

REVIEW

Properties of Floury Rice Mutant and its Utilization for Rice Flour

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Abstract

Rice flour is used to make various traditional foods and also increasingly as a substitute for wheat flour. To explore rice suitable for milling, the properties of grain and flour in floury rice mutants were investigated. Floury rice mutants commonly show loosely packed starch granules in the endosperm, which results in decreased grain hardness. The starch and amylose content of some floury mutant lines declined slightly compared to the wild-type. In wild-type rice, fine flour with low starch damage is obtained when jet-milled under wet condition; while flour which is pin-milled under dry conditions is coarser and more damaged compared to jet-milled flour. Rice flour prepared from floury mutant lines shows low starch damage and fine particle size, in both jet- or pin-milling. The differences in grain components, starch damage and particle size would lead to a characteristic RVA profile. In some mutant lines, the specific loaf volume (SLV) of rice bread made from pin-milled floury mutant rice is reportedly equivalent to that made from the jet-milled wild-type. The dry-milling process needs none of the excess water treatment required in wet-milling. Therefore, using floury mutant lines and the pin-mill, industrial production of rice flour suitable for high-SLV rice bread would be easier.

Discipline: Food

Additional key words: grain hardness, particle size, rice bread, specific loaf volume, starch damage

Introduction

Rice (*Oryza sativa* L.) flour is used to make various foods such as noodles and confectionary products, and also utilized as a substitute for wheat (*Triticum aestivum* L.) flour. The consumption of rice has been decreasing in Japan, and utilization of rice as flour has attracted considerable attention in recent years.

Rice flour is usually prepared by one of the following three milling methods: wet, semi-dry or dry milling. In wet milling, rice is soaked in water, drained, then ground with extra water added. During semi-dry milling, rice is soaked, drained, ground under wet conditions but no water is added. Currently, rice flour for bread-making is mainly produced by semi-dry jet-milling after pectinase treatment¹⁷. In dry milling, cleaned rice is milled under dry condition using various milling machines. Flour obtained by wet milling shows lower levels of starch

damage compared to dry milling²⁰. Less damaged rice flour, the particle size of which is about 40 μm , is suitable for making rice bread^{2,5}. For dry pin-milling, the resulting flour is coarser and more damaged compared to semi-dry jet-milled flour. However, wet or semi-dry milling requires milling facilities and complicated operations for flour drying and wastewater handling. Therefore, if superior rice flour were obtained by dry milling, this would make rice milling more economical. Recently, rice mutants, which improve milling properties, have been discovered, and production of fine flour with less damaged starch is expected to be feasible using mutants and dry pin-milling. This review deals with the milling and processing properties of floury mutants developed by Ashida et al.^{3,4} with reference to floury endosperm genes, *flol(flo4)*, *flo2*, *flo3* and *flo5* (Table 1)^{8,10,11,15,16,19}.

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Chalk and floury mutants

'Chalk' is a white opaque area caused by loose packing of the starch granules in rice grains. The grains appear opaque because numerous air spaces in the starchy endosperm reflect light diffusely. The structure of the opaque endosperm of chalky kernel differs from that of waxy rice grain. In waxy endosperm, the starch granules are packed tightly but their surface reveals many micropores and hollows. Chalky grains are classified by the shape and location of the opaque part in the endosperm. For example, "white-core" is a kernel with a discoidal opaque region at the center of the endosperm, while "milky-white" is a kernel with an entirely opaque kernel

Table 1. Floury mutant genes

Gene symbol	Chromosome	Gene	Characteristics
<i>fl01 (fl04)</i>	5	OsPPDK	Floury, lower amylose content
<i>fl02</i>	4	OsFLO2	Floury, low content of 16 kDa protein
<i>fl03</i>	6	-	Floury
<i>fl05</i>	-	OsSSIIIa	Floury (white-core)

when observed vertically, but a transverse section shows a few translucent parts in the peripheral endosperm. Generally speaking, rice kernels with large opaque portions such as large white-core or milky-white are known as "floury". Floury rice mutants have been obtained by inducing mutations, and the floury mutant rice has been expected to be milled easily because of the numerous air spaces in the grain.

Morphological properties of the floury rice mutant

Six floury mutant lines were developed by γ -irradiation or ethyl methanesulfonate treatments applied to a japonica rice cultivar "Koshihikari" during 1986-1987^{3,4}. Koshihikari is a non-waxy cultivar with a translucent kernel (Fig. 1A). Although the grain appearance of all floury rice mutants was opaque, the peripheral endosperm of floury mutants was translucent (Fig. 1B). In translucent grains of the parental cultivar, Koshihikari, starch granules were tightly packed in the endosperm (Fig. 1C). The starch granules were also tightly packed in the translucent part of the peripheral endosperm in floury mutant rice. In translucent rice endosperm, polyhedral compound starch granules are tightly packed, and poly-

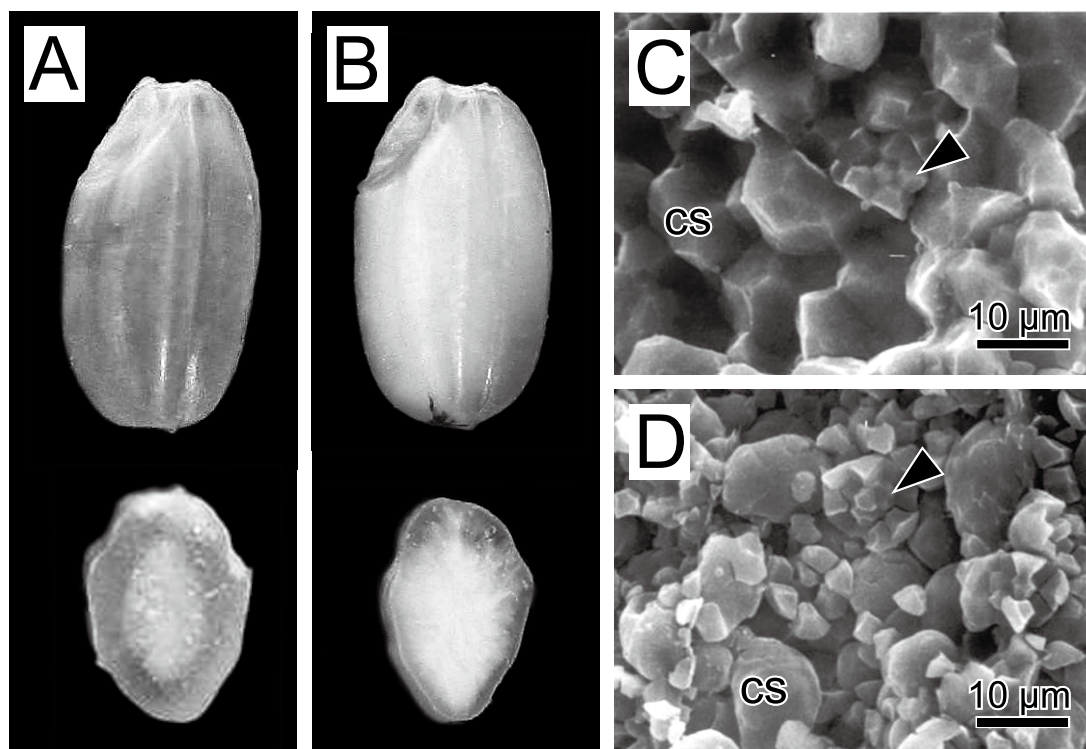


Fig. 1. Grain appearance and endosperm structure

A, grain appearance of Koshihikari; B, grain appearance of floury mutant rice; C, endosperm structure of Koshihikari; D, endosperm structure of floury mutant rice. The endosperm structure was observed using scanning electron microscopy (SEM). Arrowheads indicate single starch granules; cs, compound starch granule.

hedral single starch granules are densely packed in the compound starch granules. Conversely, compound starch granules were loosely packed in the opaque area of floury mutant rice (Fig. 1D). The compound starch granules were spherical in opaque endosperm, though single starch granules found were polyhedral. Many air spaces were observed between the starch granules in floury mutant rice, which would render the grain fragile. The grain weight of six floury mutant lines was also lighter than that of Koshihikari. The structure with numerous air spaces in the floury endosperm would be associated with grain weight. The grain appearance and structure observed in the six floury mutants resembled those reported in *flo1*, *flo2* and *flo5* mutants and chalky wild-type grains.

Grain hardness and polishing properties

The endosperm structure of floury rice mutants shows numerous air spaces and implies that the floury grain should be softer than the translucent wild-type. Using the single kernel characterization system (SKCS), which is used to evaluate the wheat kernel texture, the grain hardness of floury rice mutants can be measured. In all six floury mutant lines, the grain hardness of floury mutant rice was significantly lower than that of the translucent parent. Koshihikari showed similar grain hardness to hard wheat, whereas the grain hardness of floury mutant lines resembled those of soft to medium-soft wheat.

The low grain hardness brings about fragility in the floury mutant grain. The weight loss during grain polishing of floury mutant rice was about 30%, whereas that of the wild-type was about 10%, which indicates that a new polishing method avoiding weight loss should be developed. Furthermore, floury mutants with a kernel comprising a hard peripheral part and a soft center could decrease the weight loss more.

Chemical properties

The six floury mutant lines tended to show lower starch and amylose content than wild-type Koshihikari³. Although Kang et al. (2005) and Kaushik and Khush (1991) demonstrated that amylose contents of *flo1* (*flo4*) and *flo2* mutant were lower than those of wild-type genotypes^{8,9}, the endosperm structure of floury mutant rice differed from waxy or low-amylose rice, meaning the low amylose content itself would not be responsible for grain chalkiness in floury mutant rice. Nitrogen content and the protein composition of six floury mutant lines mentioned above do not differ significantly from those of Koshihikari.

Flour properties

1. Starch damage

In general, rice flour for bread-making is produced using jet-mill under wet conditions (jet-milling), and the resulting flour is finer and less damaged compared to pin-milled flour². In both jet- and pin-milling, the rice flour of six floury mutants showed significantly lower starch damage than Koshihikari⁴. Homma et al.⁷ also demonstrated that 'Hokuriku166', which derived from 'Kenkei2047 (*flo-1*)'¹⁰, became finer and less damaged flour than 'Koshihikari'. The friable grain structure of floury mutant rice would lead to lower damage to endosperm starch. Since rice flour with low starch damage can be produced using floury mutant rice and pin-milling, floury mutant lines are considered suitable for flour production.

2. Particle size distribution

According to particle size distribution, most rice flours on the market show a trough or border at around 30 μm between the peak and shoulder. Jet-milled Koshihikari reportedly has a shoulder at around 15–20 μm , a peak at around 50–60 μm , and median particle size (MPS) of about 40 μm ⁴ (Fig. 2). Pin-milled Koshihikari was shown to have a shoulder at around 15–20 μm , a peak at around 130 μm , and MPS of about 90 μm . On the other hand, jet-milled floury mutant rice showed two peaks at around 15–20 μm and 40–50 μm , and MPS of about 20 μm in six mutant lines⁴. The distribution pattern of pin-milled floury mutant rice was given to have two peaks around 15–20 μm and 100 μm , and MPS about 40 μm . In both milling methods, rice flour of the floury mutant was commonly shown to have a larger fraction of particles $\leq 30 \mu\text{m}$ with a peak at around 15–20 μm . The compound starch granules constituted a peak at around

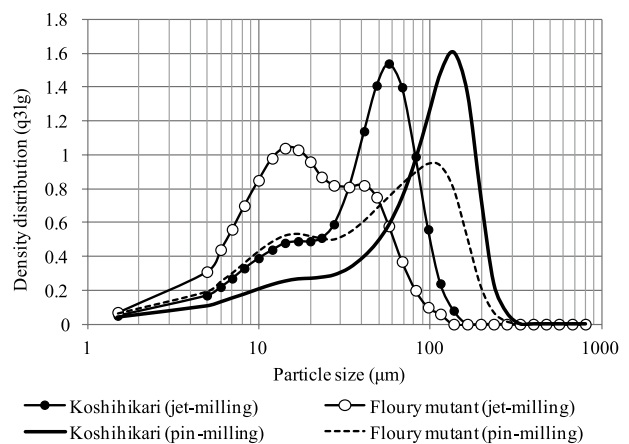


Fig. 2. Particle size distribution of rice flour

20 μm in the particle size distribution of rice flours². Since floury endosperm has numerous air spaces among compound starch granules, the endosperm cells are easily broken down into compound starch granules during milling, whereupon the peak is around 15–20 μm . Homma et al.⁷ showed that *flo1* mutant rice resulted in fine flour. As described above, endosperm structures among *flo1*, *flo2*, and *flo5* mutants are similar, meaning floury rice mutants are generally expected to be milled into rice flour, the particle size distribution of which peaks at around 15–20 μm .

3. Pasting properties

Pasting properties of rice flour can be analyzed using a viscograph or a rapid visco analyser (RVA). It was shown that the jet-milled flours have higher viscosities than pin-milled flours (Fig. 3)⁴. Washing and pectinase treatment conceivably eliminates endosperm components, which would suppress viscosity. Flours milled from floury mutant rice tended to show lower peak viscosities than those from Koshihikari, both in jet- and pin-milling, but the final viscosity was similar (Fig. 3). The difference in damaged starch content and particle size distribution between floury mutant lines and Koshihikari would affect the RVA profile, while the differences in starch properties such as amylose and amylopectin fine structure between six floury mutant lines and Koshihikari could also affect RVA viscosities.

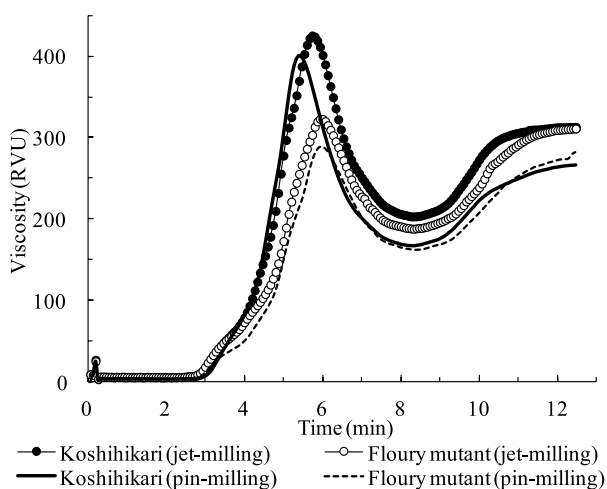


Fig. 3. RVA profile of rice flour

The pasting properties of rice flour were analyzed using a Rapid Visco Analyser (RVA3D+, Newport Scientific Pty. Ltd., Australia) according to AACC61-02.

Processing properties for rice bread with gluten

The protein content of rice is about 7% (w/w, brown rice), which is relatively low compared to other cereals, while the characteristics of rice protein differ from those of wheat (*Triticum aestivum* L.) protein. Rice protein cannot develop films capable of holding fermentation gases like wheat gluten. Rice bread is sorted into three groups based on the formula used to make it: rice bread with gluten, rice bread without gluten, and wheat/rice composite flour bread. Thickeners or pregelatinized starch are used for rice bread without gluten. In these three groups, rice bread with gluten is easy to make, and widespread in Japan.

Experiments using six floury mutant lines showed that the specific loaf volume (SLV) of rice bread with gluten made from jet-milled flour exceeded that of pin-milled flour in both Koshihikari and floury mutant rice⁴ (Fig. 4). Pin-milled Koshihikari showed lower SLV than the jet-milled one, but pin-milled floury mutant rice showed SLV equivalent to the jet-milled Koshihikari flour (Fig. 4). Therefore, the production of rice flour suitable for high-SLV rice bread would be possible by pin-milling when using floury mutant rice^{3,4}.

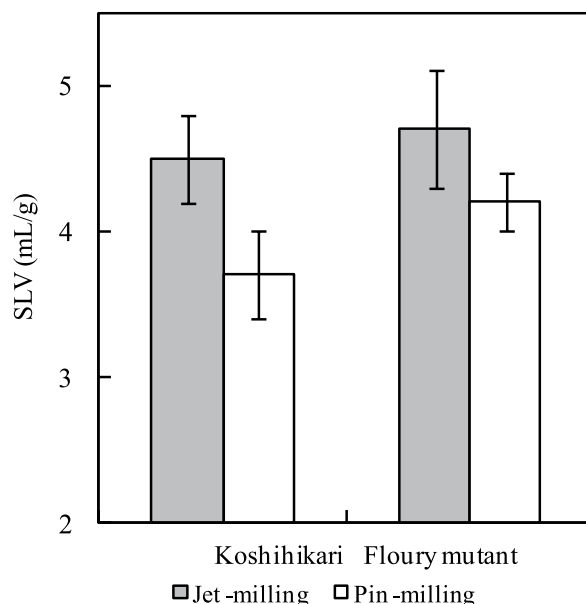


Fig. 4. Specific loaf volume of rice bread with gluten

Rice bread with gluten was made essentially as described before (Araki et al., 2009); 80 g of rice flour, 20 g of gluten, 5 g of soft white sugar, 2 g of common salt, 10 g of shortening, 1.5 g of dry yeast, and 80 mL of distilled water were used. The specific loaf volume (SLV) was calculated as the volume per weight.

Correlation between flour properties and SLV

In rice bread with gluten, there is a significant negative correlation between SLV of rice bread and the damaged starch content of rice flour². The damaged starch content of pin-milled floury mutant rice exceeded that of jet-milled Koshihikari, though the SLV of rice bread made from pin-milled floury mutant rice was similar to jet-milled Koshihikari⁴. Among rice flours used in this study, SLV was significantly correlated with starch damage, MPS, volume percent of flour particle size $\leq 30 \mu\text{m}$ ($Q_3(30)$), RVA trough, final viscosity, setback, peak time, and pasting temperature (Table 2). As well as lower starch damage, smaller particle size and RVA properties of floury mutant rice would also lead to high SLV when making rice bread with gluten. Rice bread with gluten made from *flol* mutant rice tends to be soft, and the hardening rate is low compared to ordinary cultivars¹⁸. This floury mutation could bring about not only high SLV of rice bread but also a characteristic texture.

Effects of floury mutant genes on processing properties

Although the genes responsible for the six mutant lines described in this review remain unclear, floury mutant genes are supposedly involved in the development of flour processing properties. In addition to six mutant lines, the author examined the milling properties of a mutant line 85KG-4 developed by Nishio and Iida¹³, and confirmed that the line was milled into finer and less damaged flour than Koshihikari (unpublished). The

85KG-4 have low 16 kDa protein content which accompanies the floury phenotype¹³. The phenotype of 85KG-4 coincides with those of *flol2* mutant, indicating that the responsible gene of 85KG-4 would be *flol2*. The *flol2* mutant is thus expected to be suitable for utilization as rice flour. It was shown that fine and less damaged rice flour could be obtained from *flol1* (*flol4*) rice⁷, while the processing properties of *flol3* and *flol5* mutants have not been reported. Regardless of mutant genes, the endosperm structure of floury rice mutants, which has numerous air spaces between starch granules, could make them suitable for flour processing. As well as floury rice mutants, chalky grains resulting from environmental factors can reportedly be milled into finer and less damaged rice flour than translucent grains¹².

Future perspective

Floury mutant rice is expected to help reduce milling costs. To date, chalk-free translucent kernels are desired by most of the rice industry, but floury mutant cultivars/lines are beginning to be bred for use as rice flour; 'Hoshinoko', 'Hokuriku-kona232', and 'Ouu-kona412' are floury rice varieties in Japan, although their fragile grain structure is linked to significant weight loss during polishing. The development of a new polishing process and exploration of superior floury mutant rice subject to less weight loss during polishing is needed in future. As for brown rice flour, a new milling method is being developed, and the resulting flour is reported to be suitable for making high-SLV rice bread⁶. It is interesting to see whether floury mutant rice can be adapted for use as

Table 2. Correlation coefficient

	Starch content	Starch damage	MPS	$Q_3(30)$	Peak Visco	Trough	Break-down	Final Visco	Setback	Peak time	Pasting Temp
Starch content	—										
Starch damage	-0.60**	—									
MPS	-0.22	0.83**	—								
$Q_3(30)$	0.34	-0.86**	-0.90**	—							
Peak Visco	0.77**	-0.15	0.34	-0.19	—						
Trough	0.80**	-0.76**	-0.48	0.60*	0.57*	—					
Breakdown	0.56*	0.16	0.63*	-0.50	0.93**	0.22	—				
Final Visco	0.60*	-0.79**	-0.65*	0.74**	0.29	0.94**	-0.08	—			
Setback	0.13	-0.62*	-0.76**	0.79**	-0.24	0.60*	-0.56*	0.83**	—		
Peak time	-0.24	-0.48	-0.83**	0.68**	-0.69**	0.16	-0.89**	0.43	0.76**	—	
Pasting Temp	-0.33	0.90**	0.92**	-0.90**	0.17	-0.55*	0.46	-0.68**	-0.72**	-0.67**	—
SLV	0.37	-0.90**	-0.86**	0.86**	-0.05	0.60*	-0.34	0.69**	0.66**	0.58*	-0.84**

MPS, median particle size; $Q_3(30)$, volume percent of flour particle size $\leq 30 \mu\text{m}$; Peak Visco, peak viscosity; Final Visco, final viscosity; Pasting Temp, pasting temperature; SLV, specific loaf volume. * and **, significant at 5 and 1% levels, respectively.

brown rice flour. In addition to starch damage and particle size distribution, amylose content, amylopectin structure and protein composition also affect the quality of rice bread^{14,18}. Rice with moderate amylose content (15–25%) is suitable for making rice bread with gluten¹⁸. The protein disulfide isomerase-like 1 deficient mutant, *esp2*, which deposits 57 kDa preglutelins, is also reportedly suitable for making rice bread¹⁴. Rice bread made from low glutelin rice retains softness¹⁸. A combination of floury mutation, suitable amylose content and protein composition will help promote the utilization of rice flour.

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