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The relationship between rice protein composition and nitrogen compounds in sake

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The relationship between the protein composition of rice and nitrogen compounds (amino acids and oligo-peptides) in the produced sake were investigated using endosperm protein mutant rice (LGC-1, LGC-Jun, Kx433, QA28), sake rice (*Yamadanishiki*) and cooking rice (*Nipponbare*, *Nihonmasari*, *Koshihikari*). The total nitrogen concentration, amino acid concentration and most peptide peak areas determined by RP-HPLC and gel filtration chromatography of the produced sake were lower when sake was made from a low glutelin content rice mutant compared with other rice varieties. The concentration of nitrogen compounds in the sake positively correlated with the glutelin content of the highly milled rice grains used for sake production. Sake produced using a low glutelin content rice mutant is generally evaluated as having a light taste. Our findings suggest that nitrogen compounds (oligo-peptides and amino acids) derived from rice glutelin significantly contribute to the taste of sake.

[**Key words:** Sake brewing; Rice; Glutelin; Endosperm protein mutant; Oligo-peptides]

It is recognized empirically that the rice endosperm protein gives the great effect on the quality of sake. Rice endosperm proteins are digested by sake *koji* enzymes under sake mash conditions and generate a mixture of nitrogen compounds that impart some positive characteristics to the sake, such as a superior taste and body. However, if the concentration of these nitrogen compounds is too high they can also have negative effects on the sake, such as coloration and the unpleasant tastes (1,2). Therefore, for the production of premium quality sake, highly polished or low-protein-content rice grains are preferably used.

The nitrogen compounds produced from rice protein in the sake are mainly amino acids and oligo-peptides. Amino acids are believed to affect the taste of sake, and an excess amino acid level gives the sake a rough taste (1). In another report, however, it was presumed that amino acids were not essential for the taste of the sake and that other components primarily influenced its quality (3). With regard to oligo-peptides, bitter-tasting peptides and their ethyl-esters derived from rice proteins were found in sake that was untreated with activated-charcoal (4,5). The levels of these bitter-tasting peptides in sake without activated-charcoal treatment far exceeded their sensory threshold values. Although more than 60 dipeptides are present in sake (6), the contribution of dipeptides to sake quality is not fully understood. Therefore, the relationship between nitrogen compounds derived from rice protein and the quality of sake remains unclear. The effect of these nitrogen compounds on the quality of sake, and the methods of control the formation of sensory active oligo-peptides and amino acids derived from rice protein, need to be studied more. Moreover, these results would contribute to breeding programs for sake rice aimed at producing high quality sake.

Most proteins in rice grains are found in the form of discrete particles called protein bodies (PBs). There are two types of PB, PB-I and PB-II, which consist predominantly of prolamins and glutelins, respectively (7,8). PB-I and PB-II comprise approximately 20% and 60% of milled rice proteins, respectively, although these values vary among different cultivars (9,10). In order to study endosperm protein function, endosperm protein mutant lines have been produced in which glutelin, globulin or prolamin levels were significantly lower than non-mutant lines (11–15). For example, LGC-1 has a low content of glutelin and high content of 13 kDa prolamin and 26 kDa globulin (11), whereas Kx433 lacks 26 kDa globulin (13). LGC-1-Jun, a crossbred double mutant line, shows a low content of glutelin and no 26 kDa globulin (14). QA28, a crossbred quadruple mutant line, has a low glutelin content, and lacks the glutelin a-2 subunit, glutelin a-3 subunit and 26 kDa subunit (15). Moreover, genes of the glutelin family and prolamin family have recently been cloned and their expression patterns during grain maturation have been determined along with the localization of their gene expression in the rice grains (16–20).

During the fermentation of sake mash, the degree of digestion differed between PB I and PB II (21–24). While prolamin is rarely

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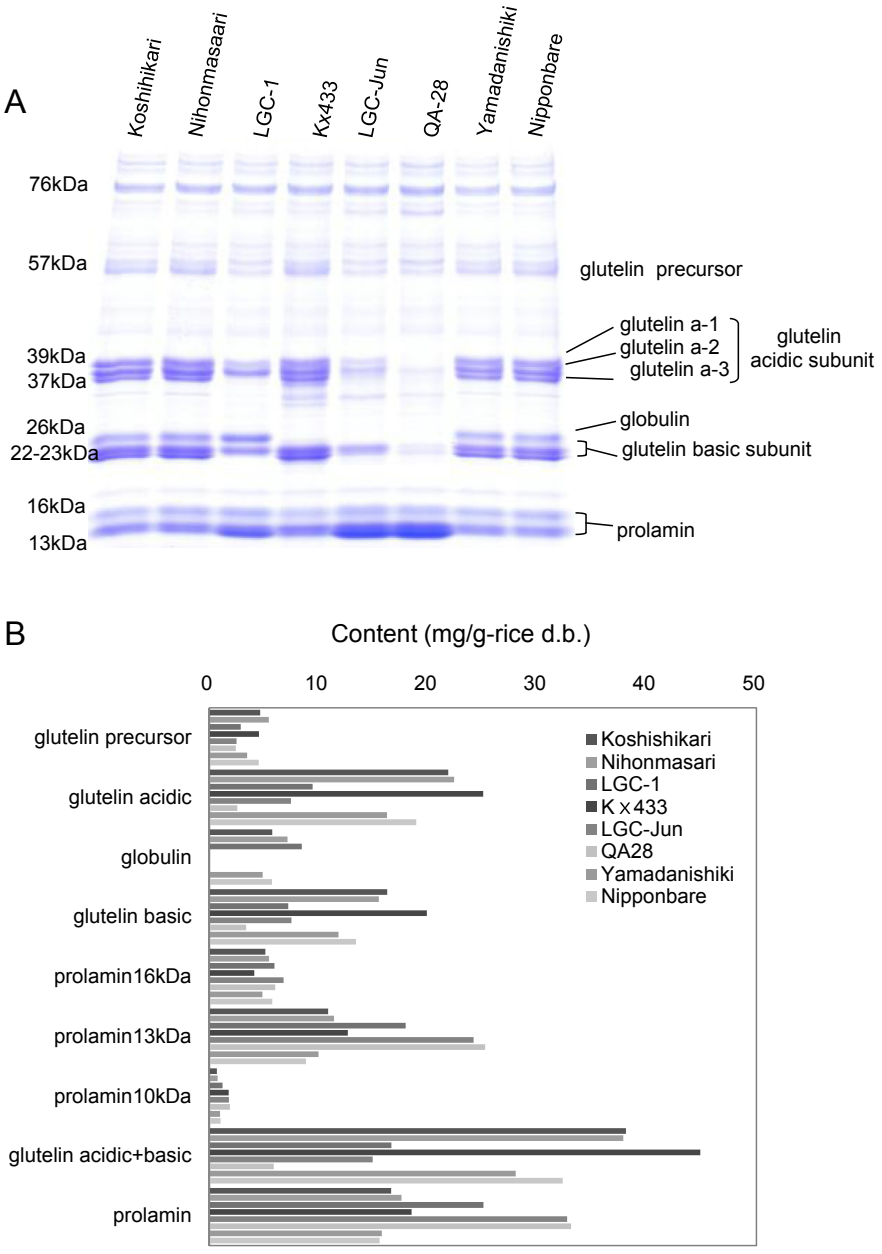

FIG. 1. Polypeptide of the rice endosperm mutant. (A) SDS-PAGE of the extracts from milled rice grains. (B) Contents of polypeptide bands. The milling ratio (%), which is the ratio by weight of milled rice to the original brown rice, of the rice grain sample was 70%.

digested in sake mash, glutelin is highly susceptible to digestion under these conditions. Furthermore, some of the digestion products derived from glutelin are thought to have a negative effect on sake quality. Therefore, the use of low-glutelin rice for sake production has recently been studied (25–31). Sake produced using low-glutelin rice grains had a reduced amino acid concentration and gave a light taste compared with sake manufactured from normal glutelin rice (25–27,29,31). The difference in the taste of the sake produced from low-glutelin rice and normal glutelin rice are thought to be based on the difference in oligo-peptides and amino acids, which are derived from rice proteins. However, oligo-peptides in sake produced using low-glutelin rice have not previously been studied. Detailed analysis of nitrogen compounds, such as oligo-peptides, in sake produced using low-glutelin rice is expected to contribute to our understanding of the relationship between rice protein and the taste of the sake.

In the present study, we used endosperm protein mutant rice (LGC-1, LGC-Jun, Kx433, QA28), sake rice (*Yamadanishiki*) and cooking rice (*Nipponbare*, *Nihonmasari*, *Koshihikari*) to investigate the relationship between protein composition and nitrogen compounds, such as amino acids and oligo-peptides, in the produced sake. Initially, we analyzed the composition of mutant protein in the highly milled rice and then evaluated its suitability for the manufacture of sake. Next, we produced sake from mutant rice and analyzed the nitrogen compounds in the final product. Lastly, we analyzed the relationship between the glutelin content in milled rice grains and the concentration of nitrogen compounds, such as amino acids and oligo-peptides, in the produced sake.

MATERIALS AND METHODS

Rice samples Four endosperm protein mutant varieties of rice, LGC-1 (11), Kx433 (13), LGC-Jun (14) and QA28 (15), and their parents, *Nihonmasari* and