Effect of endogenous enzymes on sugar production during cooking of mixed barley and rice

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Barley grain contains β-glucan and is currently in focus for its health benefits, such as rapidly reducing serum cholesterol and suppressing increases in blood sugar levels. A previous study determined the boiling characteristics, amount of water added, barley-to-rice ratio, mechanical properties, and taste characteristics when cooking rice with barley. Barley has different milling yields and heating requirements depending on the processing method, such as normal barley, steamed pressed barley, and cut and polished barley. However, there have been no detailed reports on the differences in the processing methods and the physical properties and chemical composition of barley. The sugar content of rice changes according to the cooking conditions and is greatly influenced by enzymes; however, such changes have not been investigated in barley.

Therefore, we aimed to examine the influence of cooking conditions and barley processing methods on the chemical composition and mechanical properties of cooked rice with barley and the behavior of enzymes in rice and barley to elucidate the mechanisms for sugar production.

First, normal barley obtained using different processing methods was compared and examined by assessing the boiling characteristics, amount of reducing sugar, amount of glucose, amount of amino acid, and physical properties and by sensory evaluation. Next, 90% milled pearl rice (Nipponbare) and 75% milled pearl waxy barley (Motchiriboshi) were used as samples. We conducted sensory evaluation (FACT scale of 1–9) to determine the acceptability of the mixing ratio for barley and the amount of water added.

Extracts of raw and cooked samples were prepared using the concussion extraction method with a final concentration of 50% ethanol solution, and various saccharides were measured using the Somogyi–Nelson method, phenol–sulfuric acid method, and high-performance liquid chromatography.

For western blot analysis, rice and barley grains were mixed and soaked for 1 h at 20°C and heated to a temperature of 60°C. Barley grains, rice grains, and cooking water were separated at the following three different time points: after soaking (20°C) and when the temperature of cooking water reached 40°C and 60°C. Crude enzymes were extracted from all the samples collected. To examine the presence or absence of enzyme (barley β-amylase and α-glucosidase), polyclonal antibodies against the enzyme were used.

For immunostaining, rice and barley were mixed and cooked; then, rice grains were fixed using 4% paraformaldehyde, frozen, and observed by immunoblot analysis using polyclonal antibodies against β-amylase.

Crude enzymes were extracted from barley flour using phosphate buffer, and the hydrolysis activity of soluble starch was examined at temperatures of 4°C–80°C.

In normal barley, the boiling characteristics, water content, and amount of sugar production during cooking differed depending on the processing method.

The results of sensory evaluation showed that cooking rice mixed with waxy barley at a ratio of 50% required a specific amount of water to be added by weight to the mixture (1.5 for rice and 1.8 for barley.)

The expected amount of saccharides in cooking mixed barley and rice were obtained from the experimental values for each barley and rice. The experimental values of reducing sugar, glucose, and maltose exceeded the calculated values as expected. Therefore, the effectiveness of cooking mixed barley and rice for glycogenesis was confirmed.

Western blot analysis and immunostaining showed that β-amylase transferred from the barley grain to the cooking water and rice grain during soaking for 1 h (20°C). Furthermore, β-amylase was present in the rice grains even when the temperature reached 40°C and 60°C during cooking. The behavior of rice α-glucosidase differed during rice cooking depending on the rice variety.

From the results of hydrolysis activity of rice and barley, the peak temperatures for producing sugars were 65°C and 60°C, respectively. It was confirmed that rice enzyme acts on barley starch, and barley enzyme acts on rice starch. Sugar production during cooking rarely occurred in the cooking water, but did occur in rice and barley grains, and the increase in maltose and glucose was remarkable.

In this study, we investigated sugar production during the process of cooking mixed barley and rice. We confirmed that, compared with single cooking, sugars increased remarkably by cooking rice with barley, and the mechanisms were elucidated from the behavior and characteristics of the enzymes during cooking.

To date, there have been no reports showing the localization of enzymes in the rice cooking process by immunostaining methods, and this study contributes important knowledge to the field.