Reducing the Stale Flavor of Cooked Rice by Treating with Cells of Acetic Acid Bacteria

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Japonica-type rice grains stored at 40°C for 2 months were treated with a freeze-dried powder of acetic acid bacteria and then cooked. It was found that the originally existing n-hexanal were decreased by more than 35%. Moreover, the stale flavor had been significantly reduced compared to that of non-treated rice grains. Simultaneous treatment with freeze-dried cells of the acetic acid bacteria and a surfactant was 1.3-1.9 times more effective for reducing the amount of n-hexanal than with the cells of acetic acid bacteria alone.

The optimum conditions for reducing the stale flavor were investigated by using a combination of the acetic acid bacterial cells and the surfactant. The optimum conditions were as follows: soaking temperature, 50°C; soaking time, 30-180 min; pH 4.5-7.0, optimally at pH 6.0.

Treating Indica-type rice grains with the freeze-dried cells of acetic acid bacteria was also effective for reducing the specific off-flavor of the rice.

Rice is the principal foodstuff in Japan, and it is known that the quality of rice, especially its flavor, is easily damaged by oxidative deterioration during storage.

The trigger for the deterioration of rice flavor is a change of the spherosome in the aleurone cells. Most of the neutral lipids of rice grains exist in the spherosome of embryos and aleurone cells which are surrounded by a double monolayer of phospholipid.1)

When the spherosome membrane is broken during storage, the neutral lipids are hydrolyzed by lipase which is located in the aleurone cells, allowing an increase of free fatty acids. The free unsaturated fatty acids thus produced are further converted into low-molecular-weight volatile products via hydroperoxides by the action of lipoygenase and succeeding autoxidation.2) Yasumatsu et al.3) have elucidated n-hexanal to be the main component of the stale flavor (komai-shu) which is formed from unsaturated fatty acids.

In an earlier study on the elimination of this stale flavor, Yamamoto et al.4) have reported that the addition of L-lysine hydrochloride to boiling rice was effective. However, their method resulted in some differences among the kinds of rice grains with respect to eliminating the effect of the stale flavor.

Our previous paper5) has reported a new method for reducing the off-flavor originating from such aldehydes as n-hexanal by using the cells of acetic acid bacteria which oxidize aldehydes into their corresponding carboxylic acids, whose threshold level is much higher than that of the aldehydes.

In this present paper, the use of acetic acid bacteria to reduce the stale flavor of rice is examined in more detail.

Materials and Methods

Materials. Commercially available Japonica-type milled rice grains stored at 40°C for 2 months and Indica-type milled Thailand rice grains were used in this study. Rice powder was prepared from the rice grains by grinding each type of rice grains with a caking machine and then passing through a 710-μm sieve. Sugar ester, monoglyceride, and sorbitan mono-ester were obtained from commercial sources and used as food additives for their nonionic surfactant action. The other reagents were obtained from commercial sources and were of analytical grade.

Microorganisms. The bacterial strains used were Acetobacter acetii IFO 3284 and Glucomacter suboxydans IFO 12528. The medium and cultivation conditions for the bacteria were the same as those described in the previous paper.3) Harvested wet cells (35 g) were dispersed in 250 ml of distilled water, and the solution was lyophilized to make a freeze-dried powder.

Cooking the rice. The rice grains were cooked by the following procedures.

1) Standard method. Five grams of milled rice grains were washed in tap water and placed in a 50-ml glass tube (3.5 × 10.5 cm), 2 mg of the cells of acetic acid bacteria and 7.5 ml of distilled water then being added to the tube. The glass tube was immediately sealed with a silicon stopper. After soaking at 50°C for 1 h, the glass tube was kept at 90°C for 15 min to complete the cooking process. A similar procedure was carried out in the absence of acetic acid bacteria for the control experiment.

2) Cooking in an electric rice cooker. Three hundred and ten grams of milled rice grains were washed with water, soaked in 460 ml of distilled water containing 120 mg of the acetic acid bacterial cells at 50°C for 1 h and then cooked in an electric rice cooker. A similar procedure without the bacterial cells was used as a control.

Determination of n-hexanal. n-Hexanal was determined as the 2,4-dinitrophenylhydrazone derivative by high-performance liquid chromatography (HPLC) according to the method of Matoba et al.6) N-Octanal was used as the internal standard.

Twenty-five ml of n-hexane, 20 ml of 0.1% 2,4-dinitrophenylhydrazine in 1.1 n H3PO4, 0.3 n HCl, 0.1 ml of a 50-ppm internal standard and 12 g of cooked rice grains were put into a 50-ml glass tube with a stopper as already described. The mixture was shaken for 1 h at room temperature. After centrifuging at 3000 rpm for 5 min at 25°C, the n-hexanal layer was collected, washed with distilled water, and then evaporated to dryness under reduced pressure. The resulting residue was dissolved in 0.5 ml of methanol for an HPLC analysis, which was carried out by using Shimadzu SPD-6A-EV equipment with a UV detector. ODS (4.6 × 250 mm) was used with a protective column, and elution was by 75:24:1 of acetonitrile–water–tetrahydrofuran (v/v/v). The temperature of the column was 25°C, the flow rate was 1.0 ml/min, the sample injection volume was 2 μl, and the eluate was detected by absorption measurement at 350 nm.
Enzyme activity of aldehyde dehydrogenase (ALDH). Enzyme activity was assayed by the method of Wood et al.,39 using potassium ferricyanide as an electron acceptor as described in the previous paper.93

Sensory evaluation. The flavor of cooked rice grains was evaluated by 10 members of our laboratory according to Yoshikawa's methods.93 Scores of 5 and 5 are for the highest and lowest in quality in comparison with the reference. Significant differences between the reference data and those of the samples (acetic acid bacteria-treated) were analyzed by a t-test. Untreated Japonica and Indica Thailand rice grains were used as references.

Results and Discussion
Reduction of the stale flavor of Japonica-type rice grains by the freeze-dried cells of acetic acid bacteria
Japonica-type rice grains stored at 40°C for 2 months, the same samples treated with the cells, and Japonica-type rice grains stored in a refrigerator for 2 months (reference) were cooked as described in the Materials and Methods section and evaluated by the sensory test. The results are shown in Table I. The sensory scores for the cooked rice grains stored at 40°C for 2 months were inferior to the reference in terms of their appearance, flavor, taste, stickiness, and total judgement. All the sensory parameters were significantly different from the reference at the 1% or 5% level, and the stale flavor could be sensed significantly.

On the other hand, the cooked rice grains treated with the bacterial cells gave favorable evaluation in terms of flavor and total judgement in comparison with the reference. Moreover, the stale flavor was significantly reduced. Judging from the data obtained, it was concluded that treatment with the bacterial cells was effective for reducing the stale flavor of Japonica-type rice grains stored at 40°C for 2 months.

The results, expressed as the reduction of n-hexanal, are shown in Table II. There was a more than 35% decrease in the original n-hexanal after the rice grains had been soaked with the bacterial cells at 50°C for 1 h. Moreover, the stale flavor was significantly reduced when compared to that of the non-treated rice grains (Japonica-type stored at 40°C for 2 months contained about 0.13 ppm n-hexanal). Additionally, when the rice grains were soaked in water containing both the bacterial cells and one of the tested commercial surfactants—(sugar ester, monoglyceride, and sorbitan monoester), the reduction of n-hexanal was increased by about 1.3–1.9 times that with the bacterial cells alone as shown in Table II. By using sugar ester S 1670, which has shown the lowest inhibition of ALDH activity, the reduction in n-hexanal was increased by about 1.7–1.9 times. Therefore, sugar ester S 1670 was used in all the subsequent experiments.

We then investigated the reason why n-hexanal was reduced much more by using the combination of the bacterial cells and the surfactant. As shown in Fig. 1, in the presence of the surfactant, the amount of n-hexanal was increased by 1.8 times in the aqueous phase during soaking, the optimum diffusion being observed when a 0.5% concentration of the surfactant was used. The increase in the amount of n-hexanal in the soaking water seems to have been closely related to the reduction of n-hexanal in the rice grains. This implies that the action of the surfactant depended on the migration of n-hexanal from the aleurone cells in the rice grains to the soaking aqueous phase.

![Fig. 1. Effect of the Sugar Ester Concentration on the Migration of n-Hexanal from Rice Grains into the Soaking Water.](image)

The reaction mixture consisted of 5g of Japonica-type rice grains washed with tap water, 70ml of distilled water and various concentrations of sugar ester S 1670 in a 50-ml glass tube left to soak at 50°C for 1 h. n-Hexanal in the resulting supernatant was determined by HPLC as described in Materials and Methods section.
Optimization of the reduced stale flavor

1) Immersion temperature. Figure 2(A) shows the relationship between the reduction of n-hexanal and the immersion temperature. The rice grains were soaked in water for 1 h at 10, 20, 30, 40, and 50°C with both 120 mg of the bacterial cells and 0.3% of the sugar ester, and then cooked in an electric rice cooker. The amount of n-hexanal in the cooked rice was measured by HPLC as 2,4-dinitrophenyl-hydrazon as described in the Materials and Methods section. It was found that a relatively high immersion temperature was most effective, the maximum reduction in stale flavor being observed at 50°C. An immersion temperature of 50°C seems to be reasonable, because the optimum temperature for ALDH has been found to be around 50°C.51

2) Immersion time. Rice grains containing 120 mg of the bacterial cells and 0.3% of the sugar ester were kept at 50°C for 30, 60, 120, and 180 min, before cooling in a similar manner. As can be seen in Fig. 2(B), n-hexanal decreased gradually until 120 min of treatment, and the reduction in stale flavor reached around 70% after immersion for 120 min at 50°C.

3) Immersion pH. With additions of 120 mg of the bacterial cells and 0.3% of the sugar ester, the reduction in n-hexanal was examined against a McIlvaine buffer at various pH values. The optimum pH for the reduction of n-hexanal is shown in Fig. 2(C), more than 50% reduction being apparent in the range from pH 4.5 to 7.0, with pH 6.0 the optimum.

4) Effect of the amount of freeze-dried cells of acetic acid bacteria. Figure 3 shows the effect of the amount of acetic acid bacterial cells on the reduction of n-hexanal. The rice grains were soaked for 1 h at 50°C with various concentrations of the cells as indicated and with or without the surfactant, and were then cooked in the way already mentioned. In the absence of the sugar ester, the reduction of n-hexanal reached an equilibrium level of about 40% when using 0.3-1.0 mg of bacterial cells per 1 g of rice grains. On the other hand, in the presence of the sugar ester, 0.1-1.0 mg of the bacterial cells per 1 g of rice grains resulted in a

redaction of about 60%. The addition of a 0.3% concentration of the sugar ester achieved about a 1.5 times greater reduction in n-hexanal than without the sugar ester.

5) Effect of sucrose and salt. In the previous paper,51 we reported that the ALDH activity was not influenced when sucrose or salt was added to a final concentration of 0.1 or 0.5% of the reaction mixture. Similar results were obtained with the bacterial cells (data not shown), indicating that this treatment would still be useful when cooking rice with seasoning.

Reduction of the stale flavor of Japonica-type rice powder

Figure 4 shows the dependence of the added amount of bacterial cells on the reduction of n-hexanal with or without a surfactant when rice powder was used instead of rice.
grains. Without the sugar ester, the reduction of \( n \)-hexanal reached about 60%, which was about 1.7 times higher than that for the rice grains. This indicates that increasing the surface area facilitated the liberation of \( n \)-hexanal and that ALDH could easily degrade it. This suggests that \( n \)-hexanal adsorbed hydrophobically to the surface of the rice grains migrated to the soaking aqueous phase by treating with the surfactant. Furthermore, it was found that when 0.3% of the sugar ester was also present in the soaking water, the reduction of \( n \)-hexanal was increased by about 1.3 times that with the bacterial cells alone. In the absence of the sugar ester, the reduction of \( n \)-hexanal gradually decreased when the bacterial cells were added the range from 0.1 to 1.0 mg per 1 g of rice powder. In the presence of 0.3% of the sugar ester, the reduction reached 80% with 0.6–1.0 mg of the bacterial cells per 1 g of rice powder.

Scraped rice grains are widely used by the food industry to make rice cake, miso, sake, beer, and so on. However, when old scraped grains have been used in these foods and for alcoholic brewing, the resulting quality of the product was decreased by the stale flavor. Thus, treatment with the cells of acetic acid bacteria while processing such foods and beverages could improve the stale flavor. Furthermore, it is possible that less-expensive materials with a stale flavor such as old rice grains could be used for these purposes.

**Reduction of the cooked flavor of Indica-type rice grains**

Indica-type rice grains (310 g) were washed in tap water, soaked in 530 ml of deionized water containing 120 mg of the bacterial cells at 40 °C for 1 h, and then cooked in an electric rice cooker. Non-treated Indica-type rice grains were also cooked for use as a control reference in the sensory test. As shown in Table III, cooked Indica-type rice grains treated with the bacterial cells received a favorable evaluation for all the sensory parameters in comparison with the control. The flavor and total judgement of treated rice grains were significantly different from the reference at the 1% level, while the appearance and taste were superior at a 5% significance level.

Judging from the data obtained, it is concluded that treating Indica-type rice grains with the bacterial cells was effective for reducing the specific off-flavor of Indica-type rice grains.

Volatile carbonyl compounds from cooked Indica-type rice grains treated with the bacterial cells were analyzed, and the results are shown in Table IV. It was found that various kinds of aldehydes had been decreased in the cooked Indica-type rice grains. ALDH easily oxidized the C5 or C6 aldehydes, and the result obtained here is reasonable from the substrate specificity of the enzyme. Moreover, the volatile amount of the peaks measured by gas chromatography (GLC) was almost eliminated when Indica-type rice grains were cooked with a 0.1% hydroxylamine solution, which means they were carbonyl compounds (data not shown). Considering the data obtained here, it can be concluded that the unique off-flavor of Indica-type rice grains comes from aldehydes, although the components of this off-flavor have not been completely identified.

**References**