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Research Paper

Analysis of the relationship between bread-making quality and dough stress during the proofing process using near-isogenic lines of 'Harunoakebono'

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The physical properties of various white bread doughs made from the flours of 'Harunoakebono' and 10 genotypes of its near-isogenic lines with different compositions of high molecular weight glutenin subunit (HMWGs) were measured with the Creep method based on a Maxwell–2–element model. The expansion stress in the proofing process of various doughs was obtained by a numerical calculation method. The results indicated that doughs with high elastic characteristics, namely large relaxation time (τ_0) and regularity coefficient of viscosity (η_N), have high dough stress throughout the proofing process and high stress at the proofing end (σ_{end}) and conversely, the low elastic dough with the small τ_0 and η_N has the completely opposite tendency. This study also showed that there are significantly high correlations between the calculated σ_{end} and bread-making quality (BMQ) such as gas retention of dough and specific loaf volume (SLV). These results showed that BMQ, represented by SLV, of various white bread doughs were greatly influenced by the dough's physical properties, especially τ_0 and η_N, which change with differences in the compositions of the HMWGs.

Key Words: wheat (*Triticum aestivum* L.), high molecular weight glutenin subunit (HMWGs), near-isogenic line (NIL), bread-making quality, physical properties, simulation.

Introduction

It is empirically known that the bread-making quality (BMQ), such as gas retention of dough in proofing end (GRD) and specific loaf volume (SLV), are related to the physical properties of dough (PPD). Previous studies have also confirmed that the GRD and SLV of dough and bread are largely influenced by the PPD (Janssen *et al.* 1996, Kawai *et al.* 2006a, 2006b, Rao *et al.* 2000, Takata *et al.* 2000, 2003, Yamauchi *et al.* 2003). It has also been reported that high molecular weight glutenin subunit (HMGWs) compositions, which are wheat seed storage proteins, are closely related to PPD as well (Takata *et al.* 2000, 2003) and greatly affect BMQ (Krattiger *et al.* 1987, Lawrence *et al.* 1987, Takata *et al.* 2000, 2003). They are encoded by the *Glu-1* loci on the long arms of group 1 chromosomes (Payne *et al.* 1980). The alleles at these three loci have different effects on BMQ. However, low molecular weight glutenin subunit (LMWGs) and gliadin, which are also wheat seed storage proteins except for HMWGs, are also related to the PPD (Benedettelli *et al.* 1992, Gupta *et al.* 1994, Ito *et al.* 2011, Jin *et al.* 2013, Zhang *et al.* 2012).

In addition, lipids and pentosans seem to be related to BMQ (Morrison *et al.* 1989, Roels *et al.* 1993). Previous studies have found that HMWGs compositions are a major factor affecting BMQ of wheat flour dough, but there are many other factors that affect BMQ, such as wheat flour characteristics. Therefore, to exclude factors other than the compositions of HMWGs that might have an impact on bread-making properties, 'Harunoakebono' (HA) and 10 genotypes of its near-isogenic lines (NILs), whose genetic background except for the differences in HMWGs compositions is almost the same, were used in these experiments. By using the NILs, it is possible to clearly analyze the relationship between the differences in HMWGs compositions (differences in PPD) and their impact on BMQ.

Previous studies have been done on the relationship between PPD and BMQ, but the behavior of the PPD during the proofing process of bread-making has not been extensively studied. Therefore, it is not clear how the PPD just after preparation affect its behavior in the proofing process

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and how GRD and SLV change by its behavior.

Bloksma (1957) studied the mechanism of dough expansion with an Alveographe, which measures the expansion of dough, using a Maxwell-2-element model (Maxwell model) and calculated stress behavior during dough expansion using numerical analysis. He reported that the stress relaxation time was the most important factor for the expansion ability. Matsumoto (1981) also studied the stress of dough in the fermentation process theoretically using the Maxwell model under the condition of constant cross-section and nominal strain. The study showed that stress during dough expansion changes qualitatively depending on the initial stress. Although these papers explained the stress behavior in the expansion process to some extent, they were not applied to the actual bread making process. These previous studies did not also examine the influence of the initial PPD on the behavior of PPD in the actual expansion process of bread-making. In addition, the correlation between the final stress (σ_{end}) of PPD in expansion process and BMQ has not been analyzed.

In this study, dough samples without yeast with various PPDs were prepared using flours of HA and 10 genotypes of its NILs with different HMWGs compositions. The PPD was expressed with a Maxwell model and the coefficients of physical properties of these various doughs were measured by the Creep method. Subsequently, the changes in various doughs stresses in the proofing process were simulated by numerical analysis, assuming that the PPD did not change in the bread-making process, using the initial PPD data. Based on the obtained results, the relationship between the simulation results and the actual GRD and SLV was analyzed in detail. Specifically, we analyzed the correlation between σ_{end} and BMQ such as GRD and SLV and examined how the initial PPD affects σ_{end}. Finally, the effect of HMWGs compositions on BMQ was discussed.

Materials and Methods

Analytical model of PPD

The deformation model of dough for the numerical analysis is shown in **Fig. 1**. It was assumed that the dough is an aggregate of many cylindrically shaped doughs (left side in **Fig. 1**) and that the dough is stretched in a longitudinal direction by gas inflation at a constant expanding speed of C (m/s) with time t (s), but the total volume is kept constant during the expansion. The initial height of dough are defined as a_0 (m) (right side in **Fig. 1**). From the measurements of gassing power (GP) of actual doughs, the GP of carbon dioxide that the yeast generates is almost constant. Then, assuming that little gas is lost from the doughs, the expansion rate of each dough was set as constant. As will be described later, the expansion rate (C (m/s)) of the dough was obtained from the GP of dough and the average area of the bread pan.

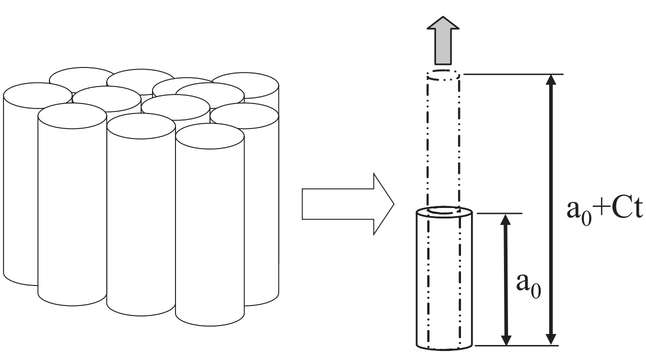

Fig. 1. Dough expansion model for dough stress simulation during the proofing process. a_0: initial height of dough, C: expansion rate of dough, t: time.

By using the data of the actual dough strain rate of longitudinal direction, the strain rate of dough ($\gamma_{(t)}$ (1/s)) at t (s) is defined as Eq. (1).

$$\dot{\gamma}_{(t)} = \frac{1}{a_{(t)}}\frac{da_{(t)}}{dt} = \frac{C}{a_0 + Ct} \qquad \text{Eq. (1)}$$

Here, $a_{(t)}$ (m) is the height of the dough at t (s). From this equation, $\gamma_{(t)}$ is not constant although the expanding speed of the dough is constant.

Next, the Maxwell model was used to represent the PPD as indicated in **Fig. 2**. In this figure, E_0 (Pa), η_N (Pa·s), γ_0 (–), and γ_N (–) are the instantaneous elasticity, the regularity coefficient of viscosity, the strain of instantaneous elasticity region and the strain of regularity coefficient viscosity region, respectively. In addition, the relaxation time (τ_0 (s)) is defined as $\tau_0 = \eta_N/E_0$. The strain rate of dough ($\gamma_{(t)}$) is represented using a Maxwell model as Eq. (2).

$$\dot{\gamma}_{(t)} = \frac{1}{E_0}\frac{d\sigma_{(t)}}{dt} + \frac{\sigma_{(t)}}{\eta_N} \qquad \text{Eq. (2)}$$

Here, $\sigma_{(t)}$ (Pa) is the stress of dough at t during expansion. Equation (3) is obtained by substituting the right side of Eq. (1) for the left side of Eq. (2) and arranging the equation. Equation (3) was obtained to show the stress changes in the

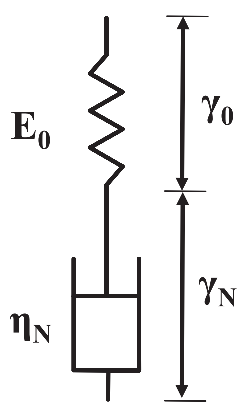

Fig. 2. Maxwell model for analysis of physical properties of dough. E_0: instantaneous elasticity, η_N: regularity coefficient of viscosity, γ_0: strain of instantaneous elasticity region, γ_N: strain of regularity viscosity region.

expansion process of dough.

$$\frac{d\sigma_{(t)}}{dt} = E_0 \frac{C}{a_0 + Ct} - \frac{E_0}{\eta_N}\sigma_{(t)} \qquad \text{Eq. (3)}$$

Here, $\sigma_{(0)}$ (Pa) is the initial stress, which was set as 0 Pa in the numerical analysis of $\sigma_{(t)}$. The $\sigma_{(t)}$ of several doughs with various physical properties was simulated with Eq. (3) by Euler's method.

The main reasons for using the Maxwell model as the PPD model in this research are as follows. (1) Previous reports show that the behavior of PPD to changes during the expansion process can be represented by this model (Matsumoto 1981). (2) This model is simple and it is easy to numerically calculate the stress changes of dough in the expansion process (Bloksma 1957, Kawai *et al.* 2006a). (3) In the expansion process of dough at a very slow strain rate, it is reasonable that the retardation elasticity region, which is normally present in the condition of rapid deformation of dough, is included in the instantaneous elasticity region.

Plant and flour materials

The NILs substituted for single and double HMWGs were used in this study, which were developed by Takata *et al.* (2000, 2003). The NILs were developed by crossing the recurrent parent, HA, and the donor parents, Haruyutaka, Norin 61, Takunekomugi and Chihokukomugi (**Table 1**). The names of various HMWGs encoded by *Glu-1* loci in **Table 1** were the same as those of the report of Takata *et al.* (2003), which is based on the band position of SDS-PAGE. Takata *et al.* (2000, 2003) reported that HMWGs 1 has almost the same properties as HMWGs 2* of HA and has an intermediate effect on the improvement of the strength of dough, that HMWGs 17 + 18, 7 + 8, 6 + 8 and 20 have a slightly stronger, equal, slightly weaker, and very weakening effect on dough strength compared to HMWGs 7 + 9 of HA, respectively and that compared to HMWGs 5 + 10 of HA, HMWGs 2 + 12, 2.2 + 12 and 4 + 12 have the effect of significantly reducing the strength of the dough.

The homozygous genotypes of the NILs were checked for glutenin by SDS-PAGE (Nakamura *et al.* 1990) and for gliadin components by acid polyacrylamide gel electrophoresis (A-PAGE) (Bushuk and Zillman 1978). Eight kinds of NILs substituted for single HMWGs, two kinds of NILs for double HMWGs, and HA, a recurrent parent, were cultivated according to the conditions reported by Yamauchi *et al.* (2007).

Table 1. HMWGs compositions of recurrent parent and donor parents

Cultivar	HMWGs			
	Glu-A1	*Glu-B1*	*Glu-D1*	
HA	2*	7 + 9	5 + 10	recurrent parent
Haruyutaka	<u>1</u>	<u>17 + 18</u>	<u>2 + 12</u>	donor parent
Norin 61	2*	<u>7 + 8</u>	<u>2.2 + 12</u>	donor parent
Takunekomugi	1	<u>6 + 8</u>	<u>4 + 12</u>	donor parent
Chihokukomugi	1	<u>20</u>	<u>2 + 12</u>	donor parent

HMWGs: high molecular weight glutenin subunit, HA: Harunoakebono. The underline shows HMWGs introduced to HA.

The obtained grain samples to which water was added so as to make their moisture content 16% were milled with a Bühler test mill (Bühler Inc., Uzwil, Switzerland) and flours of 60% extraction rate (flours) were obtained. The protein and ash contents (w/w, 13.5% moisture base) were respectively measured using a near-infrared reflectance instrument (Inframatic 8120, PerCon Co., Hamburg, Germany) and the method of the American Association of Cereal Chemists (AACC) as reported by Yamauchi *et al.* (2014).

Dough preparations and bread-making tests

The bread-making tests were done using the no-time method and standard white bread formulation. The tests were partially modified from the method reported by Yamauchi *et al.* (2001). Namely, the proofing temperature in this study was changed from 38 to 30°C. The proofing process was also done until the top height of the dough reached 1 cm from the top edge of the pan.

The optimum amounts of water for dough preparations and bread-making tests were determined with a Farinograph at 500 BU according to the AACC method (1991). The optimum water absorption of HA and NILs flours were 59.4 to 62.0% for dough preparations and bread-making tests.

The GRD was evaluated by measuring the maximum expansion volume of 20 g of dough proofed at 30°C and 85% relative humidity in a cylinder under low pressure, following steps outlined by Yamauchi *et al.* (2000).

The GP of 20 g of dough after bench time was measured at 30°C for 3 hrs at 5 min intervals using a Fermograph II (ATTO Co., Ltd., Tokyo, Japan) as reported by Santiago *et al.* (2015). The GP rates of each 100 g of dough used for bread making in the proofing process were calculated by assuming that the GP rates are constant. Using the GP data, the vertical expansion rates in the proofing process of various doughs were calculated to take into consideration the volume of 100 g of dough after molding and the average area of a baking pan, assuming that the shape of dough after molding was a rectangular parallelepiped.

The SLV of various breads cooled at room temperature for 1 h after baking was measured by the AACC International (AACCI) rapeseed-displacement method (2000). Individual slices from the breads were photocopied using a copy machine to evaluate the size and crumb grain of each bread.

Measuring conditions of PPD

The measurements of PPD were established using the doughs just after mixing. The doughs were divided into 30 g pieces, rounded to a spherical shape, compressed to 1.5 cm thickness using a flat box, put inside a polyethylene bag to prevent water evaporation, and then kept in an incubator at 30°C for 60 min to ensure a flat surface and the stress relaxation of the dough. The actual PPD of various samples were measured via the Creep method using a Rheoner (Model

Table 2. HMWGs compositions, flour protein content, and ash content of HA and NILs of *Glu-1* alleles

Flour samples	HMWGs			Properties of flours	
	Glu-A1	*Glu-B1*	*Glu-D1*	Protein (%)	Ash (%)
HA	2*	7 + 8	5 + 10	10.1 ± 0.2cd	0.39 ± 0.00a
NIL1	1	7 + 9	5 + 10	10.4 ± 0.2abc	0.38 ± 0.01a
NIL6 + 8	2*	6 + 8	5 + 10	9.9 ± 0.2d	0.41 ± 0.01a
NIL7 + 8	2*	7 + 8	5 + 10	9.3 ± 0.1e	0.39 ± 0.02a
NIL17 + 18	2*	17 + 18	5 + 10	10.1 ± 0.1cd	0.41 ± 0.02a
NIL20	2*	20	5 + 10	10.2 ± 0.0bcd	0.39 ± 0.01a
NIL4 + 12	2*	7 + 9	4 + 12	10.5 ± 0.4abc	0.39 ± 0.01a
NIL2 + 12	2*	7 + 9	2 + 12	10.7 ± 0.2a	0.38 ± 0.01a
NIL2.2 + 12	2*	7 + 9	2.2 + 12	10.4 ± 0.1abc	0.39 ± 0.01a
NIL17 + 18/2.2 + 12	2*	17 + 18	2.2 + 12	10.6 ± 0.1ab	0.41 ± 0.01a
NIL20/2.2 + 12	2*	20	2.2 + 12	10.3 ± 0.1abcd	0.40 ± 0.02a

HMWGs: high molecular weight glutenin subunit, HA: Harunoakebono, NILs: near-isogenic lines, NIL: near-isogenic line. The letters of underline shows substituted HMWGs. Data are shown in mean ± SD (n = 3). Protein and ash contents are based on 13.5% moisture content. The values followed by different letters within column are significantly different ($p < 0.05$). The analysis of variance between the data was evaluated using Tukey's multiple range test of Excel statistical software 2012.

RE33005, Yamaden Co., Ltd., Tokyo, Japan) as reported by Kawai *et al.* (2006a). The load for the Creep measurement was 499 Pa. This value was determined to accurately measure the physical property values of various doughs within the range where the relationship between the load and the strain is almost linear. Each coefficient, E_0 and η_N, of the Maxwell model was determined with the automatic analytical software of the Rheoner. Each τ_0 was calculated using the PPD coefficients.

Simulation of dough stress during the proofing process

The simulations of the stresses of various doughs during the proofing process were carried out using Eq. (3) by Euler's method. The boundary and initial conditions for the simulations were $\sigma_{(0)} = 0$ Pa, $a_0 = 1.4 \times 10^{-2}$ m, and C at 0 s = 6.50×10^{-6} m/s, respectively. Since there was almost no difference in GP in all doughs, the average value thereof was used as the C value in all stress simulations. The calculating interval used in Euler's method was 20 s. The value of a_0 was calculated using the volume (98.1 ml) of 100 g of dough just after molding and the average area (68.3 cm^2) of the baking pan, assuming that the dough was a rectangular parallelepiped.

Statistical analysis

All experimental data are shown as mean ± SD, except for τ_0. Significant differences of the data in **Tables 2** and **3** were evaluated using the analysis of variance at 5% level of p-value and Tukey's multiple range test using Excel statistical software 2012 (SSRI Corporation, Tokyo, Japan). Correlation analyses between various data were performed using Microsoft Excel 2012 software.

Results

Characteristics of HA and NILs flours

HMWGs compositions, flour protein content and ash content are shown in **Table 2**. Except for the introduced subunits, these HA and NILs showed the same electrophoresis pattern by SDS-PAGE and the same gliadin bands by A-PAGE (data not shown). All samples, including HA, had hard grain. The flour protein and ash contents ranged 9.3 to 10.7% and 0.38 to 0.41%, respectively. The protein contents of partial NILs (NIL7 + 8, NIL2 + 12 and NIL17 + 18/2.2 + 12) were significantly different from that of HA. Especially, the protein content of NIL 2 + 12 was significantly higher than that of HA and NIL 7 + 8 was significantly lower than HA. However, the range of protein contents of HA and its NILs was 1.4% and not much different. On the other hand, the ash contents were not significantly different among all the samples and the range was also small. The GP from the bread dough did not significantly differ among all samples (data not shown).

Table 3. Physical properties of various bread doughs

Flour samples	E_0 (kPa)	η_N(Pa·s × 10^5)	τ_0 (s × 10^2)
HA	3.64 ± 0.32ab	10.96 ± 2.31abc	3.01
NIL1	3.16 ± 0.35bcd	11.85 ± 2.42ab	3.76
NIL6 + 8	3.60 ± 0.76abc	12.48 ± 2.30a	3.46
NIL7 + 8	3.95 ± 0.28a	13.24 ± 1.90a	3.35
NIL17 + 18	3.29 ± 0.33bcd	12.88 ± 3.02a	3.94
NIL20	3.08 ± 0.34bcd	8.39 ± 1.40cd	2.72
NIL4 + 12	3.60 ± 0.51abc	8.80 ± 1.48c	2.44
NIL2 + 12	3.28 ± 0.39bcd	8.37 ± 2.29cd	2.55
NIL2.2 + 12	2.98 ± 0.23de	5.88 ± 0.37de	1.98
NIL17 + 18/2.2 + 12	3.05 ± 0.47cd	9.39 ± 0.99bc	3.07
NIL20/2.2 + 12	2.04 ± 0.30e	4.66 ± 0.17e	1.94

E_0: instantaneous elasticity, η_N: regularity coefficient of viscosity, τ_0: relaxation time, HA: Harunoakebono, NIL: near-isogenic line. Data are shown in mean ± SD (n = 10–12). The physical properties of dough were measured using no yeast doughs. The values followed by different letters within column are significantly different ($p < 0.05$). The analysis of variance between the data was evaluated using Tukey's multiple range test of Excel statistical software 2012.

Physical properties of various doughs

The doughs for the measurements of various PPDs were prepared using the HA and NILs flours shown in **Table 2**, which were produced by the same way as those used in the bread-making tests except that no yeast was added. Because when leavened doughs are used, PPD measurements are less accurate. The PPD of HA and its NILs are shown in **Table 3**. The E_0, η_N, and τ_0 values ranged from 2.04 to 3.95 kPa, 4.66×10^5 to 13.24×10^5 Pa·s and 1.94×10^2 to 3.94×10^2 s, respectively. The E_0 and η_N values of partial NILs (E_0: NIL2.2 + 12, NIL17 + 18/2.2 + 12 and NIL20/2.2 + 12; η_N: NIL2.2 + 12 and 20/2.2 + 12) were significantly different from the HA. Due to the difference of HMWGs compositions, the τ_0 value drastically changed as the E_0 and η_N values, especially the latter, of PPD changed. In terms of the η_N and τ_0, the NILs with HMWGs 5 + 10 except for NIL 20 (NILs with 5 + 10) showed clearly larger η_N and τ_0 values than the other NILs without HMWGs 5 + 10 except for NIL 17 + 18/2.2 + 12 (NILs without 5 + 10). In addition, NIL 20 and NIL 17 + 18/2.2 + 12 showed a value approximately midway between NILs with 5 + 10 and NILs without 5 + 10, and a value close to the NILs without 5 + 10 or the NILs with 5 + 10, respectively. NIL 20/2.2 + 12 also showed the lowest PPD, very low η_N and τ_0 values, among all samples. NIL 17 + 18 showed large values of η_N and τ_0 due to double strengthening effects of HMWGs 17 + 18 and 5 + 10, and NIL 17 + 18 also showed the largest τ_0 value among all samples.

Simulation results of dough stresses

The simulations of the stresses of various doughs during the proofing process were done by using the values of each coefficient of the various PPDs shown in **Table 3**. The simulation results are shown in **Figs. 3** and **4**. The proofing process on bread-making was done until the top height of the dough reached 1 cm from the top edge of the pan and the time was measured. And the each value of σ_{end} is the respective simulation dough stress in the each proofing end time. These results indicate that all stress curves sharply increased at the initial stage of expansion and then eventually decreased.

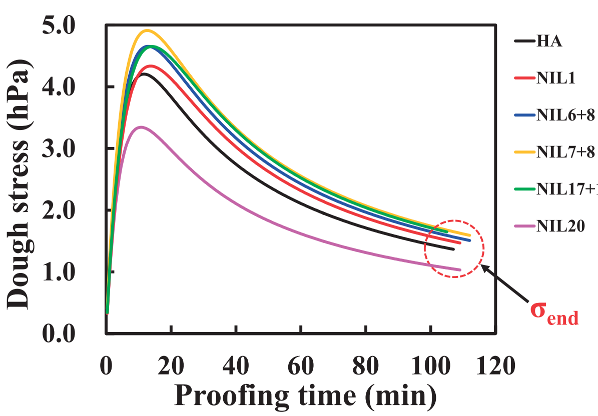

Fig. 3. The stress simulation of doughs of various physical properties in the proofing process. HA: Harunoakebono, NIL: near-isogenic line, σ_{end}: dough stress in end of proofing process.

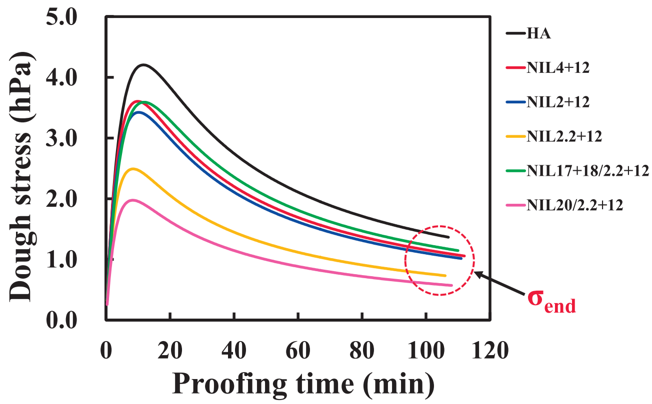

Fig. 4. The stress simulation of doughs of various physical properties in the proofing process. HA: Harunoakebono, NIL: near-isogenic line, σ_{end}: dough stress in end of proofing process.

Fig. 3 shows the simulation results for the dough stresses in the expansion process of NILs substituted for HMWGs of *Glu-A1* and *Glu-B1* alleles. The simulation results of dough stresses of all NILs except for NIL 20 did not differ much from HA. On the other hand, NIL 20 showed a considerably smaller stress peak and σ_{end} value than the HA.

Fig. 4 shows the simulation results of the dough stresses in the expansion process of NILs substituted for HMWGs of *Glu-B1* and *Glu-D1* alleles. Regarding the HA and its NILs in **Fig. 4**, the stress peaks and σ_{end} of all NILs showed lower values than the HA. In particular, those of NIL 2.2 + 12 and NIL 20/2.2 + 12, especially the latter, showed the very low values compared to the other NILs.

Relationship between various PPDs, GRD and SLV

It was found that PPD changed substantially with substitutions of HMWGs (**Table 3** and **Figs. 3**, **4**) and the dough stress in the proofing process also decreased largely due to the weakening of the PPD (mainly η_N and τ_0). The bread-making tests were carried out using the HA and NILs flours shown in **Table 2** and the BMQ data such as GRD and SLV were obtained. The correlations between PPD, GRD and SLV were analyzed. The results are shown in **Table 4**. All PPD were correlated significantly with GRD and SLV in a logarithmic approximation and the correlations between PPD except for E_0, GRD and SLV were extremely

Table 4. Correlation coefficients between PPD, GRD and SLV

	GRD (ml)	SLV (ml/g)
E_0 (kPa)	0.853***	0.646*
η_N (Pa·s × 10^5)	0.951***	0.904***
τ_0 (s × 10^2)	0.851***	0.902***
σ_{end} (daPa)	0.948***	0.916***

The correlation coefficients are a logarithmic approximation coefficient. PPD: physical properties of dough, GRD: gas retention of dough, SLV: specific loaf volume, E_0: instantaneous elasticity, η_N: regularity coefficient of viscosity, τ_0: relaxation time, σ_{end}: dough stress in end of proofing process, *,***: significant level; $p < 0.05$ and $p < 0.001$, respectively.

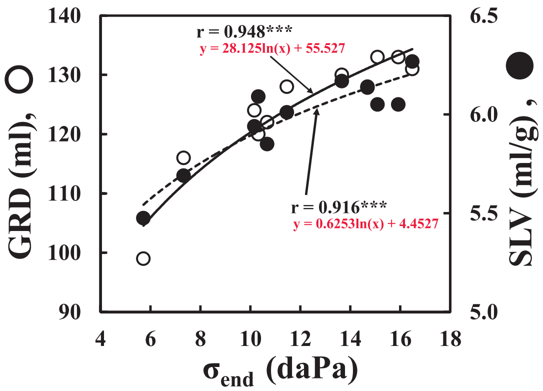

Fig. 5. Correlations between σ_{end}, GRD and SLV. The lines are log correlation curves. GRD: gas retention of dough, SLV: specific loaf volume, σ_{end}: dough stress in end of proofing process, r: correlation coefficient, ***: significant level ($p < 0.001$), y: GRD or SLV, x: σ_{end}.

Table 5. Correlations coefficients between σ_{end} and various values of physical properties

	E_0 (kPa)	η_N (Pa·s × 10^5)	τ_0 (s × 10^2)
σ_{end} (daPa)	0.736**	0.997***	0.946***

The correlation coefficients are a coefficient of linear approximation. E_0: instantaneous elasticity, η_N: regularity coefficient of viscosity, τ_0: relaxation time, σ_{end}: dough stress in end of proofing process, **,***: significant level; $p < 0.01$ and $p < 0.001$, respectively.

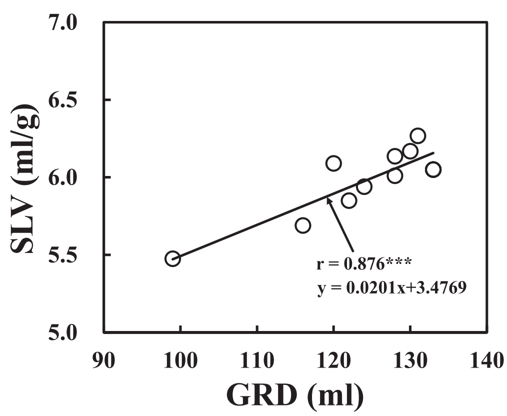

Fig. 6. Correlation between GRD and SLV. The line is a straight line of linear correlation. GRD: gas retention of dough, SLV: specific loaf volume, r: correlation coefficient, ***: significant level ($p < 0.001$), y: SLV, x: GRD.

high and significant at a less than 0.1% level of p-value.

Fig. 5 shows the correlations between σ_{end}, GRD and SLV, which have comprehensively the highest correlation coefficients in **Table 4**. It can be seen that GRD and SLV can mostly be estimated by the value of σ_{end}.

In order to clarify PPD which greatly affects σ_{end} value, the correlations between σ_{end} and various PPDs were analyzed. **Table 5** shows the correlation coefficients between σ_{end} and various PPDs values. It was found that the correlations between σ_{end}, η_N and τ_0 are extremely high and that σ_{end} is mostly determined by the η_N and τ_0 values of the

Fig. 7. Size and crumb grain images and SLV of breads made from doughs of HA and NILs. HA: Harunoakebono, NIL: near-isogenic line, NILs: near-isogenic lines, SLV: specific loaf volume.

doughs in this study.

Fig. 6 shows the correlation between GRD and SLV. There was a significant correlation between GRD and SLV at less than 0.1% level of p-value. This indicates that GRD has a great influence on SLV.

Evaluation of size, crumb grain and SLV of breads

The photocopies and SLV of the breads obtained from 11 HA and its NILs are shown in **Fig. 7**. The biggest bread was obtained from the dough of NIL 17 + 18 and the bread obtained from the dough of NIL20/2.2 + 12 was the smallest. **Fig. 7** also shows that the NILs with 5 + 10 including NIL20, especially NIL17 + 18, had the largest SLV and the best crumb grain and that the NILs without 5 + 10, especially NIL 20/2.2 + 12, had a low SLV and rougher and non-uniform crumb grain. On the other hand, NIL 17 + 18/2.2 + 12 showed a large SLV and relatively good crumb grain among the NILs without 5 + 10, which had characteristics similar to those of HA.

Fig. 7 also showed that the crumb grain of NILs having weak elastic properties (low τ_0), especially that of NIL20/2.2 + 12, exhibited crumb grain with very non-uniform and large bubbles, which indicates that the bubbles in the dough broke and coalesced in the proofing process.

Discussion

Characteristics of flours and physical properties of doughs

Table 2 showed that the protein content of HA and its NILs were somewhat different and that the ash content and GP were almost the same among all samples. These results suggest that the differences of protein and ash contents of flours and GP of doughs among the HA and the NILs hardly influence the differences in PPD and BMQ and that the flour compositions of all samples except for HMWGs compositions were nearly same. **Table 3** showed that the PPD of HA and the NILs, especially η_N and τ_0, greatly change due to the difference of HMWGs compositions. The values of η_N and τ_0 are indicators of the elasticity of PPD and the larger values show that the dough has the stronger elastic properties. The values of η_N and τ_0 of HA and its NILs based on the Maxwell model in this study showed a similar tendency compared to the breaking forces of doughs measured with the same HA and its NILs by Takata *et al.* (2000, 2003). They also examined the influence of various HMWGs on the PPD and reported that HMWGs 5 + 10 and 17 + 18 had the largest and second largest effects on strengthening of the PPD, respectively and that HMWGs 20, 4 + 12, 2 + 12 and 2.2 + 12 had very strong effect to weaken the elastic properties of dough. The data in **Table 3** were mostly consistent with the results reported by Takata *et al.* (2000, 2003).

In addition, much knowledge has been accumulated about the influence of differences in HMWGs compositions on the physical properties of dough (gluten), which is because research on the alleles of three loci (*Glu-A1*, *Glu-B1* and *Glu-D1* locus) of HMWGs has progressed rapidly. Basically, HMWGs, except for HMWGs encoded by *Glu-A1* locus, are composed of two subunits of x-type and y-type encoded by two genes in each locus (Payne 1987). It is known that the y-type subunit has more cysteine residues related to the disulfide bond in its molecular than the x-type subunit (Megan and Skerritt 1999, Morel and Bonicel 1996). Models have also been proposed for glutenin polymers composed of HMWGs and LMWGs that are believed to form a gluten backbone. In these models, HMWGs, especially y-type HMWGs, play a major role in the polymerization of glutenin, and LMWGs bind as branches to the backbone of HMWGs polymers (Shewry *et al.* 2002, Wieser 2007). In these structural models, y-type HMWGs can bind to many HMWGs molecules using many cysteine residues. On the other hand, it is thought that the contribution of x-type HMWGs to the glutenin polymerization in these models is limited, because most x-type HMWGs have only two cysteine residues related to the interchange disulfide bond (Pirozi *et al.* 2008, Shewry *et al.* 2003, Wieser 2007). However, only HMWGs 5 among the x-type HMWGs have three cysteine residues related to interchange disulfide bond and it is known that the contribution to glutenin polymerization is specifically large. On the other hand, x-type HMWGs 20 have only two cysteine residues in the molecule (The other x-type HMWGs except for HMWGs 5 usually have four cysteine residues), so it is believed that the contribution to glutenin polymerization is very small (Megan and Skerritt 1999, Morel and Bonicel 1996, Pirozi *et al.* 2008). Furthermore, it is also reported that the polymerization of glutenin polymers significantly enhances the physical properties of the dough (Orth and Bushuk 1972). From these findings, it is seemed that the remarkable strengthening effect of the dough's physical properties by HMWGs 5 + 10 and the strong weakening effects by HMWGs 20, 4 + 12, 2 + 12 and 2.2 + 12 in this study can be reasonably explained.

Simulation behaviors of dough stresses

Figs. 3 and **4** indicated that all stress curves of doughs of HA and its NILs sharply increased at the initial stage and then decreased. The reason is that the height of the dough is low during the initial stage, the strain rate is high and the increase of stress occurs more rapidly than the relaxation of stress in the initial stage. On the other hand, it seems that the decrease in stress after the peak is because as the height of the dough increases with the fermentation period and causes a decrease in the strain rate, the relaxation of dough stress becomes dominant compared to the increase of stress.

Fig. 3 showed the simulation results for the dough stresses of NILs substituted for HMWGs of *Glu-A1* and *Glu-B1* alleles. As described above, HMWGs 5 + 10 coded by *Glu-D1* allele shows the largest reinforcing effect of the PPD among all HMWGs. Since all HA and its NILs shown in **Fig. 3** have this HMWGs 5 + 10, the simulation results of dough stresses of all NILs except for NIL 20 did not differ much from HA. On the other hand, NIL 20 showed a considerably smaller stress peak and σ_{end} value than the HA. This is considered to be related to that HMWGs 20 coded by *Glu-B1* allele as well as HMWGs 4 + 12, 2 + 12 and 2.2 + 12 coded by *Glu-D1* allele shows the largest weakening effect on the PPD among all HMWGs. In terms of the substitutions of HMWGs of *Glu-A1* and *Glu-B1* alleles, these results suggest that the substitution of only HMWGs 20 greatly affected the PPD of the NIL and the substitution of other HMWGs had little effect on the PPD of NILs.

Fig. 4 showed the simulation results of the dough stresses of NILs substituted for HMWGs of *Glu-B1* and *Glu-D1* alleles. The stress peaks and σ_{end} of all NILs showed lower values than the HA, because all NILs did not have HMWGs 5 + 10. The particular low values of NIL 2.2 + 12 and NIL 20/2.2 + 12, especially the latter, seem to be related to HMWGs 20 coded by *Glu-B1* allele and HMWGs 2.2 + 12 coded by *Glu-D1* allele. HMWGs 2.2 + 12 had the largest weakening effect on the PPD among all of the HMWGs of NILs in **Fig. 4**. NIL 20/2.2 + 12 also showed extremely weak PPD due to double weakening effect of HMWGs 20 and 2.2 + 12 and, as a result, had very low peak stress and σ_{end}.

Relationship between various PPDs, GRD and SLV

Table 4 showed that the correlations between PPD except for E_0, GRD and SLV are extremely high. Since η_N, τ_0 and σ_{end} are the PPD that relate highly to the elastic properties of dough, those results show that good bread having large GRD and SLV, which are indicators of BMQ, can be obtained from dough exhibiting more elastic physical properties.

Fig. 5 showed the high correlations between σ_{end}, GRD and SLV. These results indicated that the more elastic dough, in which the overall dough stress and σ_{end} in the proofing process are higher, shows the higher GRD and that the bread of larger SLV is obtained from the dough. These results basically corresponded to reports by Bloksma (1957) and Matsumoto (1981), which showed that dough exhibiting more elastic properties had better expansion characteristics and BMQ (mainly the size of SLV).

Table 5 showed that the correlations between σ_{end}, η_N and τ_0 are extremely high. Both values of η_N and τ_0 are physical properties that relate to the elastic properties of dough. When those values are high, it is more likely to have elevated dough stress than the relaxation of the stress during the dough expansion process. Therefore, it is reasonable that the correlations between σ_{end}, η_N and τ_0 are high. In addition, although the PPD value that most influences the rise and relaxation of the stress in the dough expansion process is generally considered to be τ_0, the correlation coefficient between σ_{end} and η_N was higher than that between σ_{end} and τ_0 (**Table 5**). This seems to be related to the fact that the E_0 values of PPD in HA and its NILs do not show a large change, but the change of the value of η_N is large. It seems that the main cause is that the η_N values consequently varied in conjunction with τ_0. These results are basically consistent with those reported by Bloksma (1957) that stress relaxation time greatly affects dough expansion stress.

Fig. 6 showed that the very high correlation between GRD and SLV. Yamauchi *et al.* (2000) reported that there was a high correlation between GRD and SLV. However, the correlation between GRD and SLV has not been studied using HA and its NILs flours, which are almost identical in genetic background except for HMWGs compositions. Therefore, they did not find the high correlation between GRD and SLV that can be seen in **Fig. 6**. This suggests that the high correlation between GRD and SLV in **Fig. 6** is mainly caused by that the factors except for the differences of PPD do not almost affect this correlation, which was only influenced by the differences in HMWGs compositions. It was also found that when using the doughs like HA and its NILs, the SLV of the breads is mostly determined by the value of GRD, which is related to the differences in the PPD.

In order to increase the value of this GRD, it is so important to make the PPD more elastic by optimizing the HMWGs compositions (**Figs. 3–6** and **Tables 4**, **5**). Namely, the values of η_N and τ_0 of the PPD are improved by optimizing the HMWGs compositions and, thereby, the σ_{end} value increases. Since σ_{end} is highly correlated with GRD and SLV, better bread having higher SLV is obtained from dough with a higher σ_{end} value, meaning that the dough has more elastic properties. The HMWGs showing the greatest positive effect on this increase in the σ_{end} value (improvement of the PPD) was 5 + 10 and followed by 17 + 18.

Overall BMQ of various doughs of HA and NILs

Fig. 7 showed that the size and crumb grain images and SLV of the breads made from 11 HA and its NILs doughs. These results mostly corresponded to those of the bread-making tests using the same HA and NILs reported by Takata *et al.* (2000, 2003). The photocopies of NILs having weak elastic properties (low τ_0), especially that of NIL20/2.2 + 12, exhibited the crumb grain with very non-uniform and large bubbles. These results were mostly consistent with those reported by Matsumoto (1981, 1991). He reported that when a soft dough with weak elastic properties (low τ_0) expands with yeast fermentation, the bubble membrane in the dough breaks and coalesces during the expansion process.

From the data of the experiments using HA and its NILs doughs with various PPD, it was observed that the σ_{end} value obtained by simulation significantly correlated with the GRD and SLV. Therefore, to obtain a bread having a large SLV and good crumb grain, it is necessary to keep dough stress high and suppress the coalescence of gas bubbles in the dough during the proofing process. BMQ, such as GRD and SLV, are also strongly related to the PPD, especially η_N and τ_0. Among HA and its NILs, NIL 17 + 18 having the most overall elastic PPD showed the best BMQ and NIL 20/2.2 + 12 which had the completely opposite PPD showed the lowest BMQ.

In this study, the HA and its NILs flours with a mostly homogeneous background except for HMWGs compositions were used, and the preparation of the various doughs was strictly controlled and bread-making conditions except for the HMWGs compositions of the flours were all performed under the same conditions. Therefore, the data concerning BMQ in this study are more reliable than those of normal bread-making tests. It is expected to study whether similar results can be obtained using different bread-making conditions and dough compositions with the HA and its NILs flours used in this experiment.

Author Contribution Statement

K. T. and H. Y. conceived and planned this study, and performed the analytic calculations and the numerical simulations. K. M., D. G. and T. N. carried out the experiments. K. T. and H. Y. also took the lead in writing the manuscript.

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