

Effects of ball-milling on the glass transition of wheat flour constituents

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Abstract

BACKGROUND: Starch and gluten, the major components of wheat flour, greatly influence the structural characteristics of food products made with wheat flour. The effects of ball-milling on the change in the semicrystalline structure of starch granules to the amorphous state have been reported. However, the effects of ball-milling of native wheat flour on physicochemical changes in wheat flour constituents have not been elucidated. Therefore in this study the effects of ball-milling on the glass transition of wheat flour constituents were investigated.

RESULTS: Crude gluten, non-gluten proteins and separated starch were obtained from wheat flour ball-milled for 0–10 h, and the glass transition temperature (T_g) of these constituents was evaluated. The T_g of all wheat flour constituents decreased with increasing ball-milling time. Sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed that changes in band position and intensity did not occur for gluten but did occur for non-gluten proteins. X-ray diffraction revealed decreased crystallinity and greater plasticisation by water in separated starch as the ball-milling time was prolonged.

CONCLUSION: The results showed that the ball-milling process decreased the T_g of wheat flour constituents as a function of milling time. The decrease in T_g was probably due to changes in conformation of protein subunits in gluten and depolymerisation of the non-gluten protein fraction. The information obtained here about the physical alteration of wheat flour constituents may enhance the ability to successfully use ball-milled wheat flour in food applications.

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Keywords: wheat flour; wheat flour constituents; ball-milling; glass transition temperature

INTRODUCTION

Wheat flour is a major ingredient in many food products such as breakfast cereals, extruded snacks, baked goods and fried foods. Starch and gluten, the major components of wheat flour, govern important physical properties of products made with wheat flour, e.g. dough rheology (viscoelasticity and extensibility), and consequently the structural characteristics of such foods. These characteristics are important criteria for food processing and food quality. Physical state changes such as gelatinisation or glass transition of the major functional flour components control product processing and quality.¹ For instance, when a wheat flour/water system was used in a frying process, gluten governed the development of porosity in fried dough, while starch governed this process in fried batter.² To optimise the functionality of wheat protein, the addition of various substances to wheat flour has been studied. Li and Lee³ found that adding cysteine to wheat flour markedly decreased the expansion ratio of wheat flour extrudate. Tseng and Lai⁴ studied the addition of microbial transglutaminase to modify the viscoelastic characteristics of wheat flour dough and found that the enzyme affected the extensibility and stickiness of dough because of crosslinks that occur within wheat gluten. In addition, chemical modifications have been used to improve the properties of wheat starch.^{5,6}

Originally, ball-milling was a metallurgical process for reducing the size of mined ore by grinding the ore in a rotating cylindrical mill containing steel balls of different sizes. Today, this process

is applied to modify starch mechanically. Published studies have reported that ball-milling starch at room temperature promotes the change in the semicrystalline structure of starch granules to the amorphous state^{7–10} by the force generated inside the mill, from ball to ball and from ball to wall. Most ball-milling research has focused on the changes in physicochemical properties of various kinds of starch; however, no studies on the physicochemical changes in wheat flour constituents caused by ball-milling action on the native wheat flour have been reported.

There are only a few reports on the use of ball-milled wheat starch and flour for food applications. Schlesinger¹¹ reported that the alteration of the chemical composition of wheat flour by ball-milling had an effect on bread quality related to textural characteristics. There have been no detailed reports on the physical and chemical changes in each flour component. Donelson and Gaines¹² used starch that was separated from ball-milled wheat

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flour to produce completely damaged starch and then mixed it with native wheat flour in a sugar-snap cookie formulation. They found that using a high proportion of ball-milled starch resulted in cookies with a smaller diameter. However, ball-milled wheat flour has seen limited use in food applications probably owing to the lack of information on its physicochemical properties. The present study aimed to elucidate the alteration of wheat flour constituents by ball-milling, focusing on the glass transition of the main wheat flour constituents, in order to provide information helpful in the use of ball-milled wheat flour in food applications.

MATERIALS AND METHODS

Sample preparation

Ball-milling was carried out by using a rolling-type ball-mill (Irie Shokai V-I, Kanagawa, Japan). Samples of 20 g of wheat flour (75 g kg⁻¹ gluten, 120 g kg⁻¹ moisture content; Pioneer Co. Ltd, Kanagawa, Japan) were placed in a stainless steel cylindrical container with a diameter of 9 cm and a height of 9 cm. Each sample was milled with 50 stainless steel balls, 1 cm in diameter. The cylindrical mill was tumbled at room temperature for 0, 2, 5 or 10 h at 90 rpm. The fraction of ball-milled wheat flour was separated into wheat starch, crude gluten and non-gluten proteins, based on the method of Addo *et al.*¹³, using the separation procedure shown in Fig. 1. A 30 g sample of wheat flour was mixed with 10 g of distilled water to form a dough, which was aged for 30 min. The dough was then washed in 100 mL of distilled water five times. The wash water (the mixture of separated wheat starch and non-gluten proteins) was centrifuged at 5000 × *g* for 10 min. The supernatant, i.e. the non-gluten protein fraction, was collected. The non-gluten protein fraction consists of many compounds; however, water-soluble albumins and salt-soluble globulins are considered to be the main components in this fraction. The crude gluten and non-gluten proteins were freeze-dried. The separated starch portion was collected and then left to dry at room temperature (25 °C).

X-ray diffraction (XRD)

XRD measurement of wheat flour was performed using a RINT-UltimaIII diffractometer (Rigaku, Tokyo, Japan). XRD was carried out at 40 kV and 40 mA with Cu K α radiation of wavelength 1.54 Å at a scanning rate of 2° (2 θ) min⁻¹ with a 4–40° scanning range of the diffraction angle at 0.1° intervals.

Differential scanning calorimetry (DSC)

The glass transition temperatures (T_g) of wheat flour, crude gluten, separated starch and non-gluten proteins were examined by DSC (DSC-50, Shimadzu, Kyoto, Japan). For calibration of temperature and heat capacity in DSC measurements, indium (Wako Pure Chemicals, Osaka, Japan) and distilled water were used. α -Alumina was used as the reference material. The moisture content of all samples was adjusted by exposing them to saturated salts. Samples of 20 mg were weighed into the DSC pan. Scanning temperatures of 0–100, 150 and 200 °C with a heating rate of 5 °C min were used for non-gluten proteins, gluten and wheat starch or wheat flour respectively. The DSC pan was punctured after the second scanning to determine the moisture content by drying at 105 °C for 24 h. The glass transition temperature was determined from the onset of an endothermic shift of the baseline using TA-60 software (DSC-50, Shimadzu, Kyoto, Japan) interfaced with the DSC-50.

The T_g curves of wheat flour and all wheat flour components were fitted based on the Gordon–Taylor equation, an empirical

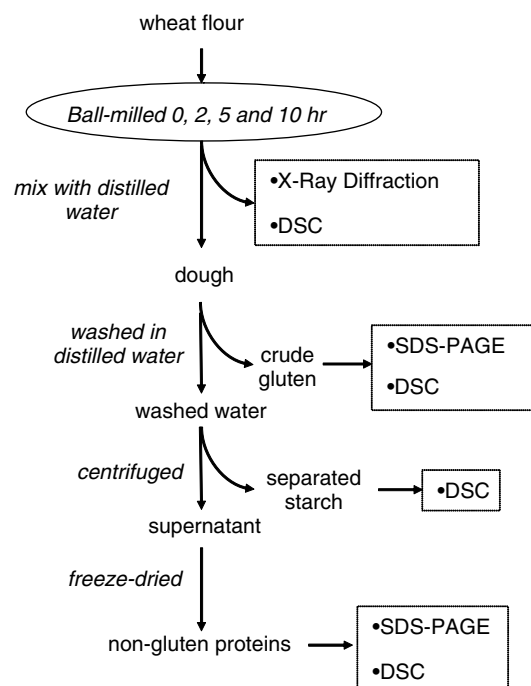


Figure 1. Schematic diagram of sample preparation.

equation for predicting the T_g of mixtures from the T_g values of their components, to determine the increase in T_g with decreasing water content:

$$T_g = (w_1 T_{g1} + k w_2 T_{g2}) / (w_1 + k w_2)$$

where T_{g1} is the T_g of anhydrous solute, T_{g2} is the T_g of amorphous water and k is a constant. A T_g of -135 °C was used for water. The value of k was the mean derived from experimental data on T_g and water content.

Extension of dough

The extension of dough made of 0, 2, 5 and 10 h ball-milled wheat flour was assessed. Dough was sheeted until a final thickness of 1.8 mm was achieved. The sheeted dough was cut into 20 mm wide strips. The strips of dough were folded around a holder and stretched (TTP-50BX Tensipresser, Taketomo, Tokyo, Japan) at 5 mm s⁻¹. Two main parameters were measured: maximum resistance force (R_{max}) and extension.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Samples of 1 mg of freeze-dried crude gluten and 5 mg of freeze-dried non-gluten proteins were weighed into 2 mL Eppendorf™ tubes. A 100 μ L aliquot of phosphate-buffered saline (PBS) and 100 μ L of 2× sample buffer solution (2-Mercaptoethanol (2 ME+); Wako Pure Chemicals) containing 0.125 mol L⁻¹ Tris–HCl, 40 g kg⁻¹ SDS, 200 g kg⁻¹ glycerol, 0.02 g kg⁻¹ bromophenol blue and 100 g kg⁻¹ 2-mercaptoethanol were added and the tube contents were mixed for 2 min. In order to increase the solubility of gluten in the sample buffer, the sample solutions of both glutes were preheated in a water bath at 50 °C for 3 h. All samples were heated at 100 °C for 30 min. Aliquots of 20 μ L of all sample solutions and 5 μ L of protein standard marker (Precision Plus Protein™ Standards,

Bio-Rad, Hercules, CA, USA) were loaded on 80 g kg⁻¹ SDS gel with running solution (Wako Pure Chemicals) at 20 mA for 100 min. After that time the gel was stained for 24 h and de-stained with de-stain solution.

RESULTS

XRD

The XRD patterns of wheat flour ball-milled for various times are shown in Fig. 2. Although there are many components in wheat flour, preliminary tests indicated that there were no peaks for gluten, which was separated from wheat flour, in the observed scanning range. Hoseney *et al.*¹⁴ suggested that gluten is an amorphous random polymer lacking long-range order. Therefore the obtained peaks depict the effect of the wheat starch component. The native wheat flour showed peaks at approximately 15–27° (2θ) in the XRD pattern, which represent A-type crystallites. The XRD pattern of ball-milled wheat flour exhibited a broader shallow peak with increasing ball-milling time. This indicated that the semicrystals in ball-milled wheat flour became partially amorphous.

DSC and glass transition

T_g values of wheat flour and wheat flour constituents were determined by DSC. Typical DSC thermograms are shown in Fig. 3. Enthalpy relaxation in glassy amorphous regions, displayed as an endothermic peak, was observed in the first scan. However, the T_g in the first scan could not be seen clearly owing to the sub-endothermic peak and its thermal history, so the onset temperature in the second scan was considered as the T_g in this study. T_g curves were constructed from T_g and moisture content values as shown in Figs 4 and 5. Since the T_g of the anhydrous polymer cannot be determined owing to thermal decomposition, the Gordon–Taylor equation was used to predict the T_g values of anhydrous wheat flour constituents. This equation is generally used for binary systems and was applied in this study for predicting water plasticisation of wheat flour constituents. This was done using our data, assuming that the T_g of water is -135 °C. The predicted T_g values and corresponding k values for wheat flour and wheat flour constituents are shown in the insets of Figs 4 and 5. The predicted T_g and k values of anhydrous native wheat flour were 160 °C and 4.06 respectively (Fig. 4). The predicted T_g

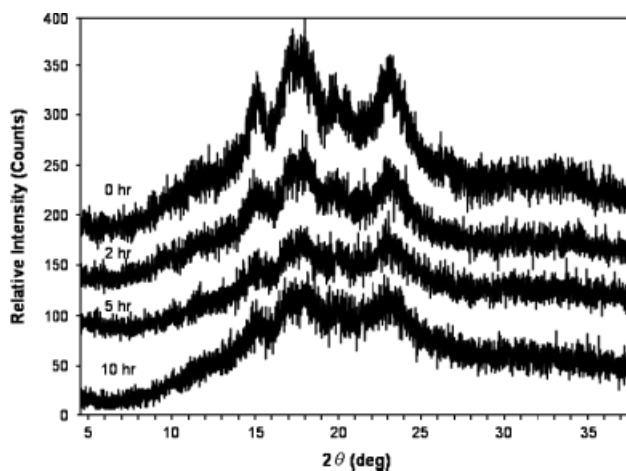


Figure 2. XRD patterns of wheat flour ball-milled for various times.

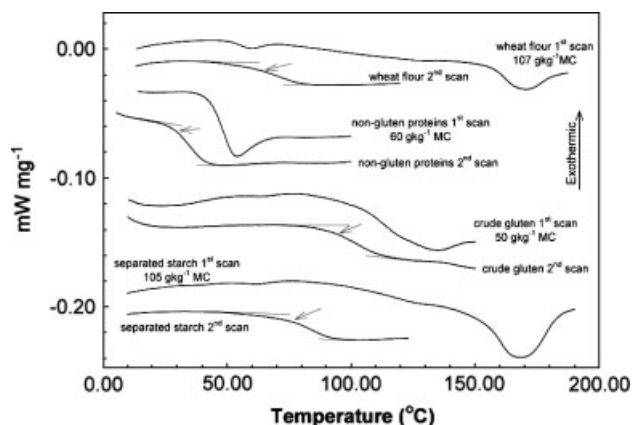


Figure 3. Typical DSC thermograms of wheat flour, crude gluten, separated starch and non-gluten proteins.

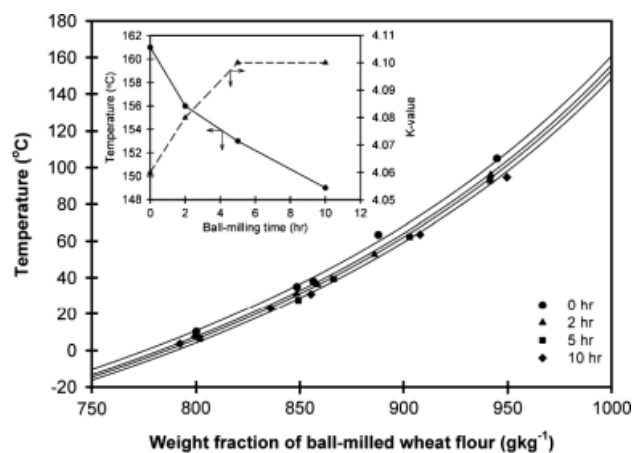


Figure 4. T_g curves of wheat flour ball-milled for various times.

and k values of anhydrous crude gluten obtained from native wheat flour were 155 °C and 5.84 respectively (Fig. 5(a)). The T_g and k values of anhydrous non-gluten proteins obtained from native wheat flour were 100 °C and 7.22 respectively (Fig. 5(b)). In the case of separated starch the predicted T_g was 193 °C and the k value was 4.77 (Fig. 5(c)). It was noticeable that the T_g of wheat flour was lower than that of separated starch but higher than that of crude gluten. This may be due to the greater heterogeneity of the molecular arrangements in the native flour. T_g decreases with decreasing molecular weight.¹⁵ In a previous study,² two different T_g values were also identified in the second scan of defatted wheat flour, indicating that these two transitions probably correspond to a starch-rich phase for the higher-temperature transition and to a gluten-rich phase for the lower-temperature transition.^{16–20} The oil removal from wheat flour in our previous study probably induced the immiscibility of gluten and the starch fraction, making it possible to observe two different glass transitions. In the present study, however, only a single apparent T_g for wheat flour was observed by DSC. This result indicates that the composition of wheat flour affected T_g .

The T_g values of crude gluten, non-gluten proteins and separated starch tended to decrease as the ball-milling time increased. However, the k values of these components had a tendency to increase with increasing ball-milling time. The parameter k in the Gordon–Taylor equation expresses the

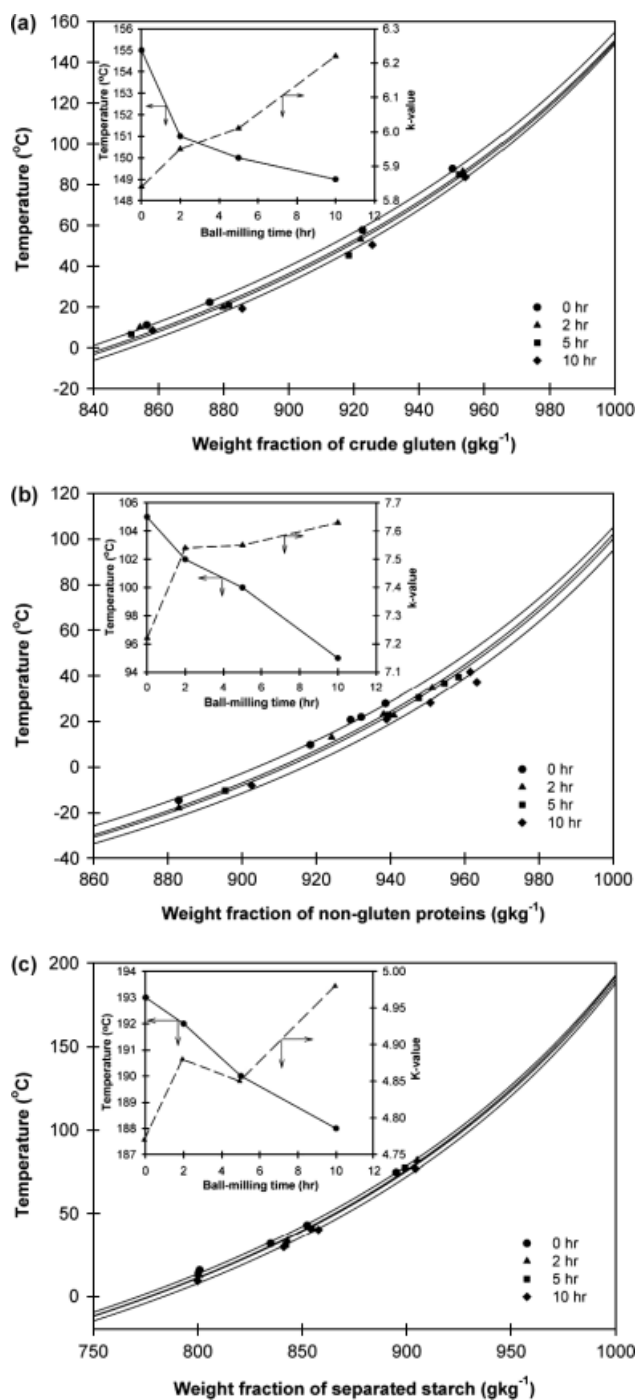


Figure 5. T_g curves of (a) crude gluten, (b) non-gluten proteins and (c) separated starch from wheat flour ball-milled for various times.

curvature of the T_g composition dependence and can be semiquantitatively related to the strength of the interaction between two polymers. It can be expected that a higher k value indicates better plasticisation.¹⁷ This finding suggests that the ball-milling process promotes an increase in the water plasticisation effect in separated starch, crude gluten and non-gluten proteins. This is probably due to an increase in hydrophilic groups in these compounds as a result of the ball-milling process. Water molecules can form hydrogen bonds with these hydrophilic groups.²¹ In addition, non-gluten proteins had higher k values than the other

components with increasing ball-milling time and consequently were more sensitive to plasticisation by water.²²

Extension of dough

Gluten has been reported to be the main substance influencing the structure and quality of wheat flour dough and thus governing desirable properties of baked goods and extruded products. The extension of dough is considered to reflect the gluten functionality of wheat flour and to consequently influence the characteristics of the final product. The main parameters characterising the qualities of gluten in dough are the extension of dough and the maximum resistance to extension (R_{max}). Their values are shown in Table 1. The extensibility results revealed that the ball-milling process affected the rheological properties of wheat flour dough. Dough made from flour that had undergone a longer ball-milling time had lower extensibility and higher maximum resistance to extension. Dough extensibility has been widely reported to be related to high-molecular-weight glutenin subunits.²³

SDS-PAGE

Figure 6 depicts the changes in molecular weight of wheat flour proteins due to ball-milling as assessed by SDS-PAGE. The SDS-PAGE patterns of crude gluten indicated an apparent molecular weight in the range 60–90 kDa. No changes in gluten subunit bands of wheat gluten with increasing ball-milling time were observed. With regard to non-gluten proteins, it was found that the intensity of the 50–75 and 15–20 kDa bands decreased as the ball-milling time increased. This suggests that the action of ball-milling affects non-gluten proteins in these molecular weight ranges.

DISCUSSION

Gluten and non-gluten proteins

A T_g alteration in crude gluten and non-gluten proteins obtained from ball-milled wheat flour was observed. The results showed that ball-milling did not change the molecular weight but did influence the T_g of crude gluten. Ball-milling affected both the molecular weight and T_g of non-gluten proteins.

Wheat gluten proteins are generally divided into glutenin and gliadin fractions. Glutenins form the multichain structure of polypeptides held together by disulfide bonds. Gliadins are single polypeptide chains (monomeric proteins). The associations between gliadins and glutenins are mostly non-covalent bonds, although covalent bonding is possible through disulfide interchange reactions.²⁴ Disulfide bonds play a role in the association of glutenin subunits to form glutenin polymer and also establish gluten by combining glutenin polymer with gliadin. The results

Table 1. Extension and maximum resistance force (R_{max}) of dough made from wheat flour ball-milled for various times

Ball-milling time (h)	R_{max} (kg)	Extension (mm)
0	0.0544 ± 0.017b	56.88 ± 2.07a
2	0.0893 ± 0.017b	53.43 ± 9.52a
5	0.1173 ± 0.036a	42.48 ± 9.89b
10	0.1557 ± 0.023a	34.85 ± 3.21b

Means with the same letter within a column are not significantly different ($P < 0.05$).

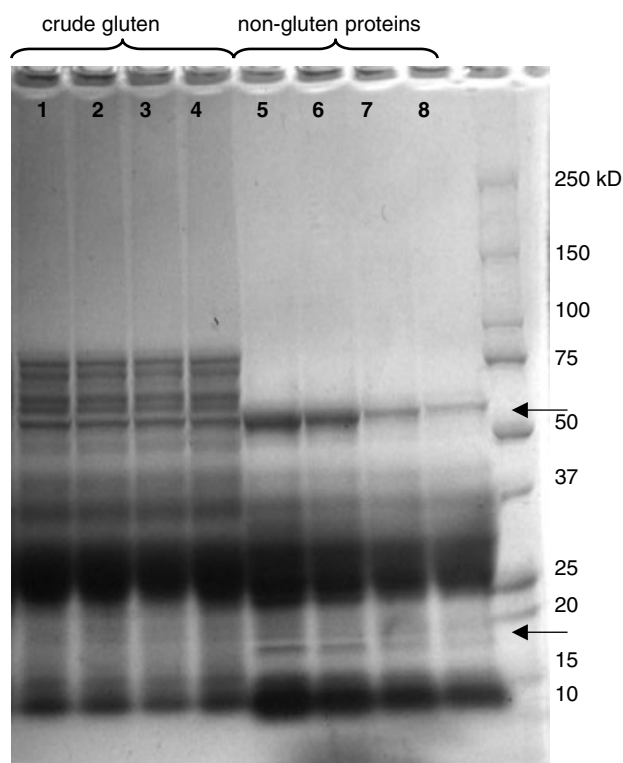


Figure 6. SDS-PAGE patterns of crude gluten (lanes 1–4) and non-gluten proteins (lanes 5–8) from wheat flour ball-milled for 0, 2, 5 and 10 h respectively.

of SDS-PAGE indicated that the band position and intensities of gluten in all treatments were not changed. Kieffer *et al.*¹⁸ reported that the cleavage and rearrangement of disulfide bonds, which were confirmed by SDS-PAGE, did not occur when gliadin was subjected to high pressure (0.1–800 MPa).

T_g depends more on the average degree of polymerisation of polypeptide chains than on whole protein molecules.²⁵ During ball-milling, the impact force may randomly attack covalent bonds and change the conformation of protein subunits in gluten. Conformational changes of protein subunits in gluten macropolymer due to the impact force from ball-milling may also be possible. These probably lead to interrupted thermoset network formation via disulfide crosslinks during heating, with consequent reduction in the interaction between chains of gluten macropolymer. Kieffer *et al.*¹⁸ reported that increased pressure and heating had strong effects on the rheological properties of gluten and led to an increase in gluten strength via the cleavage of intra-chain disulfide bonds and their arrangement into inter-chain bonds. The decrease in the interaction between polymer chains and network cohesion results in T_g depression.²⁶ Moreover, the cleavage of covalent bonds that had linked subunits may be considered to be the reason for the depression of T_g of ball-milled gluten. Some studies have reported that glutenin macropolymer, which is composed of glutenin subunits, is easier to cleave during mechanical mixing. Low-molecular-weight glutenins are more susceptible than high-molecular-weight glutenins to cleavage from the polymer.²⁷ In addition, Singh and MacRitchie¹⁹ reported that disulfide bonds linking subunits of glutenin macropolymer will be broken preferentially by the effects of sonication on wheat gluten, similar to the subunit cleavage during dough mixing. However, in the present study the proportion of unextractable

polymeric protein was not determined. Thus it is difficult to clarify the mechanism and kinetics of covalent bond disruption by ball-milling.

The non-gluten protein fraction of wheat flour is considered to consist mainly of water-soluble albumins and salt-soluble globulins.²⁸ The high-molecular-weight globulins have molecular weights of 52–58 and 22–23 kDa and the high-molecular-weight albumins have molecular weights of 69, 63, 60 and 45 kDa.²⁹ Thus the bands that appeared in the SDS-PAGE patterns (Fig. 6) reflect a mixture of globulins and albumins. The results of SDS-PAGE indicated that the impact force from the balls led to the disappearance of bands at 50–75 and 15–20 kDa. Hence the depression of T_g is considered to be due to the increasing depolymerisation of non-gluten proteins during the ball-milling process. However, it is difficult to identify which proteins correspond to the disappearance of bands in these ranges. Gomes *et al.*³⁰ reported that the effect of high pressure above 400 MPa on soluble proteins in wheat flour led to the loss of bands corresponding to a molecular weight of about 60 kDa, which may be β -amylase. However, SDS-PAGE is a qualitative method for detecting the molecular weights of proteins; more precise quantitative methods will be needed to further confirm our hypotheses regarding gluten and non-gluten proteins in future studies.

Starch

The ball-milling process has been reported to influence the crystallinity of starch. In the present study, XRD of wheat flour revealed that ball-milling increased the amorphous nature of starch granules in wheat flour subjected to the process. This result agrees with the decrease in crystallinity found in ball-milled wheat starch³¹ and ball-milled corn starch.³² Morrison and Tester⁷ found that the amylose content of wheat starch did not change with prolonged ball-milling time, while the low-molecular-weight amylopectin content increased, i.e. the starch underwent a decline in crystallinity. Hosney³³ reported that ball-milling starch at room temperature will eventually destroy completely both the birefringence and the XRD pattern. Semicrystalline starch granules absorb a relatively small amount of water and preserve their glassy state.²¹ From the results obtained in the present study, the increase in k values with increasing ball-milling time suggests that this process decreased the crystallinity of starch granules, leading to an increase in free volume and easier plasticisation by water.

Separated starch obtained from ball-milled wheat flour tended to show lower T_g values than those of native starch. This result coincides with the lower T_g in pregelatinised wheat flour reported by Pereira and Oliveira.²⁰ Chung *et al.*³⁴ also reported that the T_g of gelatinised starch is lower than that of native starch. The crystalline regions suppress the mobility of amorphous molecules because they behave as crosslinkages between amorphous regions of the polymeric network.³⁵ Nevertheless, small molecules are generated by depolymerisation.⁸ Morrison and Tester⁷ reported that amylose is barely affected by physical damage to the granule, whereas amylopectin is progressively degraded into low-molecular-weight fragments that could be considered to affect the depression of T_g in ball-milled starch. In summary, we conclude that the semicrystalline structure in wheat starch granules can become amorphous as a result of the ball-milling process regardless of whether the material subjected to ball-milling is wheat flour or wheat starch.

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