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Rice ripened at lower temperature slows firming of bread

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Key words: Amylopectin; Bread firming; Firming rate; Rice bread

Abstract

Firming of bread after baking leads to waste due to loss of palatability. Firming is affected mainly by amylopectin, a starch component. Here, we found that the firming rate of rice bread can be slowed by using flour made from rice grains which filled at lower temperatures, because temperature during grain filling (TGF) affects amylopectin chain synthesis. We found significant positive correlations between TGF and firming rate of gluten-containing rice bread (rice flour 80% + wheat gluten 20%) and rice-flour-containing wheat bread (wheat flour 80% + rice flour 20%) made from samples of rice grains filled at 21.0 to 28.3 °C. Regressions indicate that if TGF is decreased from 27 to 22 °C, the shelf life of gluten-containing rice bread will be extended by about 150%, and that of rice-flour-containing wheat bread by about 50%. The slow-firming effect was confirmed by sensory tests. This result is potentially applicable to other cereals such as wheat, whose amylopectin structure is also affected by TGF.

Abbreviations: DP, degree of polymerization; TGF, Temperature during grain filling

1. Introduction

Much bread is wasted owing to the loss of palatability after baking: in some countries, more than 20% of bread is wasted (Jörissen et al., 2015; Quested et al., 2013). Bread firming is one of the most important contributing factors. Reducing bread firming would extend bread shelf life, with great benefit to the bread industry and consumers.

Extensive research has been conducted to reveal the molecular basis of bread firming (Fadda et al., 2014). Starch, composed of amylose and amylopectin, plays a major role.

Amylose is essentially a linear molecule containing α-(1-4)-linked glucose units with few branches, whereas amylopectin is a branched molecule with linear chains of α-(1-4)-linked glucose units and α-(1-6)-linked branches (Hizukuri et al., 1989). The firming of bread during aging is attributed mainly to the retrogradation of amylopectin (Goesaert et al., 2005).

The relationship between starch and bread firming can be investigated through the comparison of wheat flour with rice flour, because rice proteins do not form a gluten matrix, and hence starch properties directly affect bread properties. We previously showed that slow-firming rice bread can be made from flour of rice mutants that have a large proportion of short amylopectin chains (Aoki et al., 2015). Biosynthesis of amylopectin in cereals is not only strictly regulated by many genes (Tetlow, 2011), but is also affected by environmental factors such as temperature during grain filling (TGF) (Beckles and Thitisaksakul, 2014; Thitisaksakul et al., 2012). Lower TGF increases the proportion of amylopectin short chains in rice starch (Asaoka et al., 1984; Inouchi et al., 2000; Umemoto et al., 1999). Thus, we hypothesized that bread firming would be slowed by using flour made from rice which has filled at lower temperatures.

In this study, we confirmed that lower TGF is associated with the short chain ratio of amylopectin, and that lower TGF greatly slows the firming of bread.

2. Materials and methods

2.1 Rice cultivars and cultivation conditions

We planted three rice cultivars on each of three sowing dates (with one exception) in 2 or 3 years (Table 1). All plants were grown in paddy fields (36°0′N, 140°1′E) in Tsukubamirai, Ibaraki, Japan. Consequently, the crops headed 2 to 4 weeks apart. TGF was defined as the mean average daily temperature during 20 days after flowering.

2.2 Preparation of rice flour

In order to produce rice flour with low damaged starch content, which is suitable for making high loaf volume of rice bread, wet milling was employed (Araki et al., 2009). Polished rice grains were soaked in a solution of 0.3% trisodium citrate dihydrate and 0.05% pectinase (Pectinase G; Amano Enzyme, Nagoya, Japan) for 1 h at 40 °C. The soaked rice was drained and then pulverized in a jet mill (SPM-R290; Nishimura Machine Works, Osaka, Japan) at 5300 rpm under wet condition without excess water. The flour, in which moisture content was about 20% (data now shown), was dried in a dryer at a controlled temperature of 60 °C overnight.

2.3 Measurements of flour properties

We measured flour particle size, damaged starch content, amylose content, protein content, pasting temperature, and degree of polymerization (DP) of amylopectin chains as described in Aoki et al. (2015). The relative ratio of each chain type of amylopectin was defined according to Hanashiro et al. (1996): A chains, $DP \leq 12$; B1 chains, $13 \leq DP \leq 24$; B2 chains, $25 \leq DP \leq 36$; longer chains, $DP \geq 37$. Amylopectin chain lengths in each sample were measured once. Particle size was measured in duplicate. Other values were measured in triplicate.

2.4 Bread making and analysis

We made two types of bread: 80% rice flour + 20% gluten ("gluten-containing rice bread") and 80% wheat flour + 20% rice flour ("rice-flour-containing wheat bread", made with rice from May and July plantings). The gluten-containing rice bread was made according to the straight dough method (Aoki et al., 2015; Yamauchi et al., 2004) using 600 g of rice flour, 150 g of vital wheat gluten (Dough Master FR; Riken Vitamin Inc., Tokyo, Japan), 52.5 g of sugar, 22.5 g of powdered skimmed milk, 11.3 g of salt, 60 g of shortening, 11.3 g of dry yeast, and 562.5g of water. The rice-flour-containing wheat bread was made using 600 g of commercial wheat flour (Camellia; Nisshin Flour Milling Inc., Tokyo, Japan), 150 g of rice flour, 52.5 g of sugar, 22.5 g of powdered skimmed milk, 11.3 g of salt, 60 g of shortening, 11.3 g of dry yeast, and 487.5g of water. We put 400 g of dough in loaf pans and allowed it to rise for about 1 h at 38 °C and 80% humidity until it reached the top of the pan, and then baked the loaves at 185 °C for 28 min.

Bread volumes and firmness were measured according to Aoki et al. (2015). Bread firmness was measured 1, 2, and 3 days after baking. Bread firming rate was defined as the increment of bread firmness per day (g/day). To evaluate the sensory firmness, around 20 people tested rice bread made from flour samples of 'Koshihikari' 2 days after baking. Bread made from commercial rice flour (Super Miracle Powder; Namisato Inc., Sano, Japan) and gluten was used as a reference for gluten-containing rice bread. Bread made from commercial wheat flour was used as a reference for rice-flour-containing wheat bread.

2.5 Statistical analyses

Regression analysis and Tukey's test were performed in R statistical software. Statistical significance was defined as $P < 0.05$ or $P < 0.01$.

3. Results

3.1 Properties of rice samples

TGF values ranged from 21.0 to 28.4 °C (Table 1). TGF was significantly negatively correlated with amylose content ($r = -0.87$, $P < 0.01$), but differences among samples were small (15.1%–22.9%). Chain length distributions of amylopectin were affected more by cultivation condition than by cultivar (Supplementary data 1). The molar ratio of DP 6 had the highest correlation with TGF among amylopectin chains (Supplementary data 2). TGF was significantly correlated with the ratios of A chains, B2 chains, and longer chains (Table 1).

3.2 Bread shape and loaf volume

Gluten-containing rice bread caved in at the sides, unlike rice-flour-containing wheat bread (Fig. 1). The shapes of rice-flour-containing wheat bread were similar among bread samples. There were no large differences in bread weights among bread samples regardless of bread type, rice cultivar, or cultivation condition (data not shown). Neither TGF nor amylose content was significantly correlated with specific loaf volumes of either bread type ($P > 0.05$, Table 2).

3.3 Bread firming rate

TGF values were significantly positively correlated with firming rates of both bread types (Fig. 2). Firming rates were significantly (positively or negatively) correlated with fractions of amylopectin chains, pasting temperature, amylose content, and protein content, with the exception of the ratio of B1 chains in gluten-containing rice bread (Table 3). It is noteworthy that amylose content was negatively correlated with firming rate. The ratio of DP 6 had the greatest correlation with firming rate among amylopectin chains (Supplementary data 2). All bread firmness scores are shown in Supplementary data 3.

3.4 Sensory test of 'Koshihikari' rice bread

Gluten-containing rice bread made from rice transplanted in July and therefore cooler TGF was significantly softer ($P < 0.05$) than that made from rice transplanted in May in each year (Fig. 3A–C). Rice-flour-containing wheat bread made from rice transplanted in July was non-significantly softer ($P < 0.05$) than that made from rice transplanted in May (Fig. 3D, E).

4. Discussion

4.1 Close relationship between TGF and bread firming rate

Our results show significant correlations among TGF, the ratios of amylopectin chain lengths, and firming of rice bread (Fig. 2; Tables 1, 3; Supplementary data 2). The relationship between TGF and amylopectin chains was consistent with other studies (Asaoka et al., 1984; Inouchi et al., 2000; Umemoto et al., 1999). The lower firming rate of rice bread might be due to the higher proportion of short amylopectin chains, because slow-firming rice bread can be made from flour of rice mutants that have a large proportion of short amylopectin chains (Aoki et al., 2015). The regression equations in Figure 2 indicate that if TGF is reduced from 27 to 22 °C, the firming rate will be reduced from 39.1 to 15.7 g/day (2.49:1) in gluten-containing rice bread, and from 87.6 to 59.4 g/day (1.47:1) in rice-flour-containing wheat bread. This means that shelf life would be increased by about 150% in gluten-containing rice bread and by about 50% in rice-flour-containing wheat bread. This would make rice grown in cool conditions, for example in northern Japan or in the highlands, valuable for making slow-firming bread.

The significant high correlations indicate that TGF or pasting temperature (Table 3) can be used as an indicator of bread firming.

4.2 Possible effects of other constituents on bread firming rate

The significant negative correlation between TGF and amylose content (Table 1) was consistent with other studies (Asaoka et al., 1985; Inouchi et al., 2000; Umemoto et al., 1999). Although Bhattacharya et al. (2002) and Takahashi et al. (2009) found that amylose has an effect on firming of bread, we found the opposite (Table 3), probably because amylopectin has greater effects than amylose. Protein content and damaged starch content were negatively correlated with bread firming rate (data not shown). Further studies are needed to clarify their relationships with bread quality. There was no significant correlation between particle size and bread firming rate (data not shown). Amylase retards bread firming (Gujral et al., 2003), but the effect of endogenous α-amylase is probably negligible because amylase activity in rice endosperm is low (Shinke et al., 1973) and it becomes lower at lower ambient temperatures (Yamakawa et al., 2007).

4.3 Potential applications of gluten-free rice bread

Rice flour is useful for making gluten-free bread, but rice bread firms faster than wheat bread (Kadan et al., 2001) and has a short shelf life (Nishita et al., 1976). We did not investigate the properties of gluten-free bread made from our rice samples, but we suppose that softer gluten-free bread can be made from rice that filled at low TGF and thus has a high amylopectin short-chain ratio, because flour from mutants with higher amylopectin short-chain ratios led to softer bread (Aoki et al., 2015), but with a smaller loaf volume than control breads. Therefore, it is possible that gluten-free breads made from low-TGS rice will also have small loaf volumes because of the high proportion of amylopectin short chains (Table 1). If high-amylose cultivars are used for making gluten-free bread, the bread may have a larger loaf volume than bread made from rice cultivars with moderate amylose contents such as 'Koshihikari', because amylose content is significantly positively correlated with the specific loaf volume of rice bread (Aoki et al., 2012).

4.4 Possibility of application to other cereals

We used rice flour, but our results could be applicable to other cereals such as wheat. Matsuki et al. (2003) similarly found in wheat that a lower TGF led to an increase in the amylopectin short-chain ratio, and a decrease in the long-chain ratio. So slow-firming wheat bread might be made with low-TGF wheat flour. As bread wheat is a hexaploid species, it is much harder to breed mutant lines with short amylopectin chains than in diploid species like maize or rice. However, TGF can be altered relatively easily by changing the sowing date or by growing cultivars with earlier flowering periods. A similar tendency between TGF and amylopectin short chains has been reported in maize (Lu et al., 1996), sorghum (Li et al., 2013), and sweet potato (Noda et al., 2001). If the shelf lives of foods can be increased by changing growing conditions, food waste will be reduced, with benefits for both the food industry and consumers.

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Tables

Figure captions

Figure 1 Shapes of loaves made from 'Koshihikari' rice flour derived from plants transplanted in the indicated months each year. Loaves made from 'Hitomebore' and

‘Takanari’ flour were similar (data not shown).

[a] 80% rice flour + 20% wheat gluten.

[b] 80% wheat flour + 20% rice flour.

Figure 2 Correlation of temperature during ripening and bread firming rate. A: Gluten-containing rice bread. B: Rice-flour-containing wheat bread.

Figure 3 Sensory evaluation of breads made with flour of ‘Koshihikari’ rice transplanted in May or July. A–C: Gluten-containing rice bread. D, E: Rice-flour-containing wheat bread. Rice bread made from commercial rice flour was used as a reference for gluten-containing rice bread. Bread made from commercial wheat flour was used as a reference for rice-flour-containing wheat bread. Softness was scored from −3 (very hard) to 3 (very soft), with that of reference bread designated as 0.

Highlights

- Slow-firming rice bread was made from rice that filled at lower temperatures.
- Temperature during grain filling was positively correlated with firming rate of bread.
- The slow-firming effect was caused by the increase of short amylopectin chains.

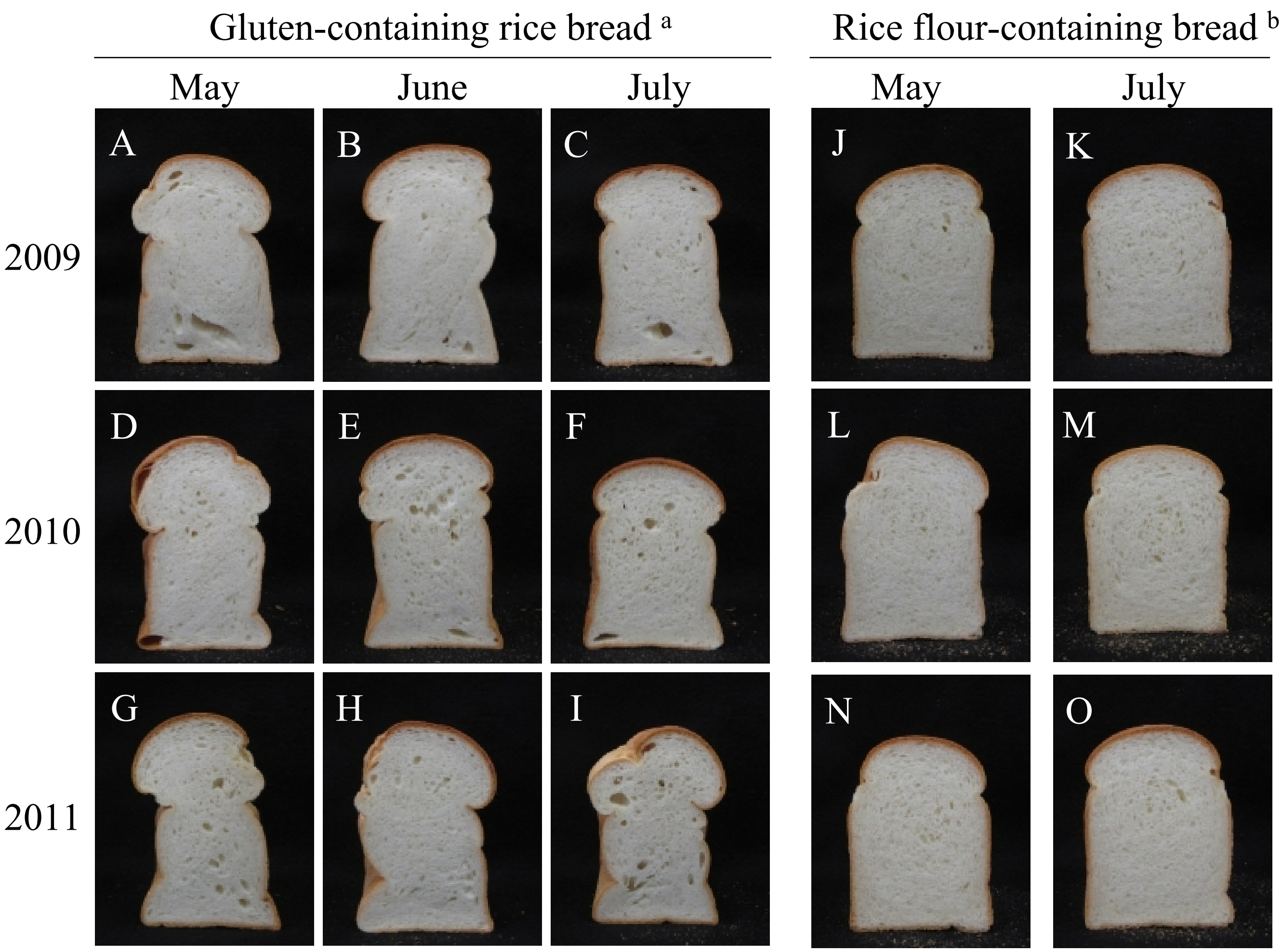
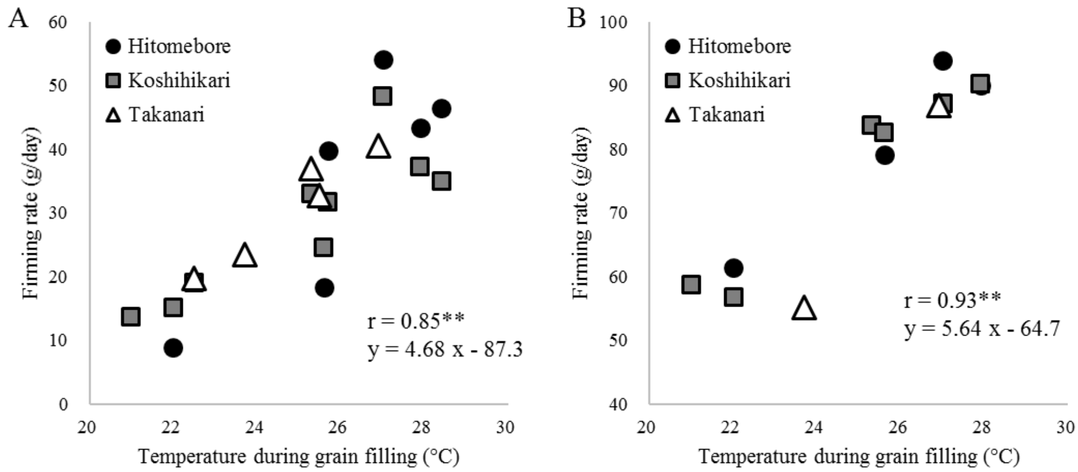

Gluten-containing rice bread [a]
Rice flour-containing bread [b]
May
June
July
May
July
A
B
C
J
K
2009
D
E
F
L
M
2010
G
H
I
N
O
2011

Figure 1



A
B
Hitomebore
Koshihikari
Takanari
Firming rate (g/day)
Temperature during grain filling (°C)
r = 0.85**
y = 4.68 x - 87.3
r = 0.93**
y = 5.64 x - 64.7

Figure 2

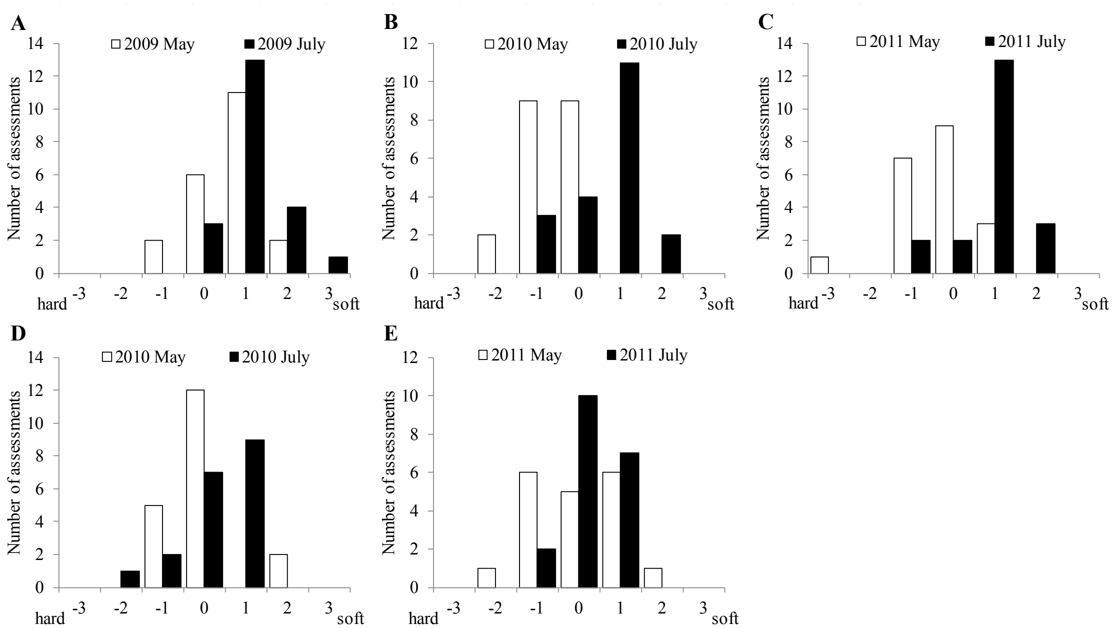

A
2009 May
2009 July
B
2010 May
2010 July
C
2011 May
2011 July
D
2010 May
2010 July
E
2011 May
2011 July
Number of assessments
hard
soft

Figure 3

Table 1 Properties of rice flour samples

Cultivar	Yea r	Sowing date	Transplanti ng date	Heading date	Temperature during grain filling (°C)[a]	Average particle size (μm)	Damaged starch content (%)	Amylose content (%)
Hitomebore	2010	16-Apr	14-May	28-Jul	27.8	26.0	2.2 ± 0.0 fh	17.0 ± 0.2 efg
		19-May	17-Jun	14-Aug	28.3	27.6	2.4 ± 0.1 cdf	16.2 ± 0.5 fh
		1-Jul	22-Jul	12-Sep	21.3	29.2	2.7 ± 0.1 bc	21.9 ± 0.2 a
	2011	16-Apr	11-May	29-Jul	27.0	32.9	2.0 ± 0.0 fh	16.1 ± 0.3 gh
		19-May	9-Jun	12-Aug	25.7	33.6	2.1 ± 0.0 fh	16.9 ± 0.7 efg
		11-Jun	9-Jul	2-Sep	25.5	37.2	2.6 ± 0.1 bd	17.3 ± 0.3 df
Koshihikari	2009	17-Apr	14-May	7-Aug	25.3	32.9	1.9 ± 0.1 gh	18.3 ± 0.2 cd
		27-May	18-Jun	24-Aug	22.5	31.2	2.4 ± 0.4 cde	20.0 ± 0.3 b
		3-Jul	29-Jul	23-Sep	21.0	33.1	3.4 ± 0.1 a	22.5 ± 0.2 a
	2010	16-Apr	14-May	1-Aug	27.9	27.4	2.1 ± 0.1 efg	17.0 ± 0.3 efg
		19-May	17-Jun	15-Aug	28.4	29.5	1.7 ± 0.0 h	15.1 ± 0.3 h
		1-Jul	22-Jul	9-Sep	22.0	38.5	2.3 ± 0.1 df	19.3 ± 0.2 bc
	2011	16-Apr	11-May	29-Jul	27.0	32.8	2.0 ± 0.0 fh	16.3 ± 0.1 fh
		19-May	9-Jun	12-Aug	25.7	34.4	1.7 ± 0.0 h	16.2 ± 0.3 fh
		11-Jun	9-Jul	26-Aug	25.6	39.7	2.2 ± 0.1 df	16.0 ± 0.6 gh
Takanari	2009	17-Apr	14-May	12-Aug	25.0	32.2	2.1 ± 0.2 efg	17.8 ± 0.2 de
		27-May	18-Jun	31-Aug	21.3	29.1	2.7 ± 0.0 bc	22.8 ± 0.3 a
	2011	16-Apr	14-May	4-Aug	26.9	28.9	2.1 ± 0.0 efg	16.6 ± 0.8 fg
		19-May	9-Jun	15-Aug	25.5	33.9	2.1 ± 0.1 efg	17.4 ± 0.6 df
		11-Jun	9-Jul	7-Sep	23.7	29.0	2.9 ± 0.1 b	22.9 ± 0.5 a
Correlation coefficient with temperature during grain filling					–	- 0.24 ns	- 0.70**	- 0.87**

[a] Mean average daily temperature during 20 days after heading.

[b] The temperature at the onset of the rise in viscosity was determined as the pasting temperature.

Means with the same letter are not significantly different at $P < 0.05$ (Tukey's range test). **$P < 0.01$.

Protein content (%)	Pasting temperature (°C)[b]
5.3 ± 0.0 n	71.9 ± 0.9 acd
6.0 ± 0.0 jkl	73.0 ± 0.5 a
8.6 ± 0.0 b	66.5 ± 0.8 hi
6.1 ± 0.1 ijkl	71.9 ± 0.5 acd
6.3 ± 0.0 ghj	70.5 ± 0.4 def
6.6 ± 0.0 fg	69.2 ± 0.1 fg
5.3 ± 0.1 n	70.3 ± 0.4 ef
6.2 ± 0.1 hjk	67.8 ± 0.0 gh
9.7 ± 0.1 a	65.3 ± 0.4 i
5.6 ± 0.2 mn	72.6 ± 0.4 ab
5.9 ± 0.0 jm	73.1 ± 0.8 a
9.6 ± 0.1 a	66.7 ± 0.4 hi
5.8 ± 0.1 lm	72.3 ± 0.4 ac
6.4 ± 0.1 ghi	71.2 ± 0.8 bce
7.2 ± 0.0 d	70.7 ± 0.7 cf
6.7 ± 0.0 ef	69.5 ± 0.5 f
7.1 ± 0.0 de	66.7 ± 0.5 hi
5.9 ± 0.1 km	70.8 ± 0.0 cf
6.4 ± 0.4 fh	70.2 ± 0.4 ef
8.1 ± 0.0 c	65.5 ± 0.4 i
- 0.78**	0.95**

Table 2 Loaf volumes of breads made with each rice sample.

Cultivar	Year	Transplanting month	Gluten-containing rice bread [a]	Rice flour containing bread [b]
Hitomebore	2010	May	4.1 ± 0.0 ac	3.7 ± 0.0 d
		June	3.9 ± 0.1 ac	-
		July	3.9 ± 0.2 ac	3.9 ± 0.1 abc
	2011	May	4.0 ± 0.3 ac	3.9 ± 0.1 abc
		June	3.9 ± 0.1 ac	-
		July	4.2 ± 0.0 ab	3.6 ± 0.0 d
Koshihikari	2009	May	4.2 ± 0.2 ac	3.8 ± 0.0 bd
		June	4.2 ± 0.1 ac	-
		July	3.8 ± 0.1 bc	3.8 ± 0.0 cd
	2010	May	4.1 ± 0.5 ac	4.1 ± 0.0 a
		June	4.2 ± 0.1 ab	-
		July	3.6 ± 0.1 c	3.9 ± 0.1 abc
	2011	May	3.9 ± 0.3 ac	4.0 ± 0.1 ab
		June	4.2 ± 0.2 ab	-
		July	3.9 ± 0.1 bc	4.0 ± 0.1 ab
Takanari	2009	May	4.5 ± 0.1 a	-
		June	3.9 ± 0.0 bc	-
	2011	May	3.9 ± 0.3 bc	4.0 ± 0.1 ab
		June	4.0 ± 0.2 ac	-
		July	3.8 ± 0.1 bc	4.1 ± 0.0 ab
Correlation coefficient with temperature during grain filling			0.36 ns	- 0.15 ns
Correlation coefficient with amylose content			-0.35 ns	- 0.08 ns

[a] 80% rice flour + 20% wheat gluten.

[b] 80% wheat flour + 20% rice flour.

Means with the same letter are not significantly different at $P < 0.05$ (Tukey's range test). *$P < 0.05$, **$P <$

Table 3 Coefficients of correlation with bread firming rate.

	Gluten-containing rice bread [a]	Rice flour containing bread [b]
A chain ratio of amylopecin	-0.82 **	-0.85 **
B1 chain ratio of amylopecin	0.39 ns	0.68 **
B2 chain ratio of amylopecin	0.81 **	0.81 **
Long chain ratio of amylopecin	0.82 **	0.80 **
Pasting temperature	0.85 **	0.97 **
Amylose content	-0.72 **	-0.90 **
Protein content	-0.74 **	-0.92 **

[a] 80% rice flour + 20% wheat gluten.

[b] 80% wheat flour + 20% rice flour.

Supplementary data 1: Relative molar ratios of amylopectin chains.

DP	Hitomebore						Koshihikari									Takanari				
	2010			2011			2009			2010			2011			2009		2011		
	May	June	July	May	June	July	May	June	July	May	June	July	May	June	July	May	June	May	June	July
5	0.10	0.13	0.32	0.11	0.15	0.21	0.19	0.16	0.22	0.11	0.19	0.46	0.12	0.19	0.22	0.11	0.10	0.11	0.11	0.42
6	0.88	0.92	1.63	0.93	1.08	1.27	1.14	1.31	1.69	0.92	1.00	1.67	0.99	1.17	1.21	1.23	1.45	1.07	1.14	1.67
7	2.29	2.40	2.91	2.32	2.48	2.69	2.44	2.55	2.89	2.25	2.42	3.01	2.41	2.51	2.54	2.58	2.71	2.51	2.52	2.91
8	4.44	4.65	4.54	4.43	4.54	4.90	4.28	4.19	4.20	4.19	4.46	4.64	4.49	4.30	4.31	4.26	4.15	4.47	4.36	4.42
9	7.04	7.18	7.25	6.95	7.07	7.29	7.09	7.01	7.01	6.84	7.01	7.34	7.16	7.03	7.08	7.01	7.04	7.13	7.06	7.05
10	8.47	8.55	8.74	8.35	8.46	8.67	8.78	8.72	8.75	8.42	8.45	8.94	8.69	8.62	8.74	8.59	8.70	8.53	8.49	8.55
11	8.80	8.84	9.10	8.68	8.86	9.25	9.15	9.12	9.17	8.77	8.77	9.27	9.01	8.96	9.17	8.91	9.12	8.92	8.95	8.98
12	8.71	8.71	8.83	8.57	8.65	8.78	9.09	9.08	9.14	8.74	8.69	9.06	8.92	8.91	9.01	8.86	9.03	8.81	8.81	8.79
13	7.77	7.80	7.91	7.67	7.76	7.91	8.09	8.07	8.18	7.84	7.81	8.16	7.94	7.99	8.05	7.99	8.17	7.89	7.95	7.97
14	7.37	7.35	7.33	7.26	7.33	7.37	7.61	7.56	7.53	7.42	7.36	7.55	7.46	7.45	7.52	7.47	7.59	7.43	7.46	7.40
15	6.78	6.76	6.68	6.70	6.75	6.74	6.99	6.94	6.91	6.85	6.77	6.86	6.84	6.85	6.90	6.85	6.96	6.86	6.91	6.78
16	5.96	5.92	5.88	5.89	5.94	5.91	6.14	6.12	6.12	6.04	5.95	6.03	6.00	6.06	6.03	6.06	6.20	6.05	6.10	6.00
17	4.88	4.83	4.89	4.85	4.89	4.84	5.04	5.09	5.12	4.98	4.91	5.02	4.91	5.01	4.99	5.06	5.20	4.98	5.05	5.04
18	3.98	3.94	4.02	3.97	4.01	3.95	4.06	4.14	4.17	4.07	4.01	4.07	3.99	4.08	4.09	4.14	4.22	4.07	4.13	4.14
19	3.37	3.33	3.30	3.37	3.37	3.28	3.27	3.36	3.31	3.38	3.36	3.21	3.33	3.37	3.36	3.40	3.38	3.37	3.40	3.35
20	2.69	2.66	2.57	2.73	2.70	2.57	2.48	2.58	2.48	2.67	2.66	2.37	2.62	2.63	2.61	2.66	2.56	2.63	2.63	2.56
21	2.14	2.12	2.00	2.19	2.15	2.02	1.90	1.98	1.85	2.10	2.10	1.75	2.06	2.05	2.02	2.08	1.94	2.05	2.04	1.96
22	1.68	1.67	1.55	1.73	1.69	1.56	1.46	1.51	1.39	1.64	1.64	1.31	1.61	1.58	1.56	1.61	1.46	1.59	1.57	1.50
23	1.31	1.30	1.21	1.37	1.32	1.22	1.13	1.16	1.06	1.28	1.29	1.02	1.25	1.22	1.20	1.25	1.11	1.24	1.21	1.15
24	1.04	1.04	0.94	1.10	1.03	0.90	0.89	0.91	0.83	1.02	1.02	0.79	0.99	0.96	0.95	0.99	0.86	0.98	0.96	0.90
25	0.83	0.83	0.74	0.88	0.85	0.82	0.70	0.71	0.65	0.81	0.81	0.61	0.79	0.76	0.74	0.78	0.67	0.78	0.75	0.69
26	0.68	0.68	0.59	0.71	0.66	0.58	0.57	0.57	0.52	0.66	0.66	0.51	0.64	0.61	0.60	0.63	0.53	0.65	0.62	0.57
27	0.56	0.56	0.49	0.59	0.55	0.49	0.47	0.47	0.43	0.54	0.55	0.41	0.53	0.50	0.49	0.51	0.44	0.52	0.49	0.45
28	0.46	0.46	0.40	0.49	0.45	0.41	0.38	0.38	0.35	0.45	0.45	0.33	0.43	0.40	0.40	0.42	0.36	0.43	0.41	0.37
29	0.39	0.39	0.34	0.41	0.38	0.35	0.32	0.32	0.30	0.38	0.38	0.28	0.36	0.34	0.33	0.35	0.30	0.36	0.34	0.32
30	0.32	0.32	0.29	0.35	0.32	0.29	0.27	0.27	0.25	0.32	0.32	0.24	0.30	0.28	0.28	0.29	0.25	0.30	0.29	0.27
31	0.28	0.28	0.25	0.30	0.28	0.25	0.24	0.24	0.23	0.28	0.28	0.21	0.26	0.25	0.23	0.26	0.22	0.26	0.25	0.24
32	0.25	0.25	0.23	0.27	0.25	0.22	0.21	0.21	0.21	0.25	0.25	0.19	0.23	0.23	0.22	0.23	0.20	0.24	0.23	0.22
33	0.23	0.22	0.21	0.24	0.23	0.21	0.20	0.20	0.20	0.23	0.23	0.18	0.21	0.21	0.20	0.21	0.19	0.22	0.21	0.21
34	0.22	0.21	0.20	0.23	0.22	0.20	0.19	0.19	0.19	0.22	0.21	0.18	0.20	0.20	0.19	0.20	0.18	0.20	0.20	0.20
35	0.21	0.20	0.20	0.22	0.21	0.19	0.18	0.19	0.19	0.21	0.21	0.17	0.19	0.20	0.18	0.20	0.18	0.20	0.20	0.20
36	0.21	0.20	0.20	0.22	0.21	0.20	0.19	0.19	0.19	0.21	0.21	0.18	0.19	0.20	0.18	0.20	0.19	0.20	0.20	0.21
37	0.22	0.21	0.21	0.23	0.22	0.20	0.20	0.20	0.20	0.22	0.21	0.19	0.20	0.21	0.19	0.20	0.20	0.20	0.21	0.22
38	0.23	0.22	0.22	0.24	0.24	0.21	0.21	0.21	0.21	0.24	0.22	0.20	0.20	0.22	0.20	0.21	0.21	0.21	0.22	0.23
39	0.24	0.23	0.22	0.26	0.25	0.22	0.22	0.22	0.22	0.25	0.24	0.20	0.21	0.23	0.21	0.22	0.22	0.22	0.23	0.24
40	0.26	0.24	0.23	0.27	0.26	0.23	0.23	0.23	0.23	0.27	0.25	0.21	0.23	0.24	0.22	0.23	0.23	0.24	0.24	0.25
41	0.27	0.25	0.24	0.28	0.27	0.23	0.24	0.24	0.23	0.28	0.26	0.21	0.23	0.25	0.23	0.24	0.24	0.24	0.25	0.25
42	0.28	0.26	0.24	0.29	0.28	0.24	0.25	0.25	0.24	0.29	0.27	0.22	0.24	0.26	0.24	0.25	0.24	0.25	0.26	0.26
43	0.29	0.27	0.24	0.30	0.28	0.24	0.26	0.25	0.24	0.30	0.28	0.22	0.25	0.27	0.24	0.25	0.24	0.26	0.27	0.26
44	0.30	0.27	0.24	0.31	0.28	0.24	0.26	0.25	0.24	0.31	0.29	0.22	0.25	0.27	0.24	0.25	0.24	0.26	0.27	0.25
45	0.30	0.28	0.23	0.31	0.28	0.24	0.26	0.25	0.23	0.31	0.29	0.22	0.25	0.27	0.24	0.25	0.24	0.26	0.27	0.25
46	0.29	0.27	0.22	0.31	0.27	0.23	0.25	0.24	0.22	0.31	0.29	0.21	0.25	0.26	0.23	0.24	0.23	0.26	0.26	0.24
47	0.29	0.27	0.21	0.30	0.26	0.22	0.25	0.23	0.21	0.30	0.28	0.20	0.25	0.25	0.22	0.23	0.22	0.25	0.25	0.23
48	0.28	0.26	0.20	0.29	0.25	0.21	0.24	0.22	0.20	0.29	0.27	0.19	0.24	0.23	0.21	0.22	0.21	0.24	0.24	0.21
49	0.26	0.25	0.18	0.28	0.24	0.20	0.22	0.20	0.18	0.28	0.26	0.18	0.22	0.22	0.20	0.21	0.19	0.23	0.23	0.20
50	0.25	0.23	0.17	0.26	0.22	0.19	0.21	0.19	0.17	0.26	0.25	0.16	0.21	0.21	0.19	0.19	0.18	0.22	0.22	0.18
51	0.23	0.22	0.16	0.24	0.20	0.17	0.19	0.17	0.16	0.24	0.23	0.15	0.20	0.19	0.17	0.18	0.16	0.20	0.20	0.16
52	0.21	0.20	0.14	0.22	0.18	0.16	0.18	0.16	0.14	0.23	0.21	0.14	0.18	0.17	0.16	0.16	0.15	0.18	0.18	0.15
53	0.20	0.19	0.13	0.20	0.17	0.14	0.16	0.14	0.13	0.21	0.20	0.12	0.17	0.16	0.14	0.15	0.13	0.17	0.16	0.13
54	0.18	0.17	0.11	0.19	0.15	0.13	0.15	0.13	0.12	0.19	0.18	0.11	0.15	0.14	0.13	0.13	0.12	0.15	0.15	0.12
55	0.16	0.16	0.10	0.17	0.14	0.12	0.13	0.11	0.11	0.17	0.16	0.10	0.14	0.13	0.12	0.12	0.11	0.14	0.13	0.11
56	0.15	0.14	0.09	0.15	0.12	0.10	0.12	0.10	0.09	0.15	0.15	0.09	0.13	0.11	0.10	0.11	0.09	0.13	0.12	0.10
57	0.13	0.13	0.08	0.14	0.11	0.09	0.11	0.09	0.08	0.14	0.13	0.08	0.11	0.10	0.09	0.10	0.08	0.11	0.11	0.09
58	0.12	0.11	0.07	0.12	0.10	0.08	0.10	0.08	0.08	0.12	0.12	0.07	0.10	0.09	0.08	0.09	0.08	0.10	0.10	0.08
59	0.11	0.10	0.07	0.11	0.09	0.07	0.09	0.07	0.07	0.11	0.11	0.06	0.09	0.08	0.07	0.08	0.07	0.09	0.09	0.07
60	0.10	0.09	0.06	0.10	0.08	0.07	0.08	0.07	0.06	0.10	0.10	0.06	0.08	0.07	0.07	0.07	0.06	0.08	0.08	0.06
61	0.09	0.08	0.05	0.09	0.07	0.06	0.07	0.06	0.06	0.09	0.09	0.05	0.07	0.07	0.06	0.06	0.06	0.07	0.07	0.06
62	0.08	0.08	0.05	0.08	0.07	0.05	0.06	0.05	0.05	0.08	0.08	0.05	0.07	0.06	0.06	0.06	0.05	0.07	0.06	0.05
63	0.07	0.07	0.05	0.08	0.06	0.05	0.06	0.05	0.05	0.08	0.07	0.04	0.06	0.06	0.05	0.05	0.05	0.06	0.06	0.05
64	0.07	0.06	0.04	0.07	0.05	0.05	0.05	0.05	0.04	0.07	0.07	0.04	0.06	0.05	0.05	0.05	0.04	0.06	0.05	0.05
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Supplementary data 2: Coefficients of correlation with relative molar ratios o

DP	Temperature during grain filling		Firming rate (gluten-containing rice bread)		Firming rate (rice flour-containing bread)	
5	-0.48	**	-0.64	**	-0.89	**
6	-0.88	**	-0.87	**	-0.99	**
7	-0.82	*	-0.84	*	-0.96	*
8	0.23		0.04		-0.14	
9	-0.31		-0.39		-0.46	
10	-0.71	**	-0.71	**	-0.61	*
11	-0.73	**	-0.80	**	-0.60	*
12	-0.71	**	-0.64	**	-0.49	
13	-0.76	*	-0.71	*	-0.66	*
14	-0.55	*	-0.48	*	-0.29	
15	-0.37		-0.27		-0.02	
16	-0.45	**	-0.30		-0.13	
17	-0.64	**	-0.50	*	-0.54	
18	-0.63	*	-0.50	*	-0.60	*
19	0.33		0.43		0.55	
20	0.70	**	0.72	**	0.74	**
21	0.75	**	0.76	**	0.78	**
22	0.78	**	0.78	**	0.79	**
23	0.78	**	0.78	**	0.80	**
24	0.80	**	0.84	**	0.80	**
25	0.79	**	0.75	**	0.82	**
26	0.84	**	0.86	**	0.83	**
27	0.83	**	0.84	**	0.86	**
28	0.83	**	0.84	**	0.85	**
29	0.83	**	0.83	**	0.83	**
30	0.82	**	0.82	**	0.81	**
31	0.79	**	0.81	**	0.76	**
32	0.78	**	0.79	**	0.75	*
33	0.74	**	0.75	**	0.67	*
34	0.68	**	0.70	**	0.57	*
35	0.59	*	0.62	*	0.47	
36	0.49	*	0.54	*	0.36	
37	0.45	*	0.49	*	0.34	
38	0.46	*	0.50	*	0.36	
39	0.50	**	0.54	**	0.42	
40	0.56	**	0.58	**	0.50	
41	0.61	**	0.63	**	0.57	*
42	0.67	**	0.69	**	0.64	**
43	0.73	**	0.73	**	0.70	**
44	0.76	**	0.77	**	0.74	**
45	0.79	**	0.79	**	0.78	**
46	0.81	**	0.81	**	0.80	**
47	0.83	**	0.82	**	0.81	**
48	0.84	**	0.83	**	0.83	**
49	0.84	**	0.83	**	0.84	**
50	0.86	**	0.84	**	0.85	**
51	0.86	**	0.84	**	0.85	**
52	0.86	**	0.84	**	0.86	**
53	0.86	**	0.85	**	0.86	**
54	0.87	**	0.85	**	0.87	**
55	0.87	**	0.85	**	0.86	**
56	0.87	**	0.85	**	0.86	**
57	0.87	**	0.85	**	0.86	**
58	0.86	**	0.84	**	0.86	**
59	0.86	**	0.84	**	0.86	**
60	0.86	**	0.85	**	0.86	**
61	0.86	**	0.84	**	0.85	**
62	0.86	**	0.85	**	0.85	**
63	0.86	**	0.84	**	0.85	**
64	0.86	**	0.84	**	0.83	**

* and ** indicates significant difference ($p<0.05$) and ($p<0.01$), respectively.

Supplementary data 3: Changes in rice bread firmness after baking.

Cultivar	Year	Transplanting month	Rice flour + gluten[a]			Wheat flour + rice flour[b]		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Hitomebore	2010	May	74 ± 4 df	115 ± 0 bd	161 ± 13 af	172 ± 17 abc	252 ± 15 a	352 ± 23 a
		June	79 ± 14 cdf	111 ± 18 bd	172 ± 28 acde	-	-	-
		July	104 ± 10 bde	104 ± 12 bd	122 ± 16 df	120 ± 10 e	173 ± 22 c	243 ± 32 cd
	2011	May	83 ± 20 bdf	125 ± 24 bd	191 ± 17 ab	165 ± 10 abd	268 ± 12 a	353 ± 09 a
		June	80 ± 7 cdf	102 ± 16 bd	159 ± 9 af	-	-	-
		July	68 ± 8 ef	88 ± 17 d	105 ± 21 f	182 ± 13 a	253 ± 19 a	340 ± 21 ab
Koshihikari	2009	May	62 ± 3 f	95 ± 15 cd	128 ± 13 cf	177 ± 05 ab	285 ± 12 a	345 ± 22 a
		June	77 ± 3 cdf	93 ± 6 d	115 ± 5 ef	-	-	-
		July	115 ± 14 bc	128 ± 9 bd	143 ± 12 af	183 ± 03 a	252 ± 07 a	300 ± 28 ad
	2010	May	84 ± 22 bdf	127 ± 31 bd	159 ± 20 af	139 ± 13 cde	239 ± 03 ab	320 ± 18 ab
		June	84 ± 8 bdf	120 ± 13 bd	154 ± 19 af	-	-	-
		July	121 ± 8 ab	132 ± 9 ad	151 ± 9 af	158 ± 10 abd	246 ± 06 ab	272 ± 24 bd
	2011	May	85 ± 3 bdf	140 ± 7 ab	182 ± 10 ac	168 ± 14 abd	287 ± 35 a	343 ± 29 a
		June	73 ± 8 ef	106 ± 12 bd	136 ± 24 bcf	-	-	-
		July	85 ± 9 bdf	97 ± 18 bd	134 ± 26 bcf	148 ± 12 be	239 ± 19 ab	314 ± 10 ab
Takanari	2009	May	103 ± 11 bde	139 ± 16 abc	177 ± 31 acd	-	-	-
		June	157 ± 37 a	176 ± 14 a	196 ± 28 a	-	-	-
	2011	May	79 ± 0 cdf	117 ± 3 bd	160 ± 5 af	139 ± 14 de	249 ± 13 ab	312 ± 32abc
		June	73 ± 9 df	95 ± 12 cd	138 ± 26 af	-	-	-
		July	114 ± 4 bd	141 ± 3 ab	161 ± 6 af	124 ± 05 e	201 ± 13 bc	235 ± 24 d
Correlation coefficient with temperature during grain filling			- 0.62 **	- 0.19 ns	0.30 ns	0.10 ns	0.47 ns	0.72 **

[a] 80% rice flour + 20% wheat gluten

[b] 80% wheat flour 20% rice flour (20%)

Means with the same letter are not significantly different at $P < 0.05$ (Tukey's range test).

** $P < 0.01$.