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Effects of Transglutaminase on the Physical Properties of Resistant Starch-added Wheat Flour Doughs and Baguettes

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Abstract Effects of transglutaminase (TG) on physicochemical properties of dough prepared with 20% resistant starch (RS)-added wheat flour were investigated. RS levels of wheat flours added with native wheat starch (NS), Hi-maize (RS2), retrograded (RS3), and cross-linked (RS4) wheat starches were 2.97, 11.88, 5.79, and 9.09%, respectively. Peak viscosity of NS-added flour was higher, whereas setback was lower, than those added with other resistant starches. TG had no effect on pasting behaviors of RS-added flours. Water absorption ranged from 66.5 to 79.0%, and development time increased with RS addition. TG increased tensile strength of dough after fermentation and bread volume, due to well-developed gluten network resulting from cross-linking facilitated by TG. Addition of TG decreased hardness of baguettes, with RS2-added baguette showing lowest value. These results indicate addition of TG enhanced eating quality of RS-added breads.

Keywords: resistant starch, transglutaminase, dough properties, bread quality, baguette

Introduction

Modification of proteins by enzymes such as transglutaminase (TG) has recently been the subject of great interest to food scientists (1-5). TG (amine γ-glutamyltransferase, EC 2.3.2.13) catalyzes the conversion of soluble proteins into insoluble high molecular weight (HMW) protein polymers via the formation of nondisulfide covalent crosslinks (6, 7). The application of this crosslinking to the wheat gluten proteins would be of particular interest due to the high glutamine content of the proteins (comprising approximately one-third of the total amino acids). In addition, stability and elasticity of dough, bread volume, and crumb texture were all positively influenced by the addition of TG (8).

Several studies have demonstrated that TG can catalyze the formation of polymers among milk, meat, soybean, and wheat proteins (9-11). Gerrard *et al.* (12) indicated that TG may exert beneficial effects, which are comparable to those associated with traditional oxidizing improvers, during bread-making. TG has also been reported to exert dramatic effects on the volume of yeast croissants and puff pastries (13).

Recent demands by the consumers for healthy foods containing functional materials such as high dietary fiber have led to the studies on the effects of dietary fiber in context of bread-making. Resistant starch (RS) is known to increase the content of dietary fiber in baked products (14). It exhibits low water-binding capacity, and its physicochemical properties are affected by the preparation methods used, as well as the formation of RS and types employed. RS is classified into four types, all of which exhibit different properties with regard to food processing (15-18). RS2 from high-amylose maize starch, RS3, and RS4 can increase the dietary fiber content of food, whereas RS1 and most RS2s generally lose their enzyme resistance after cooking and processing.

In the case of wheat flour substituted for RS, the gluten content of wheat flour was reduced, so that the making of bread was extremely difficult. TG was therefore applied to improve the properties of gluten by facilitating cross-linking among gluten proteins in the wheat flour.

The objectives of this study were to determine the effects of TG on the physical properties of bread, as well as the physicochemical and rheological properties of dough prepared with RS-added wheat flour, and to measure the textural and sensory properties of French baguettes made from this dough.

Materials and Methods

Materials Wheat starch was purchased from Sinsong Foods Company (Nonsan, Korea), and commercial hard wheat flour with 9.8% protein content was obtained from the Dongah Milling Co. (Incheon, Korea). TG (ACTIVA TG, STG-M, optimal pH and temp: 5-8 and 40°C, respectively) was obtained from Ajinomoto Co. (Tokyo, Japan). Hi-Maize (high amylose maize starch, RS2) was purchased from National Starch Chemical Co. (Bridgewater, NJ, USA). Sodium trimetaphosphate and sodium tripolyphosphate for cross-linking were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Pancreatin (from *Porcine pancreas*, P7545, Sigma Chemical Co.) and pullanase (Promozyme ID # 115193, Novo Nordisk, Denmark) were used for determination of RS levels.

Preparation of resistant starches RS3 was prepared according to the methods described by Sievert and Pomeranz (19). In brief, the starch (100.0 g) was mixed with 200 mL water, and the suspension was autoclaved for 1 hr at 121°C. The resulting starch paste was cooled at room temperature and stored overnight at 4°C. The sample was repeated the same autoclaving-cooling cycle and the sample was dried at 40°C in an incubator and ground into

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fine particles (<150 μm) in a food mixer (JAM-505. Jewoo, Korea).

The cross-linked RS4 was prepared according to the methods described by Woo and Seib (20) and Shin *et al.* (21). The wheat starch suspension (starch:water = 1:1) was annealed at 50°C in a shaking water bath (130 rpm) for 12 hr, followed by cross-linking reaction. The annealed starch suspension was then stirred with 10.0 g sodium sulfate [10%, starch basis (sb)], 12% of 99.9:0.1 (w/w) sodium trimetaphosphate (11.99 g, sb) and sodium tripolyphosphate (0.01 g, sb) mixture. The mixture was then adjusted to pH 11.5 with 1 M sodium hydroxide solution and allowed to react for 3 hr at 45°C with agitation. Hydrochloric acid (1 M) was added to neutralize the slurry to pH 6.0. The starch was then collected by centrifugation, washed with water (200 mL × 6), and dried at 40°C. Finally, the sample was ground into fine particles and passed through a 100-mesh sieve.

Determination of RS levels in RS-added wheat flours The RS-added wheat flours were prepared by adding 20% (w/w) native starch (NS), Hi-Maize RS2, retrograded RS3 or cross-linked RS4 to wheat flour. RS levels were determined using the pancreatin-gravimetric method described by Shin *et al.* (21).

Physicochemical properties of dough Farinograms of the RS-added flours containing TG were obtained according to the AACC method 54-21 (22) using a farinograph equipped with a 300-g bowl. Optimum water absorption was designated as the amount of water required viscosity of central line of farinogram upto 500 BU. The apparent gluten contents of the TG-containing RS-added wheat flours were then analyzed via the hand-washing method (AACC method 38-10). The pasting properties were assessed using a Rapid Visco Analyser (RVA, Model 3D, Newport Scientific Pty., Ltd., Narranbeen, Australia) according to the AACC method (22). The temperature was maintained at 50°C for 1 min, raised to 95°C for 3.7 min, maintained at 95°C for 2.5 min, cooled to 50°C for 3.8 min, and maintained at 50°C for 2 min. A plot of the paste viscosity, expressed in arbitrary RVA units (RVU) versus time, was then used for the determination of initial temperature, peak viscosity (P), trough viscosity (T), cold viscosity (C), breakdown viscosity (P-T), and total setback viscosity (C-T). Scanning electron microscope (JOEL, JSM-5400, Tokyo, Japan) was used to visualize the network of dough. The freeze-dried dough powders were attached to an SEM stub using double backed-cellophane tape. The stub and the sample were then coated with gold-palladium, examined, and photographed.

Baguette-making The formula for the baguettes included yeast (2.4%, fb), salt (2%, fb), and hard wheat flour (Dongah Milling Co.) supplemented with 20% (fb) native wheat starch, RS2, RS3, or RS4 and 69, 70, 80, and 75% water, respectively. Dough samples were then prepared by a straight dough method using a Hobart mixer (N50, Hobart, USA); for TG dough preparation, 0.6 mL TG solution (0.05 g/5 mL) was added during the mixing phase. The dough preparations were proofed for 1 hr at 30 °C and 75% RH in a proofing cabinet (Turbofan Back Bar Co., City, New Zealand). The 2nd proofing was repeated for 45-50 min under the same conditions as in the 1st proofing. The oven was pre-heat to 230°C, and, during the first 30 sec of heating, live steam was injected into the oven. The temperature was then decreased to 200°C. After 15 min, the baguette dough preparations were put into the oven. After baking for 25 min, the fully baked baguettes were taken out of the oven and cooled for 4 hr at room temperature.

Rheological properties of dough Tensile strength and elongation distance in tensile tests were measured with a rheometer (Compac-100, Sun Sci. Co., Tokyo, Japan). The dough was rolled into sheets of 3 mm thickness with a noodle maker (Atlas Model 150 mm Deluxe, Italy) and cut into 10 x 50 mm strips. The dough strips were then tested using a rheometer equipped with a hook-shaped accessory. The conditions for the tensile test were as follows: 1 kg load cell; table speed, 450 mm/min; and chart speed, 150 mm/sec.

Baguette properties The moisture content and the specific volume of breads were measured by AACC methods 44-15 and 72-10, respectively. Cross-section of the bread was also observed using a digital camera (cybershot 5.0 megapixels Sony, Japan). Textural properties of the bread were measured via a texture profile test using a rheometer (Compac-100, Sun Sci. Co.). The crumb portion in the center of the baguette was used as the sample. The hardness, adhesiveness, springiness, and cohesiveness were measured under the following conditions: sample size, 1 × 1 × 1 cm^3; load cell, 1.0 kg; probe diameter, 1.0 cm; deformation rate, 50%; table speed, 120 mm/min; and chart speed, 60 m/sec. Sensory evaluation of the baguettes was performed via quantitative descriptive analysis (QDA) test by 12 trained panelists. Training consisted of presenting the breads and explaining about experimental goal and evaluating to the panelists in the preliminary sessions. The whole and sliced baguettes (thickness, 1 cm) were assigned random three digit numbers and served to the panelists for evaluating bread characteristics including appearance (volume, color), flavor (roasted, yeast), texture (crumb homogeneity, hardness, elasticity, chewiness, moistness), and overall eating quality. The unstructured 15 cm line with 2.5 cm anchor point from both ends was used. All attributes represented intensity except for overall eating quality, which represented acceptance of baguette.

Statistical analysis Experiments were performed in the least duplicates. The data obtained were statistically evaluated using analysis of variance (ANOVA) and Duncan's multiple range test. The SAS package (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results and Discussion

Pasting properties by RVA The pasting properties of RS-added wheat flours containing TG are shown in Table 1. In general, the degree to which the starch gelatinizes can be affected by factors such as swelling of starch granules,

Table 1. Pasting properties of RS added wheat flours with TG using Rapid Visco Analyzer

Additives	Viscosity (RVU)				
	Peak (P)	Trough (T)	Cold (C)	Breakdown (P-T)	Total setback (C-T)
NS	180.3	115.0	233.7	65.3	118.7
RS2	83.3	56.9	122.3	26.4	65.4
RS3	109.8	76.9	156.9	32.9	80.0
RS4	94.9	66.0	145.8	28.9	79.8
NS-TG	179.9	114.4	233.6	65.5	119.2
RS2-TG	84.0	57.8	125.1	26.2	67.3
RS3-TG	110.1	76.7	157.1	33.4	80.4
RS4-TG	96.9	66.6	146.5	30.3	79.9

NS, RS2, RS3, RS4, and TG means native starch, Hi-maize, retrograded wheat starch, cross-linked wheat starch and transglutaminase, respectively.

stability of the applied heat and shear force on the swollen granules, size and shape of the starch granules, amylose and amylopectin ratios and structures, and crystallinity (23). The peak viscosity of the native starch-containing wheat flour was higher than those of the others. Peak viscosity refers to the viscosity at maximum swelling power before the rupture of swollen granules, and is highly correlated with the palatability of noodles (24). The substitution of RS for flour (20% fb) resulted in the reduction in peak, trough, and cold viscosities, as compared to native starch-substituted flour. Among the RS types tested in this study, RS2 evidenced the lowest viscosity. High-amylose maize starch did not gelatinized at temperatures below 95°C. Breakdown and setback viscosities were also lower in the RS2-added flour than in others. TG had no effect on the pasting viscosities of the RS-added wheat flour. RVA of RS-added flours remained unchanged after TG addition, possibly because RVA exhibits pasting of starch component, and not protein component, in flour. TG can induce cross-linking with glutamine of wheat flour proteins and improve the gluten functionality.

Dough properties The apparent gluten contents, farinogram characteristics, and color values of the flours, with the addition of different types of RS, and with or without TG, are shown in Table 2. The apparent gluten content of the wheat flour decreased when substituted with native starch or RS, although the gluten content of the wheat flour added with RS4 increased slightly. This difference was attributed to starch-gluten interactions occurring during the dough-making. RS was easily entrapped within gluten due to its smaller granule size (RS2), partial gelatinization (RS3), and cross-linking properties (RS4). The water absorption value of the native starch-added flour was 62.6%, and increased with the addition of RS to 66.5% in RS2, 68.0% in RS4, and 79.0% in RS3. This increase was more evident in the RS3-added flour, because RS3, composed of ~80% gelatinized fraction, which could be digested by α-amylase, exhibited a high water-binding capacity. The development times of the RS2- and RS4-added flours (2.3 min) were similar to that of the native starch-added flour (2.5 min), whereas that of the RS3-added flour was five times longer than those of other flour. This could be attributed to the different degrees of hydration between RS3 and the flour. TG showed no effect on water absorption and development time. The mixing tolerance index (MTI) of the native starch-added flour increased by 5 BU, whereas those of the RS2- and RS3-added flours decreased by 5 BU after the addition of TG. Increasing levels of TG resulted in decreased water absorption characteristics of the dough and height of the mixing curve (12,25). The lightness (L) values of the RS2- and RS4-added flours increased, while that of the RS3-added flour decreased. The ranges of redness (a) and yellowness (b) of all samples were between -0.40 ~ -0.74 and +13.74 ~ +14.17, respectively, and were not affected by the addition of TG.

Rheological properties of dough Table 3 shows the tensile strength and the maximum length of the dough strip that can be stretched. The length of the RS-added doughs increased slightly, whereas the tensile strength increased quite drastically, after the addition of TG and proofing. The RS3-added dough exhibited lower strength than the others, because RS3 possessed a large amorphous region and absorbed greater quantity of water than the

Table 2. Apparent gluten contents and Hunter L, a, b values of RS added doughs with TG

Additives	ADG (%)	Farinograph data			Hunter color value		
		WA (%)	DT (min)	MTI (Bu)	L	a	b
NS	10.1	62.6	2.5	30	88.25±0.13^{b}	-0.74±0.04^{e}	+13.74±0.18^{cd}
RS2	11.2	66.5	2.3	20	88.52±0.09^{a}	-0.58±0.03^{c}	+14.17±0.06^{b}
RS3	12.0	79.0	11.5	15	87.17±0.14^{d}	-0.40±0.01^{b}	+13.29±0.15^{e}
RS4	15.9	68.0	2.3	50	88.80±0.07^{a}	-0.64±0.05^{d}	+14.02±0.27^{bc}
NS-TG	10.5	62.8	2.5	35	88.75±0.14^{a}	-0.56±0.02^{c}	+13.39±0.07^{e}
RS2-TG	11.3	66.7	2.5	15	88.73±0.11^{a}	-0.54±0.03^{c}	+14.15±0.16^{b}
RS3-TG	11.9	79.2	11.5	10	87.66±0.14^{c}	-0.35±0.04^{a}	+13.49±0.15^{de}
RS4-TG	15.7	67.7	2.7	50	88.73±0.28^{a}	-0.70±0.03^{e}	+14.50±0.32^{a}

NS, RS2, RS3, RS4, TG, ADG, WA, and DT means native starch, Hi-maize, retrograded wheat starch, cross-linked wheat starch, transglutaminase, apparent dry gluten, water absorption, and development time, respectively.
Color values showed as L (lightness), a (redness/greenness), and b (yellowness/blueness).
$^{a-e}$Values within each column with the same superscript are not significantly different ($p<0.05$).
N=5

Table 3. Textural properties of RS added doughs with TG before and after 1st proofing by tensile test

Additives	Before 1st proofing		After 1st proofing	
	Distance (mm)	Strength (g)	Distance (mm)	Strength (g)
NS	43.85±1.24	30.54±4.33^{cd}	44.27±0.97	41.21±4.08^{e}
RS2	43.57±1.59	31.83±3.87^{cd}	44.46±1.48	56.94±7.09^{bc}
RS3	43.28±1.74	27.47±4.42^{d}	44.63±1.12	46.57±8.77^{de}
RS4	43.85±2.44	34.84±7.54^{c}	45.02±0.92	57.19±7.26^{bc}
NS-TG	44.39±0.93	47.87±5.06^{a}	44.78±1.02	64.03±5.45^{a}
RS2-TG	44.93±0.55	41.96±5.81^{b}	45.15±0.72	63.06±6.80^{ab}
RS3-TG	45.24±1.18	31.84±3.07^{cd}	44.71±0.92	52.32±7.17^{cd}
RS4-TG	44.56±0.99	46.32±3.48^{ab}	45.09±1.07	62.43±8.23^{ab}

NS, RS2, RS3, RS4, and TG means native starch, Hi-maize, retrograded wheat starch, cross-linked wheat starch and transglutaminase, respectively.
$^{a-e}$Values within each column with the same superscript are not significantly different ($p<0.05$).
N=10

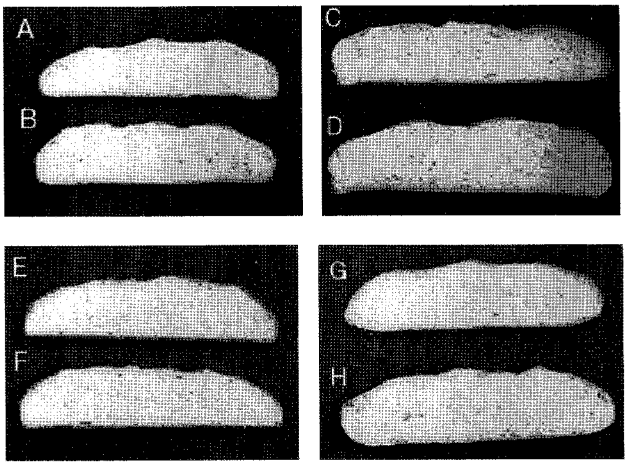

Fig. 1. Cross sections of native starch and various types of RS added French baguettes with and without TG. Baguettes were prepared with native starch (A), native starch and TG (B), RS2 (C), RS2 and TG (D), RS3 (E) RS3 and TG (F), RS4 (G), and RS4 and TG (H).

other RS variants. The ratio of the strength of the RS-added doughs before and after proofing was higher, at 1.64-1.79, than that of the NS-added dough (1.35). In addition, the strength ratios of the RS-added doughs containing TG after proofing were higher, at 1.35-1.64, than that of the NS-added dough (1.25). The addition of TG also resulted in increased strength ratios, but only slightly as compared to the increases associated with proofing. The addition of RS increased strength after the proofing of the dough, regardless of the RS type. These results indicate that both RS and TG improved the network structures of the baguettes concomitantly with increases in the strength of the dough network.

Physical properties of baguettes The specific volumes and textural properties of the baguettes containing RS and TG are shown in Table 4. The range of moisture contents of the baguettes was 42.9-47.9%, regardless of the addition of TG. In particular, the RS3-added baguette maintained higher moisture content than the others. The specific volume of the baguette made with the NS-added dough was 4.5 mL/g, which increased to 5.0-5.3 mL/g after the addition of RS. The addition of TG increased the specific volume of baguettes, but the extent of increase varied with the RS type employed. The specific volumes of the NS-added baguette increased from 4.5 to 5.1 mL/g, whereas that of the RS-added baguettes ranged from 5.0-5.3 to 5.2-5.7 mL/g. The variation pattern of the specific volume of the baguettes was similar to that observed with the tensile strength of the dough.

The crumb hardness of the baguettes decreased with the addition of TG, the RS4-added baguette with or without TG exhibited the highest values among the samples. Adhesiveness also decreased with TG addition. These results confirmed that the addition of TG to wheat flour improved the baking qualities of the baguettes. The cross-sections of the baguettes containing RS and TG are shown in Fig. 1.

The volume and air cell homogeneity of the crumbs increased with the addition of both RS and TG, especially RS2 and RS4 induced higher increases than others.

Table 4. Quality properties of baguettes prepared from RS added doughs with TG

Additives	Moisture content (%)	Specific volume (mL/g)	Texture profile analysis parameter			
			Hardness (g/cm^2 x 10^2)	Adhesiveness (g)	Springiness (ratio)	Cohesiveness (ratio)
NS	42.3±0.2	4.5±0.9	2.67±0.66^{ab}	-7.4±4.3^{a}	0.96±0.03^{bc}	0.83±0.05^{b}
RS2	43.4±0.8	5.3±1.0	2.82±0.40^{a}	-7.8±6.4^{a}	0.94±0.44^{bc}	0.76±0.03^{c}
RS3	47.8±0.9	5.0±1.1	2.36±0.26^{b}	-7.5±2.8^{a}	0.97±0.02^{b}	0.84±0.04^{ab}
RS4	44.0±0.6	5.1±0.8	2.92±0.45^{a}	-14.5±4.0^{b}	1.02±0.08^{a}	0.89±0.09^{a}
NS-TG	42.9±0.5	5.1±1.6	1.64±0.41^{c}	-5.8±3.3^{a}	0.96±0.05^{bc}	0.83±0.09^{b}
RS2-TG	43.8±1.1	5.7±0.8	1.65±0.32^{c}	-5.3±2.3^{a}	0.95±0.03^{bc}	0.81±0.04^{bc}
RS3-TG	47.9±0.6	5.2±1.9	1.62±0.49^{c}	-5.4±2.9^{a}	0.92±0.04^{c}	0.80±0.05^{bc}
RS4-TG	44.4±0.6	5.2±0.9	2.53±0.41^{ab}	-11.7±6.6^{b}	0.97±0.02^{b}	0.83±0.02^{b}

NS, RS2, RS3, RS4, and TG means native starch, Hi-maize, retrograded wheat starch, cross-linked wheat starch and transglutaminase, respectively.
$^{a-c}$Values within each column with the same superscript are not significantly different ($p<0.05$).
N=2 in moisture content and specific volume, N=10 in texture test

Table 5. Sensory evaluation data of baguettes prepared from RS added doughs with TG

Attribute	RS added baguettes				RS and TG added baguettes			
	NS	RS2	RS3	RS4	NS-TG	RS2-TG	RS3-TG	RS4-TG
Volume	6.5±1.8^{c}	8.8±2.2^{ab}	7.3±2.6^{bc}	7.3±2.2^{bc}	7.8±1.0^{bc}	10.2±1.3^{a}	7.6±2.0^{bc}	9.2±2.1^{ab}
Color	6.2±2.2^{cd}	7.6±1.9^{bc}	9.4±2.0^{a}	5.5±0.9^{d}	5.7±2.1^{d}	9.5±1.6^{a}	9.9±2.3^{a}	8.2±0.9^{ab}
Roasted flavor	7.9±2.2	8.5±1.9	8.3±2.2	9.6±1.6	9.0±2.0	8.8±2.0	10.0±1.4	9.5±1.7
Yeast flavor	6.0±1.9	5.5±1.8	6.5±1.9	6.3±2.7	5.8±2.3	6.3±2.5	5.4 ±2.7	4.7±1.8
Crumb homogenity	7.9±3.0^{b}	10.5±0.9^{a}	10.7±1.2^{a}	10.1±1.5^{a}	10.6±1.2^{a}	10.3±1.0^{a}	10.7±1.2^{a}	9.1±2.4^{ab}
Hardness	7.0±2.3	7.4±2.4	7.2±3.1	6.4±2.6	6.6±2.2	6.5±2.3	5.7±2.5	6.8±3.1
Elasticity	9.8±2.0	10.0±1.1	9.3±1.7	9.6±2.1	9.7±1.1	9.5±1.6	9.8±1.8	8.9±2.9
Chewiness	8.2±2.1	8.8±1.4	10.3±0.5	7.8±2.6	8.4±2.0	8.2±1.9	8.4±2.4	6.9±2.6
Moistness	8.3±2.1^{c}	8.8±1.4^{bc}	10.1±1.7^{ab}	9.1±1.1^{bc}	8.3±1.9^{c}	8.4±1.6^{bc}	10.8±0.8^{a}	8.8±2.2b^{c}
Overall eating quality	7.8±1.3^{c}	9.0±1.3^{ab}	8.7±1.7^{bc}	9.0±1.6^{abc}	8.1±1.1^{c}	9.9±0.9^{abc}	9.8±1.2^{ab}	10.2±1.6^{a}

NS, RS2, RS3, RS4, and TG means native starch, Hi-maize, retrograded wheat starch, cross-linked wheat starch and transglutaminase, respectively.
$^{a-c}$Values within each row with the same superscript are not significantly different ($p<0.05$).
N=12

Sensory evaluation The sensory evaluation data collected by trained graduate students are shown in Table 5. The volume, color, crumb homogeneity, moistness, and overall eating quality were significantly different among the samples ($p<0.05$). The specific volume varied in proportion to the panel scores for volume, baguettes with higher volume had leavened sufficiently, indicating a well-developed network in the dough. Colors of the NS-added baguettes, regardless of TG addition, and the RS4-added baguettes were whiter than the others. The crumb homogeneity was lowest in the NS-added baguette, because NS had no effect on the formation of gluten networks. The addition of TG improved the crumb homogeneity of NS-added baguette. As moistness is related to the moisture content of the baguette, the RS3-added baguette maintained a high level of moistness, because the retrograded RS3-added flour exhibited a higher capacity for water absorption during the dough-making. The overall eating quality of the baguettes increased due to the addition of either RS or TG. The baguettes added with TG and RS, particularly the RS4-added baguette, received higher scores than the NS-added baguettes. These results suggest that addition of RS, or some other dietary fiber materials, to wheat flour, together with the optimal amount of TG, results in the production of higher quality baguettes.

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