

Effects of Transglutaminase Enzyme on Fundamental Rheological Properties of Sound and Bug-Damaged Wheat Flour Doughs

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ABSTRACT

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Transglutaminase (TG) catalyzes the formation of nondisulfide covalent crosslinks between peptide-bound glutaminyl residues and ϵ -amino groups of lysine residues in proteins. Crosslinks among wheat gluten proteins by TG are of particular interest because of their high glutamine content. Depolymerization of wheat gluten proteins by proteolytic enzymes associated with bug damage causes rapid deterioration of dough properties and bread quality. The aim of the present study was to investigate the possibility of using TG to regain gluten strength adversely affected by wheat bug proteases. A heavily bug-damaged (*Eurygaster* spp.) wheat flour was blended with sound cv. Augusta or cv. Sharpshooter flours. Dynamic rheological measurements, involving a frequency sweep at a fixed shear stress, were performed after 0, 30, and 60 min of incubation on doughs made from sound or blended flour samples. The complex moduli (G^*

values) of Augusta and Sharpshooter doughs blended with 10% bug-damaged flour decreased significantly after 30 min of incubation. These dough samples were extremely soft and sticky and impossible to handle for testing purposes after 60 min of incubation. To test the possibility of using TG to counteract the hydrolyzing effect of bug proteases on gluten proteins, TG was added to the flour blends. The G^* values of TG-treated sound Augusta or Sharpshooter doughs increased significantly after 60 min of incubation. The G^* values of the Augusta or Sharpshooter doughs blended with bug-damaged flour increased significantly rather than decreased after 30 and 60 min of incubation when TG was included in the dough formulation. This indicates that the TG enzyme substantially rebuilds structure of dough hydrolyzed by wheat bug protease enzymes.

Modification of proteins by enzymes such as transglutaminase (TG) has recently become of great interest to food scientists (Larree et al 1993; Sakamoto et al 1994; Chobert et al 1996; Dickinson and Yamamoto 1996). TG enzyme (R-glutaminyl-peptide: amine γ -glutamyltransferase; EC 2.3.2.13) may catalyze conversion of soluble proteins to insoluble HMW protein polymers through formation of nondisulfide covalent crosslinks (Folk and Chung 1973; Folk and Finlayson 1977). Therefore, this enzymatic reaction is a promising method to modify proteins and improve their functional properties. The enzyme catalyzes acyl-transfer reactions between peptide-bound glutaminyl residues and primary amines. When ϵ -amino groups of protein-bound lysyl residues act as acyl acceptors, intra- or intermolecular ϵ -(γ -glutamyl) lysyl isopeptide crosslinks are formed by the enzyme-catalyzed reaction (Folk and Chung 1973; Folk and Finlayson 1977). Application of this crosslinking to wheat gluten proteins would be of particular interest because of their high glutamine content (approximately one-third of the total amino acids). A number of studies have shown that TG catalyzes the formation of homologous and heterologous polymers between milk, meat, soybean, and wheat gluten proteins (Motoki and Nio 1983; Kurth and Rogers 1984). Iwami and Yasumoto (1986) succeeded in introducing lysine into wheat gluten through crosslinking using TG to improve the nutritional value. Other researchers used three different TG enzymes and reported that while two of the TG enzymes were not useful for baking applications, the third one positively improved the properties of bread (*unpublished data*). Gerrard et al (1998) indicated that TG may produce beneficial effects during breadmaking that are comparable to traditional oxidizing improvers.

Preharvest bug damage to wheat caused by *Eurygaster* spp. and *Aelia* spp. occurs in many countries of the Middle East, Eastern Europe, and North Africa (Paulian and Popov 1980). In New Zealand, a bug causing similar damage has been identified as *Nysius huttoni* (Cressey et al 1987). These bugs attack developing wheat kernels, and the infested grain contains a protease that breaks down the gluten structure of dough (Cressey and McStay 1987, Sivri and

Köksel 1996, 1998; Sivri et al 1998, 1999). Dough prepared from bug-damaged flour is runny and sticky and produces poor quality bread (Kretovich 1944; Matsoukas and Morrison 1990; Every 1992, 1993).

Rheological and baking studies have shown that wheat containing >5% bug-damaged kernels is unacceptable for producing good quality bread (Karababa and Ozan 1998). There have been some attempts to use various treatments to counteract the negative effects of damage. Among those published are the use of hydrothermal and microwave treatments before milling (Ertugay et al 1995; Sivri and Köksel 1998); use of low levels of organic acids (Kretovich 1944) and calcium chloride (Diraman and Demirci 1997) in dough formulations; and utilization of shorter fermentation periods in breadmaking (Matsoukas and Morrison 1990).

The main aim of this study was to investigate the possibility of using TG to repair the structure of gluten proteins hydrolyzed by wheat bug proteases. Effects of TG enzyme on the fundamental rheological properties of doughs prepared from sound wheat flours, as well as from flours blended with bug-damaged wheat flour and soy protein isolate, were studied using an oscillatory rheometer.

MATERIALS AND METHODS

Materials

Two sound wheat cultivars (Augusta, a soft white winter, and Sharpshooter, a hard red spring) and a Suni bug-damaged wheat cultivar (Gün-91, hard red winter) were used in this study. The sound wheat cultivars were chosen to represent weak (Augusta) and strong (Sharpshooter) physical dough properties. The bug-damaged wheat sample was obtained from the Turkish Grain Board in 1996. This sample had \approx 80% damaged kernels, and it was one of the most severely bug-damaged wheats tested in the laboratories of the Turkish Grain Board that year. Straight-grade flours were obtained from these wheat samples by milling the three wheat samples in a Buhler laboratory mill. Commercial soy protein isolate (SPI, Supro 670, and bacterial TG were obtained from Protein Technologies International (St. Louis, MO) and Ajinomoto (Teaneck, NJ), respectively.

Dough samples were prepared in a 35-g bowl mixograph (National Manufacturing Co.). First, the dry ingredients (sound wheat flour, damaged wheat flour, TG enzyme, SPI) were placed into the mixograph bowl as required and mixed for 2 min, then water was added using the amount obtained from the farinograph water absorption determined according to Approved Methods (AACC 2000). Finally, the dough was mixed to optimum development. The dough samples were made from sound or blended (90% sound and 10%

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bug-damaged flours) flours treated with or without TG (1.5% w/w), with or without SPI (3% w/w), or both. The various dough samples produced were sound flours (controls), TG-treated sound flours, SPI-treated sound flours, TG-SPI-treated sound flours, blended flours, SPI-treated-blended flours, TG-treated-blended flours, and TG-SPI-treated-blended flours.

Rheological Measurements

Dynamic oscillation was performed on a Haake RS 100 rheometer (Paramus, NJ) using 20-mm diameter serrated parallel plates. Dough (1.5 g) was placed between the plates, and the gap adjusted to 1.5 mm. Outer edges were coated with corn oil to prevent drying. The dough was rested between the plates for 3 min before testing so that the residual stresses would relax (Faubion et al 1985). Samples of each dough were evaluated immediately after mixing and also after resting for 30 and 60 min. The resting (process) was performed in a bowl placed in a ziplock plastic bag at 30°C in a water bath. Doughs were tested at strain amplitudes $\leq 0.2\%$ through a frequency sweep from 0.1 to 25.1 Hz at a constant temperature of 30°C. All tests were run in the linear range of viscoelastic behavior using standard methods (Steffe 1996). Overall structure was evaluated by comparing plots of the complex modulus (G^*) as a function of frequency. Plots of the storage modulus (G') and the loss modulus (G'') showed the same frequency dependent trend as G^* . The G^* is a more convenient indicator of overall structure because it combines both parameters: $(G^*)^2 = (G')^2 + (G'')^2$.

RESULTS AND DISCUSSION

Effects of TG and SPI on Sound Wheat Flour

The effects of TG on complex moduli (G^*) of Augusta and Sharpshooter flour doughs are presented in Fig. 1A and B, respectively. (Hereafter, results mainly from one representative cultivar will be

presented.) The G^* values of Augusta and Sharpshooter control doughs decreased after 60 min of resting period, as expected. Dong and Hosney (1995) reported that dough tested immediately after mixing had a higher storage modulus (G') and a lower loss tangent than the same dough that was allowed to rest for 180 min before testing. They suggested that during resting a number of factors may affect rheological properties: relaxation of the stress introduced during mixing, redistribution of water, continued hydration of flour components, and sulfhydryl-disulfide interchange reactions. The G^* values of Augusta and Sharpshooter doughs supplemented with TG were comparable to those of respective control doughs at 0 min of incubation. However, G^* values of TG-treated Augusta and Sharpshooter doughs increased significantly after 60 min of incubation. Viscoelastic properties of dough are primarily related to its continuous protein phase. Therefore, an increase in the average molecular weight of gluten proteins due to TG activity would be

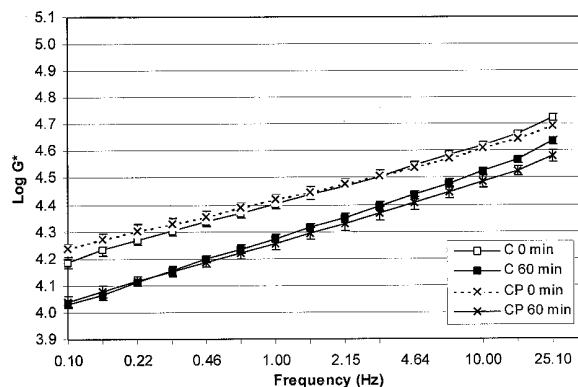


Fig. 2. Variations of G^* with frequency for Sharpshooter control and soy protein isolate (SPI) treated doughs. C = control and CP = control and SPI.

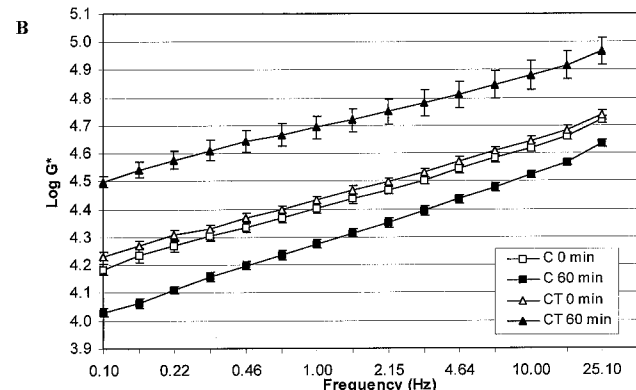
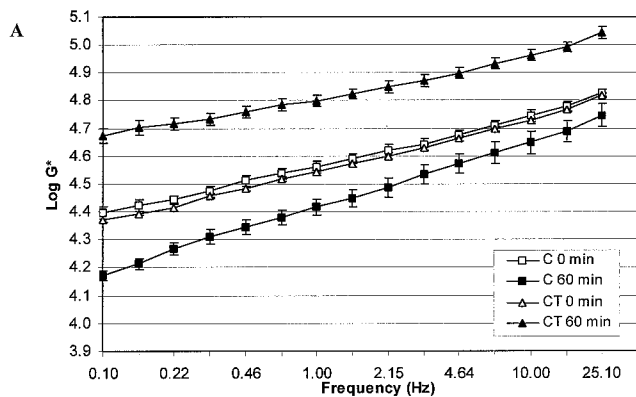


Fig. 1. Variations of G^* with frequency for Augusta control (A) and Sharpshooter control (B) and transglutaminase (TG) treated doughs. C = control and CT = control and TG.

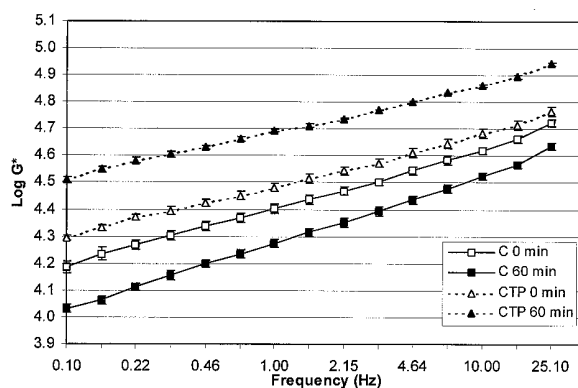


Fig. 3. Variations of G^* with frequency for Sharpshooter control and transglutaminase (TG) and soy protein isolate (SPI) treated doughs. C = control and CTP = control and TG and SPI.

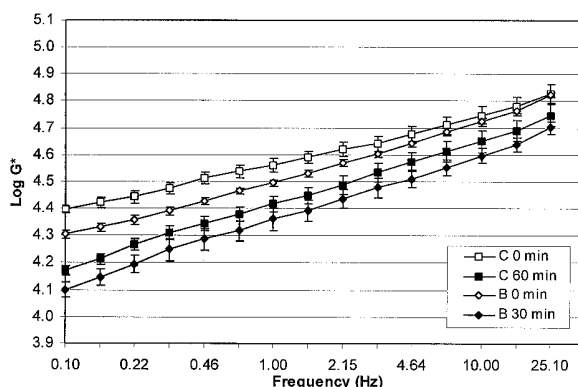


Fig. 4. Variations of G^* with frequency for Augusta control and blended doughs. C = control and B = blended.

expected to cause an increase in the complex modulus. In a recent study, Larre et al (2000) reported that G' values increased in TG-treated gluten at all frequencies tested. This is in agreement with results from our study. Using a miniature extensigraph, Gerrard et al (1998) showed that TG had comparable effects to traditional oxidizing improvers. They also reported that TG reduced the required work input and substantially increased the water absorption of the dough.

Gluten proteins are rich in glutamine but poor in lysine. The low lysine content could have been a limiting factor for the TG reaction. Therefore, a commercial SPI, Supro 670, was added at a 3% (w/w) level to increase concentration of lysyl residues and possibly enhance the extent of crosslinking of protein matrix by TG. The effects of SPI supplementation on the G^* of Augusta and Sharpshooter flour doughs were investigated. The G^* values of the Augusta (data not presented) and Sharpshooter (Fig. 2) doughs supplemented with SPI decreased significantly after a 60-min resting period. The decreases in G^* values (between 0 and 60 min) of SPI-supplemented Sharpshooter doughs were comparable to the corresponding decreases for Sharpshooter control doughs. However, the decreases in G^* values of the SPI-supplemented Augusta doughs were slightly smaller than the corresponding decreases for Augusta control doughs, especially at higher frequencies. For Sharpshooter samples, there were no significant differences in G^* values between SPI-supplemented and control doughs at either 0 or 60 min. However, for Augusta samples, SPI-supplemented doughs exhibited significantly higher G^* values than the control doughs at both 0 and 60 min of incubation.

The effects of TG on the G^* of Augusta and Sharpshooter flour doughs supplemented with SPI were examined. The G^* values of Augusta dough supplemented with both TG and SPI were comparable to those of Augusta control dough at 0 min (data not shown).

However, the G^* values of TG-SPI-treated Sharpshooter doughs were significantly higher than those of Sharpshooter control dough at 0 min (Fig. 3). The G^* values of TG-SPI-treated Augusta and Sharpshooter flour doughs increased significantly after 60 min of incubation. However, the increases were comparable to those from the respective TG-treated Augusta and Sharpshooter doughs without SPI (Fig. 1). These results indicate that addition of SPI did not significantly influence the action of TG. This might be explained by the reactivity of lysine residues in gluten proteins. Larre et al (2000) suggested that protein crosslinking by the TG enzyme was efficient in gluten despite its low lysine content. They have shown, using SDS-PAGE, that all of the gluten proteins were substrates and that the reactivity of HMW-glutenin subunits (GS) was the highest. Although the lysine content of SPI is high, TG probably did not crosslink SPI and gluten proteins extensively through lysine residues of SPI and glutamine residues of gluten proteins. Most of the available lysine present within gluten proteins might have reacted to form large gluten polymers and the remaining monomeric proteins of SPI were probably not able to participate in the TG reaction. Therefore, the added commercial SPI was not able to enhance the extent of crosslinking of protein matrix by TG to any significant extent. Although it is theoretically possible to use the TG reaction to synthesize protein conjugates by crosslinking two or more proteins, TG often does not readily crosslink heterologous proteins even though these proteins can provide both glutamine and lysine residues for the crosslinking reaction and will readily form homologous protein polymers. Studies on various binary protein mixtures indicated that, apart from (conformation-related) steric factors, thermodynamic incompatibility of protein substrates at the active site of the TG enzyme may also influence heterologous crosslinking of these proteins (Han and Damodaran 1996).

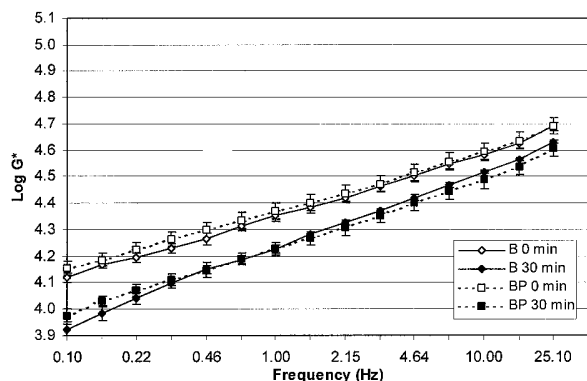


Fig. 5. Variations of G^* with frequency for blended Sharpshooter with or without soy protein isolate (SPI) treated doughs. B= blended and BP = blended and SPI.

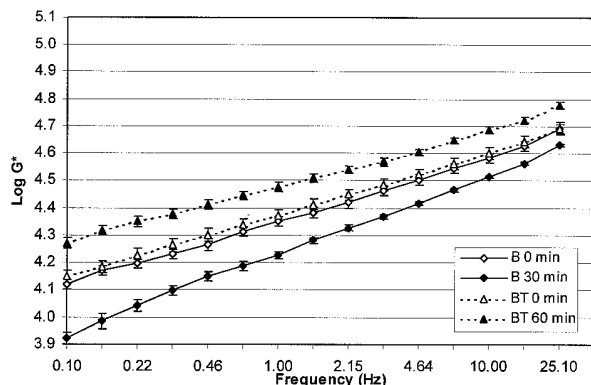


Fig. 6. Variations of G^* with frequency for blended Sharpshooter with or without transglutaminase (TG) treated doughs. B = blended and BT = blended and TG.

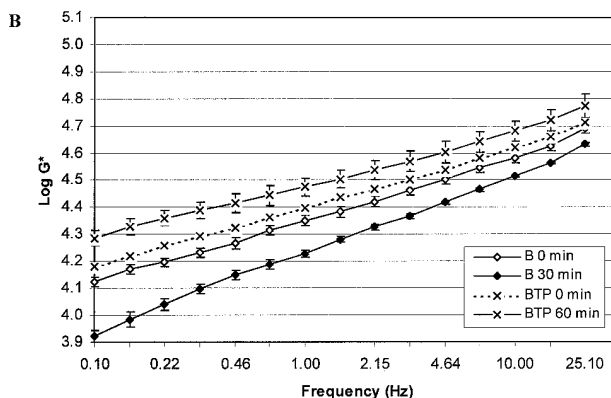
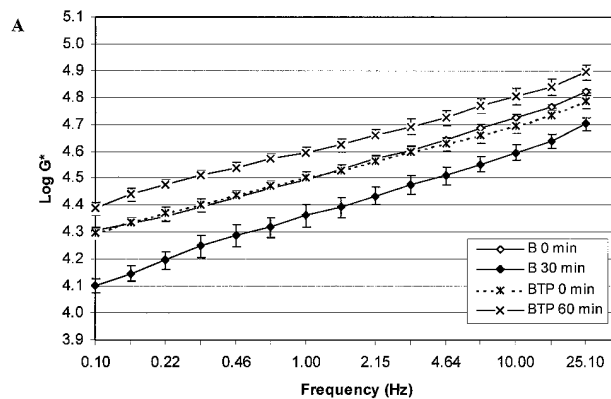


Fig. 7. Variations of G^* with frequency for blended Augusta (A) and blended Sharpshooter (B) with or without transglutaminase (TG) and soy protein isolate (SPI) treated doughs. B = blended and BTP = blended and TG and SPI.

Effects of TG and SPI on Flours Blended with Bug-Damaged Wheat Flour

Suni bug-damaged flour (BDF) was blended with Augusta and Sharpshooter flours at a 10% level to observe the effects of bug protease on rheological properties. The G^* values of Augusta and Sharpshooter doughs blended with BDF decreased significantly after 30 min of incubation (Fig. 4). The negative effects of BDF supplementation were more obvious when the loss tangent values were compared (data not shown). The dough sample was extremely soft and sticky, and it was not possible to handle for testing purposes after 60 min of incubation. This indicates that 60 min of incubation of dough samples made with 10% bug-damaged flour would result in a dough completely unsuitable for baking purposes, due essentially to the proteolytic activity of the BDF. Similar results have been reported in rheological studies by the addition of reducing agents into dough; addition of glutathione and cysteine to dough reduced G' and increased loss tangent values. Doughs became relatively less elastic with increasing reducing agent content (Berland and Launay 1995; Dong and Hosenev 1995). The results of the present study related to the doughs blended with damaged wheat flour are in agreement with the previous studies by Sivri et al (1999). These authors reported that bug damage had a great effect on the largest polymers of glutenin, which themselves have a great effect on elasticity and extensibility of dough.

Studies on the effects of supplementation of control flour doughs with damaged wheat flour and SPI were conducted. The G^* values of Augusta or Sharpshooter doughs containing 10% BDF decreased significantly even after 30 min of incubation period, as indicated earlier. At 0 min of incubation, G^* values of Sharpshooter dough supplemented with 10% BDF and 3% SPI were comparable to those of respective Sharpshooter dough supplemented only with 10% BDF (Fig. 5). On the other hand, the G^* values of Augusta dough supplemented with both 10% BDF and 3% SPI were significantly higher than those of the respective Augusta dough supplemented only with BDF at 0 min of incubation (data not shown). This difference in G^* values of Augusta became insignificant at 30 min of incubation. Again, the dough samples containing BDF and SPI were extremely soft and sticky and it was not possible to handle them for testing purposes after 60 min of incubation.

The effects of TG on G^* values of Sharpshooter flour doughs containing 10% BDF are presented in Fig. 6. Similar trends were observed for Augusta dough samples (data not shown). G^* values of Augusta and Sharpshooter doughs supplemented with both BDF and TG were comparable to those of corresponding Augusta and Sharpshooter doughs without TG at 0 min of incubation. As indicated above, the G^* values of Augusta and Sharpshooter doughs blended with 10% BDF decreased significantly after 30 min of incubation. However, the G^* values of the same dough samples (Augusta flour + 10% BDF or Sharpshooter flour + 10% BDF) did not decrease, but increased significantly after 30 and 60 min of incubation when TG was included in the dough formulations (Fig. 6).

The effects of TG on G^* values of Augusta and Sharpshooter flour doughs containing both 10% BDF and 3% SPI were also investigated. The effects of TG on the treated samples, reflected by the G^* values, were similar to those of samples containing BDF but no SPI. The G^* values of the dough samples (Augusta flour + 10% BDF + 3% SPI or Sharpshooter flour + 10% BDF + 3% SPI) did not decrease due to the action of protease, but increased significantly after 30 and 60 min of incubation when TG was included in the dough formulations (Fig. 7A and B, respectively). At 60 min of incubation, the increase in the G^* values of Augusta dough supplemented with BDF and TG was slightly lower than that of the respective Sharpshooter dough (Fig. 5). However, the increase in G^* values of Augusta and Sharpshooter doughs were comparable to each other after further supplementation with SPI (Fig. 7A and B). The lower protein content of Augusta flour might have had a limiting effect on TG activity. Further investigation into the effects of protein content on TG activity is warranted.

CONCLUSIONS

In some wheat-growing countries, considerable quantities of commercial wheat are rendered unusable in standard baking because of preharvest damage of the grain by protease-injecting bugs (Kretovich 1944; Matsoukas and Morrison 1990; Swallow and Every 1991; Sivri et al 1998). In the present study, we investigated the possibility of mitigating the detrimental effects of bug damage by using the TG enzyme. The repairing effect of the TG enzyme is evident on the dough samples hydrolyzed by wheat bug protease enzymes irrespective of SPI supplementation. It was shown in previous studies that gelation of casein and soybean globulins with TG produced a three-dimensional network structure (Nio et al 1985); this could also have great importance to dough structure. Formation of ϵ -(γ -glutamyl) lysyl crosslinks would contribute to the dough structure. This could be one of the probable mechanisms for the repairing action of TG on BDF. Sivri et al (1999) reported that wheat-bug protease caused a significant decrease in the amount of large polymeric glutenin (i.e., glutenin insoluble in 50% 1-propanol). Bug protease had a great effect on HMW-GS. Larre et al (2000) reported that HMW-GS were the gluten protein group most affected by TG. Addition of TG repairs the weakened gluten structure caused by the bug protease, probably due to its activity on HMW-GS. Based on the findings in this study, further studies on the reactions of TG and SPI as well as other protein sources in BDF are warranted so that possible ways to utilize BDF in the baking industry can be realized. The present study was mainly based on oscillatory measurements and, therefore, studies to cover other rheological tests and actual baking test are warranted.

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