05 M sodium acetate buffer, pH 4.0, and was eluted with a linear gradient of 0 to 0.4 M NaCl in 0.05 M sodium acetate buffer, pH 4.0. The resultant glucoamylase and β-glucosidase fractions were used for fermentation and decolorization tests, respectively.

Ethanol fermentation with the glucoamylase fraction

The fermentation rate of the mash that contained glucoamylase AN-2 (run 1) was higher than that of the mash that contained the fractionated glucoamylase (run 2) (Figure 3). The final amounts of CO₂ generated from the mashes in runs 1 and 2 were almost the same. Ethanol fermentation of the mashes in runs 1 and 2 was completed in 4 and 10 d.

Properties of aromatic red rice wine

Spectrophotometric analysis of the rice wines from runs 1 and 2 was performed (Figure 4). The rice wine made with the fractionated, β-glucosidase-free preparation of glucoamylase (run 2) had a characteristic peak of absorbance at 530 nm. By contrast, the characteristic absorbance at 530 nm was not observed in the rice wine made with glucoamylase AN-2

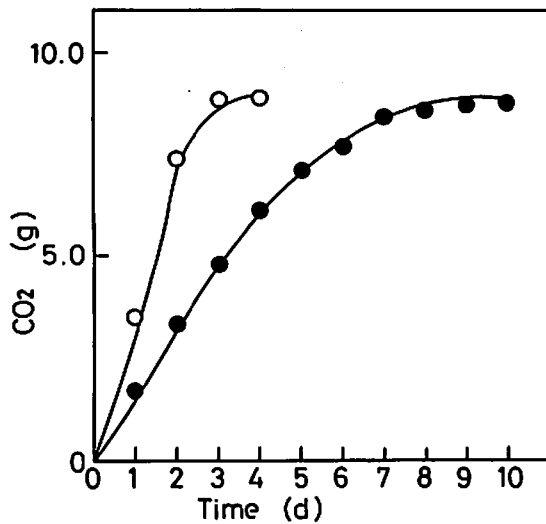


Fig. 3. Time courses of fermentation of mashes that contained glucoamylase AN-2 or the fractionated preparation of glucoamylase.

Symbols: ○, 0.2 g of glucoamylase AN-2 (run 1); ●, fractionated preparation of glucoamylase (run 2).

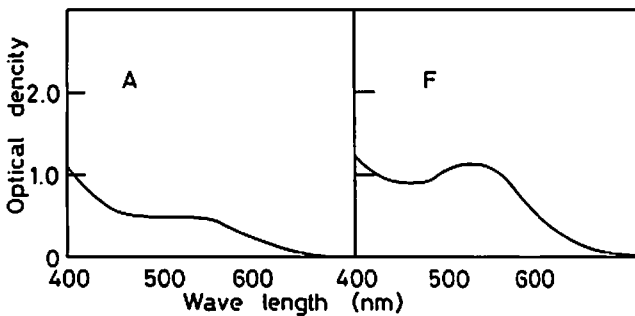


Fig. 4. Absorption curves of aromatic red rice wines made with glucoamylase AN-2 and the fractionated preparation of glucoamylase.

Rice wine made with glucoamylase AN-2 (A) and rice wine made with the fractionated preparation of glucoamylase (F).

(run 1). The red color of the rice wine from run 2 was more intense than that of the rice wine from run 1.

Decolorization test

The ruby-red color of aromatic red rice wine was dramatically decolorized by the glucoamylase preparation that contained β -glucosidase activity (3.0 U/5 ml); (Figure 5). The fractionated preparation of β -glucosidase (0.3 U/5 ml) also decolorized the aromatic red rice wine. As the red pigment contained in the red rice wine disappeared, glucose residues were released. The red pigment of the rice wine was stable in the absence of added enzyme at 30°C for 24 h.

DISCUSSION

It has been previously reported that ethanol fermentation of uncooked, unpolished aromatic red rice grain is essential for the production of aromatic red rice wine that is of refined quality, both in terms of aroma and color¹⁰. It has also reported that the preparation of glucoamylase used in the ethanol fermentation of aromatic red rice without cooking plays an important role not only in saccharification of raw starchy material but also in formation of the aroma during the brewing of aromatic red rice wine¹¹.

The commercial saccharifying agent, glucoamylase AN-2, was fractionated to yield glucoamylase and β -glucosidase

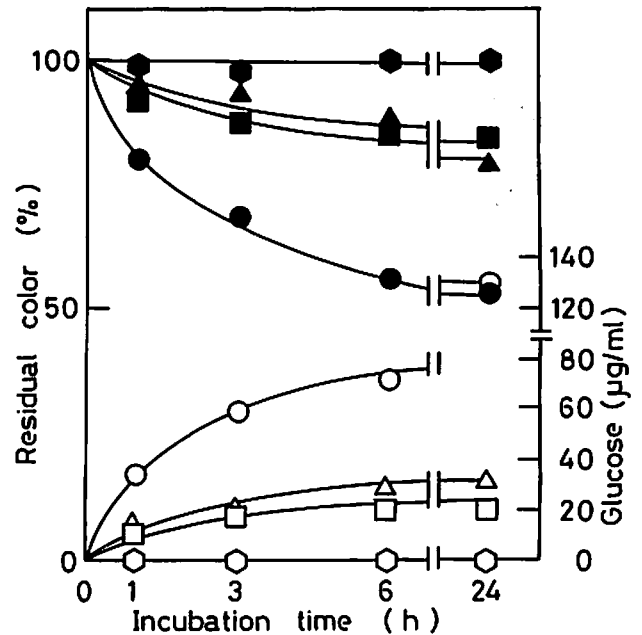


Fig. 5. Effects of various preparations of glucoamylase and β -glucosidase on the color of aromatic red rice wine.

Symbols: ●, absorbance at 530 nm of wine incubated with 1.0% glucoamylase AN-2; ▲, with 0.1% glucoamylase AN-2; ■, with the fractionated preparation of β -glucosidase; ●, with deionized water; ○, amount of glucose in the wine incubated with 1.0% glucoamylase AN-2; △, with 0.1% glucoamylase AN-2; □, with the fractionated preparation of β -glucosidase; ○, with deionized water.

activities by column chromatography on CM Sephadex C-50. The aromatic red rice wine made with the fractionated, β -glucosidase-free preparation of glucoamylase had the characteristic ruby-red color of grape wine. By contrast, the red pigment of aromatic red rice wine was actually decolorized and glucose residues were released by the action of the fractionated preparation of β -glucosidase. The red pigment of aromatic red rice wine was partially destroyed by the β -glucosidase that was present in glucoamylase AN-2.

The pigment contained in the bran layer of aromatic red rice is stable and has potential application to the brewing of red-colored alcoholic beverages and it is available as an additive for the food industry. In practice, for brewing of aromatic red rice wine β -glucosidase activity should, however, be removed from preparations of glucoamylase or β -glucosidase-free preparations of enzyme should be used as saccharifying agents in order to retain a vivid ruby-red color.

Acknowledgments. The authors are grateful to the Kumamoto Agricultural Research Institute for generously providing aromatic red rice and to Shinnihon Kagaku Kogyo Co. Ltd. for generously providing the preparations of glucoamylase. The authors also thank Bishonen Shuzo Co. Ltd. for support during this study.

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