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Effects of Transglutaminase on Pasting and Rheological Properties of Different Wheat Cultivars Blended with Barley or Soy Flour

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Abstract The effects of transglutaminase (TG) on the pasting and rheological properties of different wheat cultivars ('Sharpshooter', 'Russ', and 'AcAriss') blended with barley (40%) or soy (20%) flour were investigated. In the rapid visco-analyzer (RVA) pasting profile, the addition of barley or soy flour to wheat flour samples induced a decrease in peak, trough, final viscosity, breakdown and setback values. However, TG treatment of these blends significantly increased peak viscosity and breakdown ($p<0.05$). In particular, TG treatment greatly increased the breakdown of wheat flour blended with soy flour, indicating that the cross-linking of proteins through TG may somehow be related to an increase in starch granule rupturing in pastes. Storage (G') and loss (G") moduli of the sample pastes increased with an increase in frequency (ω), while complex viscosity (η*) decreased. In all wheat cultivars, G', G", and η were decreased by the addition of barley or soy flour, or TG treatment. Results suggest that protein cross-linking by TG can produce unique and improved properties in wheat flours blended with barley or soy flour.

Keywords: transglutaminase, wheat, soy, barley, pasting property, dynamic rheology

Introduction

Transglutaminase (TG) is the enzyme used commercially in the food industry that forms of cross-links between protein and other molecules, including the same or different proteins (1). TG as a polymerizer has been extensively studied, and is known to catalyze an acyl-transfer reaction between the γ-carboxylamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the ε-amino group of lysine residues in certain proteins (2-6). These reactions result in both inter- and intra-molecular covalent cross-links. In the absence of amine substrates, TG catalyzes the deamidation of glutamine residues during which water molecules are used as acyl acceptors. In nature, the cross-linking of proteins, with resulting formation of high molecular weight polymers, seems to be the most dominant reaction of this enzyme (1).

These TG reactions can be used to modify functional properties of food proteins in various food products. TG catalyses the formation of homologous and heterologous polymers between milk, meat, soybean, and wheat gluten proteins (5,7,8). Gerrard *et al.* (9) reported the beneficial effects of TG in the breadmaking process and in pastry and croissants, resulting in increased volumes and improved structure for these products and TG increased the amount of protein extracted in the gliadin fraction in both bread and croissant doughs and that the beneficial effects of TG may be attributed to cross-linking of the high molecular weight (HMw)-glutenin subunits, proteins known to be linked to the quality of bread flour. Gujral and Rosell (10) reported that the improvement in rice protein functionality became evident in breadmaking, since it was possible to obtain rice bread with an increased specific volume and softer crumb with inclusion of at 1% microbial TG.

Studies have conducted that wheat, soy, barley, and some of their blends cross-linked by TG were bio-chemically analyzed and the application for breadmaking was evaluated (5,6). It is beneficial study to evaluate the utilization of TG for increasing the levels of barley and soy flour incorporation in wheat flour breads, as both are good candidates for improving the nutritional and health benefits of breads. However, supplementation of wheat flour with barley or soy flour may cause problems in yeast-leavened breads by adversely affecting the rheological properties, resulting in products processing quality charac-teristics inferior to those obtained from wheat flour alone. It has been reported that the fortification of wheat flour with barley or soy was recommended to a maximum of 10 or 5% levels, respectively, due to a loss of bread quality (11). However, Basman *et al.* (6) reported that the incorpora-tion of barley or soy flours into wheat flours at higher-than-usual levels through the aid of TG-catalyzed cross-linking in breadmaking was possible, even with soft wheat cultivars. Therefore, this study was designed to investigate how cross-linking proteins affect the gelatinization and gel properties of wheat flours blended with barley or soy flours previously determined levels.

The present study was to provide the information TG effects on the pasting and dynamic rheological properties of different wheat cultivars ('Sharpshooter', 'Russ', and 'AcAriss') with a maximized level of barley or soy incorporation.

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Materials and Methods

Sample preparation Grains from 3 wheat cultivars, 'Sharpshooter' (hard type with medium gluten strength), 'Russ' (hard type with strong gluten strength), and 'AcAriss' (soft type with weak gluten strength), were milled into straight-grade flours in a Bühler laboratory mill (Bühler Bros. Inc., Uzwil, Switzerland) according to AACC method 26-10A (12). The waxy hulless barley cultivar, 'Merlin', was also milled by Bühler laboratory mill (55.4% extraction), and defatted soy flour (Nutrisoy 7B flour) was obtained from ADM Protein Specialties (Decatur, IL, USA). A bacterial TG (100 units/g) was obtained from Ajinomoto (Teaneck, NJ, USA).

Twenty g flour samples of wheat, barley, soy, or blends were evaluated; blends of flours contained 12 g wheat plus 8 g barley, or 16 g wheat plus 4 g soy. For TG treated samples, a 20 g flour sample was mixed with 0.2 g TG and 80 mL distilled water and vortexed for 5 sec. The resulting slurry mixtures were incubated at 35°C for 60 min with brief vortexing every 10 min. These incubation periods and temperature conditions were chosen based on the results of a previous study (5). The control samples did not include TG. For the termination of the enzyme reaction, the slurry was immediately cooled in ice water. The samples were then frozen and freeze-dried. The freeze-dried samples were ground using a mortar and pestle and stored at −20°C until analyzed (hereafter referred to as control or TG-treated flour samples). After freeze-dried sample preparation, sample moisture and protein contents were determined using approved methods (12).

Rapid visco-analyzer (RVA) The pasting properties of wheat flours and wheat flours blended with barley or soy were measured according to AACC approved method 61-02 (12) with a rapid visco-analyzer (RVA-4 Series, Newport Scientific Pty., Ltd., Warriewood, NSW, Australia). Value for peak, trough, and final viscosities, breakdown and setback viscosities were obtained using Thermocline software (V. 1.2, Newport Scientific).

Dynamic rheological measurement Dynamic oscillatory tests of flour pastes (8%, w/v) were performed using a controlled stress rheometer (RS 100; Haake, Karlsruhe, Germany) equipped with 35 mm diameter stainless steel parallel plates. A circulating water bath was used to control temperature. Each flour paste was prepared using the RVA after which the paste was cooled down to 25°C. The sample solution was loaded between the parallel plate and base with a 1.0 mm gap. A frequency sweep from 0.1 to 10.0 Hz was performed at a constant stress of 30 Pa at 25°C. Preliminary trials were carried out to determine the stress at which the sample remained in the linear viscoelastic region at a constant frequency of 1 Hz. From these measurements, the frequency dependence of storage modulus (G'), loss modulus (G"), and complex viscosity (η^*) were obtained.

Statistical analysis All measurements were conducted in duplicate and the data were then analyzed by SAS software (V. 8.02, SAS Institute, Cary, NC, USA). The general linear model (GLM) procedure was processed and Duncan's multiple range test was used to compare the mean values at $p<0.05$. Mean values and pooled standard errors of the mean (SEM) were recorded.

Results and Discussion

Pasting properties The protein contents of wheat cultivars, 'Sharpshooter', 'Russ', and 'AcAriss' were 12.8, 12.2, and 8.6% (wet basis), respectively. The protein contents of the wheat flours represent those of typical hard and soft wheat varieties. After TG treatment, the protein contents increased slightly to 13.6, 12.6, and 9.1%, respectively. These results were in agreement with Rosell *et al.* (13), who reported that the wet gluten content of wheat flours was slightly increased by TG treatment due to protein polymerization.

TG treatment had an effect on the pasting properties of 'Sharpshooter', 'Russ', and 'AcAriss' blended with barley or soy flour (Table 1). Peak viscosity significantly decreased with the addition of barley or soy flour to wheat flour samples compared to values for wheat flour alone, whereas TG treatment increased the peak viscosity or these wheat flours blended with barley or soy flour ($p<0.05$). In particular, the addition of soy flour to the wheat flour samples significantly decreased the peak viscosities (1,252-1,312 cp), but TG treatment increased the value (1,963-2,015 cp). Among the wheat cultivars, 'AcAriss' flour with or without barley or soy flour showed higher values in peak, trough, final, breakdown, and setback viscosities. These higher values might be due to the different protein and starch contents in the samples containing this wheat flour. It is generally accepted that the increase in viscosity during heating of flour or starch suspension is mainly due to swelling of the starch molecules (14). Based on proximate analysis (moisture and protein), it would be expected that 'AcAriss' flour contained a higher starch levels and thus a higher viscosity. Tan and Corke (15) also reported that protein content was negatively correlated to peak viscosity in flour pastes. On the one hand, this was confirmed among the 3 wheat-only samples studied, since those with higher protein contents had lower peak viscosities. But our study also suggests that cross-linking proteins by TG treatment can increase the peak viscosity in wheat flours with barley or soy flour mixtures, indicating that factors other just protein content influence peak viscosity.

The addition of barley or soy flour to wheat flour samples was associated with a decrease in final viscosity and trough viscosity. TG effects on final viscosity and trough viscosity were not shown.

The values for breakdown of wheat flour samples blended with barley or soy flour were significantly decreased as compared to the respective wheat flour alone. However, TG treatment increased the breakdown values of these blended samples to as high as the value for the respective wheat flour alone. This was especially notable for the TG-treated wheat-soy blends whose breakdown values were twice as high as those for the non-TG treated respective wheat with soy flour. Among these non-TG treated wheat flour samples, 'AcAriss' flour had a higher breakdown value than either 'Sharpshooter' or 'Russ' flour samples. Breakdown is caused by the rupture of swollen

Table 1. Pasting properties determined with rapid visco-analyzer of these wheat cultivars and their blends with barley or soy flour, with or without transglutaminase (TG) treatment

Viscosity (cp)	Wheat cultivar	Treatment[1)]					SEM[2)]
		C	B	S	B+TG	S+TG	
Peak	'Sharpshooter'	2,835a	2,158cy	1,312e	2,340by	2,015d	22.8
	'Russ'	3,020a	2,218bcy	1,252d	2,443bxy	2,014c	72.6
	'AcAriss'	3,227a	2,337cx	1,303e	2,579bx	1,963d	39.5
	SEM[3)]	96.9	17.9	62.9	33.5	24.7	
Trough	'Sharpshooter'	1,684a	1,187b	848c	1,257by	866c	25.7
	'Russ'	1,662a	1,162b	815c	1,264by	849c	44.9
	'AcAriss'	1,847a	1,255b	879c	1,375bx	837c	37.1
	SEM[3)]	60.8	26.2	43.4	19.8	9.8	
Final	'Sharpshooter'	3,046ay	2,267b	1,806c	2,300by	1,822c	43.9
	'Russ'	3,203axy	2,206b	1,691c	2,336by	1,779c	71.8
	'AcAriss'	3,492ax	2,339b	1,943c	2,467bx	1,761c	69.3
	SEM[3)]	82.6	57.9	88.9	21.9	36.4	
Breakdown	'Sharpshooter'	1,151a	971by	464c	1,083aby	1,149a	39.4
	'Russ'	1,358a	1,055cx	436d	1,179bx	1,164bc	32.4
	'AcAriss'	1,308a	1,082bcx	423d	1,203bx	1,025c	33.5
	SEM[3)]	64.4	15.8	34.4	17.1	28.1	
Setback	'Sharpshooter'	1,362ay	1,080b	957b	1,043b	956b	36.1
	'Russ'	1,541ax	1,044b	876c	1,071b	930c	30.0
	'AcAriss'	1,645ax	1,084b	1,063b	1,091b	923c	37.2
	SEM[3)]	39.6	34.5	47.7	10.8	29.9	

[1)]C, Control; B, wheat cultivars with 40% barley added; S, wheat cultivars with 20% soy added; B+TG, wheat cultivars with 40% barley added and treated with TG; S+TG, wheat cultivars with 20% soy added and treated with TG. [a-c]Values with different letters within a row differ significantly ($p<0.05$); [x-y]values with different letters within a column differ significantly ($p<0.05$).
[2)]Standard error of the mean (n=10).
[3)]Standard error of the mean (n=6).

starch granules (14). Baxter *et al.* (16) reported that the addition of prolamin to rice flour increased the breakdown of the pastes by an increase in the rate of water absorption by starch granule. Results suggest that TG-cross-linked proteins lead to increased rupturing of starch granules in the pastes, because our previous study indicated that wheat flours treated with TG had increased water holding capacity (17).

Setback is the difference between the peak viscosity and the viscosity at the cooling stage (50°C). This value is related to the retrogradation behavior of starch, which in turn is related to amylose helix interaction, and is one of the most important pasting parameters in predicting bread characteristics (18). The phenomenon of amylose retrogradation is responsible for the firming of the bread crumb during the first hours after baking (19). TG treatment did not significantly affect the setback of all the samples; just the addition of barley or soy flour to wheat flours led to pastes with decreased setback viscosities. Therefore, the addition of barley or soy flour rather than TG treatment to wheat flour samples might affect the pasting properties.

Dynamic shear properties The frequency (ω) dependence of storage modulus (G') and loss modulus (G") of the sample pastes (8%, w/v) with or without TG treatment is shown in Fig. 1. The magnitudes of G' and G" increased with increase in ω with the high frequency dependency. 'Sharpshooter', 'Russ', and 'AcAriss' non-blended flour samples showed behaviors known for concentrated flexible polymer solutions; G' is less than G" at lower ω, and G′ is greater than G" at higher ω (20). However, the addition of barley or soy flour to wheat samples changed the pattern to a semidilute polymer solution; that is G" predominated over G' at low frequencies, and there was a crossover between G' and G" in the high frequency range examined. Ikeda and Nishinari (20) reported that the crossover of G' and G" in starch or flour paste occurred due to changes in paste concentration. On occasion, a lower G' value than G" is observed in starch or flour paste. Hirashima *et al.* (21) reported that cornstarch paste (3.0 wt %) showed higher G" than G', while the increases associated with adding citric acid (lower pH) led to a higher G' than G". Therefore, the magnitudes of G' and G" are influenced by sample concentration or pH.

Regardless of the wheat cultivar samples, the addition of barley or soy flour led to decreases in G' and G" compared to the counterpart values for the respective wheat sample alone. Also, in all cases, TG treatment slightly decreased the values of G' and G". Chang *et al.* (22) reported that the addition of sugar to corn starch decreased the G', indicating that sugar had the ability to inhibit retrogradation. In the present study, TG-crosslinked proteins may have an effect of slightly decreasing the retrogradation values of paste made from TG-treated flour. The dynamic rheological data

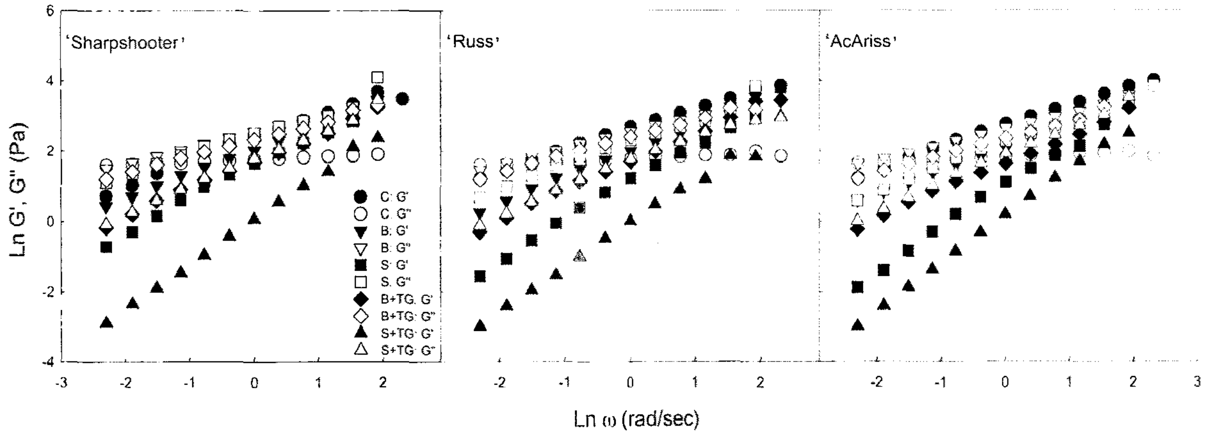

Fig. 1. Ln frequency (ω) dependence of ln G' and ln G'' for 8%(w/v) pastes of these wheat cultivars and their blends with barley (40%, w/w) or soy (20%, w/w) flour with or without transglutaminase (TG) treatment at 25°C. C, Control; B, wheat cultivars with 40% barley added; S, wheat cultivars with 20% soy added; B+TG, wheat cultivars with 40% barley added and treated with TG; S+TG, wheat cultivars with 20% soy added and treated with TG.

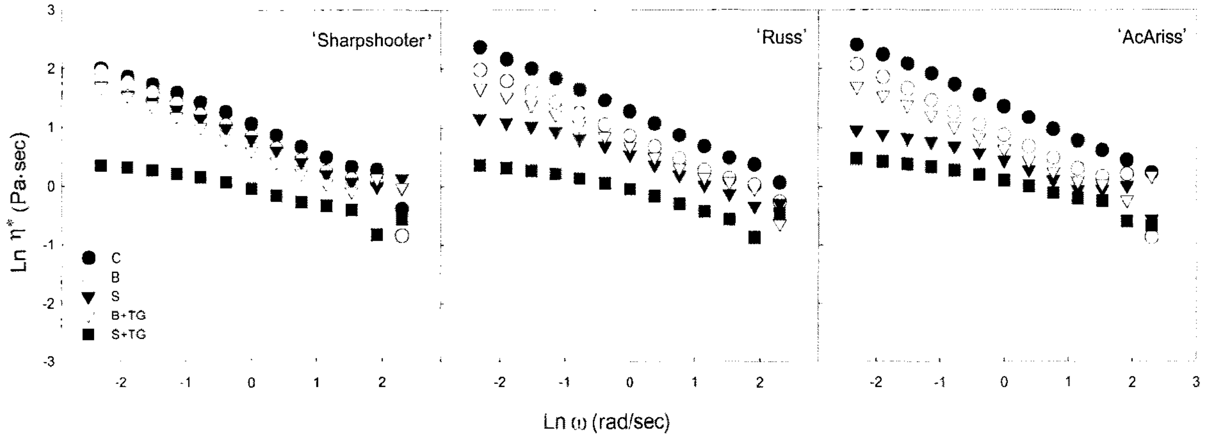

Fig. 2. Ln frequency (ω) dependence of ln η* for 8%(w/w) paste of these wheat cultivars and their blends with barley (40%, w/w) or soy (20%, w/w) flour with or without transglutaminase (TG) treatment at 25°C. C, Control; B, wheat cultivars with 40% barley added; S, wheat cultivars with 20% soy added; B+TG, wheat cultivars with 40% barley added and treated with TG; S+TG, wheat cultivars with 20% soy added and treated with TG.

of ln (G', G'') versus ln ω (rad/sec) were subjected to linear regression (23). Table 2 shows the magnitudes of slopes (n' and n''), intercepts (K' and K''), and R^2 in the following equations:

$$G' = K'(\omega)^{n'}$$
$$G'' = K''(\omega)^{n''}$$

From these dynamic rheological data, the pastes of wheat flour samples blended with barley or soy flour calculated the cross-points of G' and G'', which was due to the higher G' slopes (n'), as shown in Fig. 1. At low frequencies, the flow behavior is controlled by the translational motion of the macromolecules, and the G'' is usually higher than G' (24). At higher frequencies, on the other hand, the value G' increases due to macromolecular distortion and it is higher than that of G''. In this study, the magnitudes of G'' (K'= 0.93-11.13) are lower than those of G' (K'=5.26-20.58). These results suggest that all the paste samples are more viscous than elastic.

The frequency dependence results of complex viscosity (η*) of 8% flour pastes with or without TG treatment are shown in Fig. 2. The addition of barley or soy flour decreased the complex viscosity, and TG treatment also led to a decrease in complex viscosity of these samples. Ln η* vs. ln ω plots also show shear thinning behavior following the power law model, and such behavior is in good agreement with that found in macromolecule dispersions (25). Among the wheat cultivars, complex viscosities of 'AcAriss' containing pastes were relatively higher than that of 'Sharpshooter', and 'Russ'. This tendency is similar to the trend seen for peak viscosity from RVA of these 3 wheat flours.

In conclusion, the cross-linking of proteins by TG treatment affected the pasting and rheological properties of wheat flours blended with barley or soy flour. The addition of barley or soy flour to wheat flours caused a decrease in viscosity; however, TG treatment significantly increased the viscosity in these wheat and blended samples. Dynamic rheology indicated that TG treatment had an ability to inhibit retrogradation of starch gelatinization. Many researches indicated that TG provides unique texture (26-28). These altered characteristics via TG treatment provide

Table 2. Slopes (*n'* and *n''*) and intercepts (*K'* and *K''*) of ln (G' and G'') vs. ln frequency (ω, rad/sec) data for 8%(w/v) pastes of these wheat cultivars and their blends with barley or soy flour, with or without transglutaminase (TG)

		Wheat cultivar	Treatment[1)]					
			C	B	S	B+TG	S+TG	SEM[2)]
G'	n'	'Sharpshooter'	0.81bc	0.67dy	0.92by	0.79cd	1.27a	0.035
		'Russ'	0.75d	0.73dx	1.11bxy	0.82c	1.24a	0.014
		'AcAriss'	0.78c	0.66dy	1.19bx	0.78c	1.28a	0.022
		'SEM[3)]	0.027	0.013	0.042	0.015	0.019	
	K'	'Sharpshooter'	9.22a	7.60ab	4.78bx	5.47b	1.03cxy	0.826
		'Russ'	10.60a	7.41b	3.09dy	5.46c	0.93ey	0.183
		'AcAriss'	11.13a	8.25b	2.60dy	5.24c	1.11ex	0.312
		SEM[3)]	1.047	0.429	0.265	0.062	0.038	
	R^2	'Sharpshooter'	0.994	0.997	0.985	0.995	0.998	-
		'Russ'	0.995	0.993	0.991	0.994	0.987	-
		AcAriss	0.994	0.997	0.993	0.994	0.997	-
G''	n''	'Sharpshooter'	0.49c	0.46cxy	0.57by	0.50c	0.75axy	0.011
		'Russ'	0.46c	0.48cx	0.64bx	0.50c	0.70ay	0.012
		'AcAriss'	0.46d	0.45dy	0.67bx	0.50c	0.77ax	0.009
		SEM[3)]	0.013	0.004	0.011	0.003	0.014	
	K''	'Sharpshooter'	18.00a	12.71b	11.85bx	10.26by	5.71cy	1.083
		'Russ'	19.49a	12.77b	9.54dxy	10.91cx	5.26ez	0.346
		'AcAriss'	20.58a	14.10b	9.17cy	10.73cx	6.47dx	0.051
		SEM[3)]	1.360	0.595	0.593	0.101	0.080	
	R^2	'Sharpshooter'	0.992	0.998	0.993	0.996	0.998	-
		'Russ'	0.996	0.994	0.993	0.995	0.995	-
		'AcAriss'	0.995	0.999	0.993	0.996	0.997	-

[1)]C, Control; B, wheat cultivars with 40% barley added; S, wheat cultivars with 20% soy added; B+TG, wheat cultivars with 40% barley added and treated with TG; S+TG, wheat cultivars with 20% soy added and treated with TG. [a-e]Values with different letters within a row differ significantly ($p<0.05$); [x-z]values with different letters within a column differ significantly ($p<0.05$).
[2)]Standard error of the mean (n=10).
[3)]Standard error of the mean (n=6).

great opportunities for the wheat industry to not only improve and tailor end-use product quality but also enable supplementation with plant material other than wheat for a specific food industry.

Acknowledgments

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