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Analytical Methods

Hard Red Spring wheat/C-TRIM 20 bread: Formulation, processing and texture analysis ☆

Abdellatif Mohamed [a,*], Patricia Rayas-Duarte [b], Jingyuan Xu [a]

[a] Cereal Products and Food Science Unit, NCAUR, Agriculture Research Service, USDA, 1815 N. University Street, Peoria, IL 61604, United States
[b] Food and Agricultural Products Research Center, Oklahoma State University, Stillwater, OK 74078-6055, United States

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Abstract

C-TRIM, a β-glucan-rich fraction, was added to Hard Red Spring wheat (HRSW) flour to increase soluble fiber content of bread, and to obtain a minimum of 0.75 g/bread serving (0.75 g/30 g or 2.5%) required by FDA for health claim. Three treatments or blends FGT0 (100% wheat flour – control), FGT1 (58% flour, 25% gluten and 17% C-TRIM) and FGT2 (60% flour, 22.5% gluten, and 17% C-TRIM) were used in the study. The total amount of soluble fiber from C-TRIM in FGT1 and FGT2 was 4.07–4.17% which was more than the amount required by FDA. The presence of C-TRIM increased both, the Farinograph water absorption and the arrival time. The dough mixing tolerance index (MTI) was also increased by C-TRIM. The FGT1 had higher stability than FGT2, whereas, the loaf volume of FGT1-B was also significantly higher than FGT0-B control and FGT2-B bread. The DSC results indicated that the amount of freezable-water in C-TRIM treated bread (FGT1-B and FGT2-B) was significantly higher than the control wheat flour bread (FGT0-B). This may be attributed to the higher amount of water absorbed by C-TRIM during bread dough (FGT1-D and FGT2-D) preparation and trapped or bound within the bread matrix after baking as compared to the control. After storage of FGT0-B, FGT1-B, and FGT2-B breads 2, 5, and 7 days storage at 25 °C, 4 °C, and −20 °C, the texture of bread were measured with a Texture Analyzer and the data analyzed statistically. The FTG0-B control bread firmness was significantly higher than FGT1-B and FGT2-B C-TRIM treated breads after 7 days storage at 25 °C. The amount of 0.1 M acetic acid-extractable protein was lower in FGT1-B than the control wheat flour (FGT0-B) sample. In addition, more protein was extracted at pH 7.0 than pH 4.5 because of less charges at neutral pH than pH 4.5. The free zone capillary electrophoresis analysis showed obvious differences in the protein charge and size between the dough and bread.

Keywords: β-Glucan; Soluble fiber; Suspension rheology; Bread texture; Freezable water; DMA; DSC

1. Introduction

The health benefits of fiber (soluble and insoluble) in preventing cardiovascular diseases, colon cancer and reducing cholesterol have been reported in the literature (American Heart Association, 1986; National Institute of Health, 1985; Van Horn et al., 1986). Because of health benefits of fiber, the food industry and researchers are constantly looking at ways of delivering fiber to consumers in bread and other food products. β-Glucan is a soluble fiber or gum found in a layer between seed cover cells of barley and oats. The C-TRIM product is a β-glucan concentrate from oats and barley, which is a good source of soluble fiber or gum. Pomeranz, Shogren, Finney, and Bechtel (1977) reported that addition of different fibers to bread increased dough water absorption and mixing time, and decreased bread loaf volume. Prentice and D'Appolonia (1977) reported that bread loaf volume was reduced in samples prepared by adding different levels of brewer's spent grain, the solid material remaining after malt brewing. It was reported that bran from oats meal had little

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

* Corresponding author. Tel.: +1 309 681 6331; fax: +1 309 681 6685.
E-mail address: a.mohamed@ars.usda.gov (A. Mohamed).

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effect on flour dough stability and water absorption as compared with oat bran from oats-groats (D'Appolonia & Youngs, 1978). Pre-hydration of plant fiber was reported to improve dough water absorption and high fiber bread loaf volume (Sosulski & Wu, 1988). Researchers indicated that hull-less barley with 100% amylopectin was a good source of soluble dietary fiber (Aman & Graham, 1987; Bhatty, 1987; Newman & Newman, 1991). Berglund, Fastnaught, and Holm (1992) and Marlett (1991) reported that substantial amounts of Soluble fiber in barley. Barley flour also contains β-glucan in the form of (1–3)(1–4)-β-glucans. The addition of barley flour fraction, rich in β-glucan to bread formula increased the fiber content of bread (Wang, Miller, & Hoseney, 1998).

Several reports indicated that the addition of components with high water-binding capacity have an effect on flour water absorption, dough mixing, rheological properties and bread quality (Holas & Tipples, 1978; Jelaca & Hylnka, 1972; Kim & D'Appolonia, 1977b; McCleary, 1986; Shelton & D'Appolonia, 1985; Michmiewicz, Biliadires, & Bushuk, 1990). It was reported that Dynamic Mechanical Analysis testing of bread exhibited various changes in viscoelastic properties at very low (−70 °C) temperatures (Hallberg & Chinachoti, 1992; Vittadini & Vodovotz, 2003; Vodovotz, Hallberg, & Chinachoti, 1996).

β-Glucan is the main soluble fiber component of C-TRIM (Inglett, 1991). Scientists have linked the soluble fiber component of β-glucan to cholesterol lowering when consumed in a low fat diet (Wu, Stringfellow, & Inglett, 1994). A health claim can be made as allowed by FDA when β-glucan is consumed at 3 g per day (Food & Drug Administration, 1997) or 0.72 g per serving (one bread serving is 30 g as is or 21 g dry basis). The objectives of the study were (1) to increase the amount of soluble fiber from C-TRIM in bread to a minimum of 0.75 g per serving (2.5% as is or 3.5% dry basis), and (2) to investigate the effect of C-TRIM on the dough characteristics and bread quality.

2. Materials and methods

2.1. Materials

Hard Red Spring wheat (HRSW) flour was obtained from Dakota Miller's Choice (Fargo, ND), Vital wheat gluten was obtained from Midwest Grain (Pekin, IL), C-TRIM (a hydrocolloid composition with 6.7% moisture, 2.2% ash, 1% ether extracted fat, 9.7% protein, and 20% β-glucan) was obtained from Van Drunen (Momence, IL), ascorbic acid and α-amylase were obtained from Doh-tone American Ingredients (St. Louis, MO), and instant dry yeast was obtained from Lallem Company (Derry, NH). Other bread ingredients were sodium chloride, sugar, non-fat dry milk, and Crisco vegetable oil were obtained from the local supermarket. Three treatments or blends FGT0 (F = flour, GT = gluten and 0 stands for 100% wheat flour – control), FGT1 (F = 58% wheat flour, GT = 25% vital gluten and 1 = 17% C-TRIM) and FGT2 (F = 60% wheat flour, GT = 22.5% vital wheat gluten, and 2 = 17.5% C-TRIM) were used for the study.

2.2. Methods

2.2.1. Dietary fiber

Total, soluble, and insoluble dietary fiber content was determined using AOAC Official Method No. 985029(A-J), (Horwitz, 2000) while protein and moisture were determined according to AACC methods (39-11) and (39-06), respectively.

2.2.2. Farinograph testing

The (HRSW) flour and blends (FGT0, FGT1, and FGT2) were tested utilizing the Farinograph according to AACC Approved Method No. 54-21 (AACC, 2000). The FTG0-D, FTG1-D, and FTG2-D dough water absorption, mixing tolerance index (MTI), and stability profiles were calculated.

2.2.3. Rheological measurements

Wheat (HRSW) flour and blend samples (FGT0, FGT1 and FGT2) were suspended in a 0.05 M sodium phosphate buffer, pH 7.0 and 25 °C (Xu, Bietz, Felker, Carriere, & Wirtz, 2001) at 20% (wt.%) concentration using Polytron PT10-35 homogenizer with a "low-foam" mixing head PTA 20TS (Kinematica AG, Switzerland). The flour and blend samples were well dispersed and stored at 4 °C and used within 2 days after preparation to avoid sample degradation. At least two suspensions of each sample were prepared for testing. Rheological properties of the sample suspensions were measured with a Rheometrics ARES strain-controlled fluids rheometer (TA Instruments Inc., DE) using a 50-mm diameter cone-plate geometry (Xu et al., 2001). The angle of the cone was 0.04 rad. The sample chamber was enclosed in a humidity chamber to prevent evaporation of the solution. The temperature was controlled at 25 ± 0.1 °C in the experimental chamber using a water circulation system. Prior to dynamic rheological measurements, a strain-sweep experiment was conducted to ensure that the experiment was conducted in a linear viscoelastic range. Linear viscoelasticity indicated that the measured parameters were independent of shear strains. Below 1% strain, all measured samples in the study were within the linear range. Small-amplitude oscillatory shear experiments (shear strain = 0.5%) were conducted over a frequency (ω) range of 0.1–100 rad/s, yielding the shear storage G' and loss G'' moduli. The storage modulus represented the non-dissipative component of mechanical properties. The viscoelastic solid or "rubber-like" behavior was suggested if the G' spectrum was independent of frequency and greater than the loss modulus over a certain range of frequency. The loss modulus represents the dissipative component of the mechanical properties and is characteristic of viscous flow. The phase shift (δ) is defined by $\delta = \tan - 1(G''/G')$, and indicates whether a material is

solid ($\delta = 0$), or liquid ($\delta = 90°$), or something in between. Non-linear rheological measurements were conducted as steady shear in the range of shear rate of 0.001–400 s^{-1}. Each measurement was repeated at least two times with different samples. The relative errors were all within the range of ±11%.

2.2.4. Baking procedure

The flour (50 g) was mixed with 1.5% instant dry yeast, 6% Crisco vegetable shortening, 4% non-fat dry milk, 25 ml ascorbic acid solution (500 ppm), 5 ml α-amylase solution and 55 ml sugar/salt solution. The water absorption, as determined by mixing and feeling the dough, was 17.7, 49.2, and 47.7 ml for the FGT0-control, FGT1 and FGT2, respectively. Mixing times were 8 and 12 min for control and C-TRIM treated samples, respectively. Punching and proofing times were (a) 7.9 mm gap after 105 min; and (b) 7.9 and 4.8 mm gaps after 25 min. The sheeted dough was rolled to fit the pan after the last punch. The dough was proofed for another 30–80 min prior to baking. Loaves were baked at 425 °F for 24 and 45 min for pup and large loaves, respectively. Baking performance was analyzed, in triplicate, on pup and 1 lb loaves using a modified AACC method (10-09). Dough height, loaf weight and loaf volume were recorded.

2.2.5. Bread firmness

Bread firmness test was performed on bread loves that were stored for 2, 5, and 7 days at 25, 4, and −20 °C, using a TA-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with a 6 mm cylinder probe and a 5 kg load cell. Bread samples were analyzed using a modified AACC Method (74-09) with a standard 25 mm probe and 5 kg load cell with three center slices from the pup loaves (10 mm each). The bread macro software provided by the texture analyzer was used to collect the data.

2.2.6. Differential scanning calorimetry (DSC) and freezable water

Bread samples were analyzed using a DSC Instrument, Modulated MDSC™ 2920 (TA Instruments, New Castle, DE). Bread samples were (20–25 mg) placed in a hermetically-sealed aluminum pans and quench cooled to −90 °C using the refrigeration system connected to the DSC. Throughout the run, the cell was conditioned with nitrogen flow at a rate of 24 cm^3/min. The samples were then heated from −90 to 110 °C at 5 °C/min. The onset and peak temperatures were determined by the tangent method utilized by the instrument software, which minimizes error committed by the operator in determining the onset temperature. The amount of freezable water was determined from the DSC data according to the method of Vittadini and Vodovotz (2003).

2.2.7. Dynamic mechanical analysis (DMA)

Samples were weighed in 25.4 × 50.8 × 3 mm stainless steel windows and put in carver press at room temperature for 10 min and 2000 lbs force. The samples were removed and cut into stripe and placed in torsion rectangular fixture for the TA ARES LS2 controlled strain rheometer. Each sample had different dimensions, which were recorded before testing. The samples were clamped in the fixtures using a torque wrench set at 20 cN m. The bread samples were cooled to −60 °C and measured to as high as possible temperature before samples slipped out of grips due to change in dimensions. The temperature ramps were 2 °C/min, 0.1% strain and 1 rad/s. Storage, loss modulus, and tan δ were characterized, and the storage modulus was fitted into the Fermi equation.

2.2.8. Color analysis

After sheeting and baking, four (∼10 cm^2) samples were saved in zip locked plastic bags and stored at room temperature (25 °C) for color measurement. The dough and bread L^* (Lightness), a^* (Redness) and b^* (Yellowness) color values were measured utilizing Minolta Spectrophotometer Model CM-3500d with a D65 light source (Minolta Ltd., Osaka, Japan) in triplicates.

2.2.9. Acetic acid protein extraction

Freeze-dried control, blends, dough and bread samples were ground using a coffee grinder. Fifty grams of each treatment was suspended in 500 ml 0.1 M acetic acid. After stirring for 2 h, a portion of the suspension (250 ml) was neutralized to pH 7 using 0.1 N NaOH and stirred for another 30 min. The protein content of the supernatant was determined by using nitrogen-combustion analysis method of LECO CHN-2000 instrument (Leco Corporation, St. Joseph, MI 49085). The suspension and neutralized samples were centrifuged at 3000*g* for 20 min and the supernatant was freeze-dried.

2.2.10. Free zone capillary electrophoresis (FZCE)

FZCE analysis of the blends, dough and bread loaves were determined utilizing a Beckman P/ACE 2000 with a 60 cm fused-silica capillary (50 cm to the detector × 50 μm ID). The CElixir Solutions buffer kit and standard mesityl oxide (10 mg/mL) were supplied by MicroSolv Technology Corp (Long Branch, NJ). Acetic acid protein extract, was defatted by suspending 10 mg in 10 ml chloroform and stirred for 1 h, centrifuged at 3000*g* and the precipitate was air dried. The defatted material was suspended in 1.0 ml CElixir Solution B, pH 2.5 buffer. The mixture was sonicated for 5 s and filtered through a 45 μm nylon membrane (Titan filter, Sun SRI, Wilmington, NC). An aliquot of 50 μl of the protein solution and the neutral marker mesityl oxide (0.5 mg/ml) were used for the FZCE analysis. The column was rinsed at 20 psi pressure for 1 and 2 min with 0.1 N NaOH and CElixir Solution A, respectively. The column was saturated for 2 min and 20 psi pressure with CElixir Solution B pH 2.5, which served as the running buffer. After 10 s injection of the sample and at 2.0 psi pressure, the analysis of protein extracts at 25 kV and 214 nm was carried out.

2.2.11. Statistical analysis

The statistical analysis of the bread samples data was carried out using PROC GLM in SAS Version 8.2 for PC Windows. A completely random design (CRD) was used to compare the texture data of FGT0-B control, FGT1-B, and FGT2-B bread loaves stored for 2, 5 and 7 days at 25, 4, and −20 °C. From the ANOVA, *F*-test value was obtained and a multiple comparison test was performed on the means, using Duncan's multiple range test at 0.05 level.

3. Results and discussion

The proximate composition, soluble fiber, insoluble fiber and total dietary of wheat flour (HRSW) were 0.66%, 0.97%, and 1.63%, respectively. The fiber content of FGT1 and FGT2 are as follows: Soluble, 4.00% and 4.1%; Insoluble, 0.42% and 0.43%; Total, 4.42% and 4.4%, respectively. All these dietary-fiber percentage are based on dry basis. The final soluble fiber content in bread, dry basis was 0.150 g/serving, 0.915 g/serving, and 1.01 g/serving for the FGT0-B, FGT1-B, and FGT2-B, respectively. The term as is when it is used for bread, it means that one bread serving is 30 g or 24 g dry basis considering the bread moisture content is 30% on the average. Based on these results, the requirements set by the FDA (0.75 g/serving as is or in 21 g dry basis) were accomplished without compromising the final quality of the bread such as, loaf volume. These requirements can be met by adding only 14.5% C-TRIM instead of 17% or 17.5% dry basis. The reason for reporting the data in dry basis and not in as is, because the moisture content of C-TRIM may vary. Of course, that will require changes in the amount of wheat gluten and water in the bread formula as well as the mixing time. The HRSW flour had 12.7% moisture, 10.7% protein, fat 1.6% and 0.39% ash. The C-TRIM hydrocolloid had 6.7% moisture, 1.1% fat (ether extraction), 9.7% protein (nitrogen × 6.25), 0.25% crude fiber, and 20.0% β-glucan. The pH of the C-TRIM (10% slurry in water) was 5.5–6.5 (Mohamed, Rayas-Duarte, Xu, Palmquist, & Inglett, 2004).

The control HRSW flour (FGT0) required 60.1% water to produce a dough with an optimum consistency as recorded by the Farinograph. The C-TRIM treated blends (FGT1 and FGT2) increased the Farinograph water absorption and the arrival time as compared with the FGT0 control. The arrival time increased from 2.4 min in the FGT0 (control) to 17.8 min in FGT1 and 15.4 min in FGT2. The dough mixing tolerance index (MTI) was also increased in the C-TRIM treated blends, where FGT1 (17% C-TRIM) showed higher stability than FGT2. The dough mixing tolerance index (MTI), which is the difference in Brabender Units (BU) between the top of the peak and after 5 min, where differences were observed between C-TRIM treated blends (FGT1 and FGT2) and the control. Dough stability is the difference in minutes between the time where the top of the curve reaches 500 BU and the time where it leaves the 500 BU. The increase in C-TRIM by 0.5% decreased the dough stability by 30%, whereas, MTI of the control (32 BU) was increased by 60%. The dough stability of FGT0 control was 18.5 min, whereas, the FGT1 and FGT2 stabilities were 3.4 min and 2.5 min, respectively. The effects of C-TRIM on the overall mixing properties of the dough may be attributed to the stickiness of β-glucan and the competition with the wheat gluten for water.

The strain sweep measurements results at 1 rad/s frequency for the blends (FGT0, FGT1 and FGT2) are presented in Figs. 1a and b. The linear ranges of the three samples were similar with less than 1%. The elastic or storage modulus, G', for the FGT0 control flour suspension was higher than FGT1 and FGT2 C-TRIM treated blends (Fig. 1a). The G' of FGT1 suspension was higher than FGT2 (Fig. 1b). This may be attributed to higher gluten content in FGT1 suspension than FGT2. Both G' and G'' were linear until they were flat or plateau, after that the G' was slightly higher than G'' and they moved parallel to each other over some frequencies (Fig. 1b). The storage

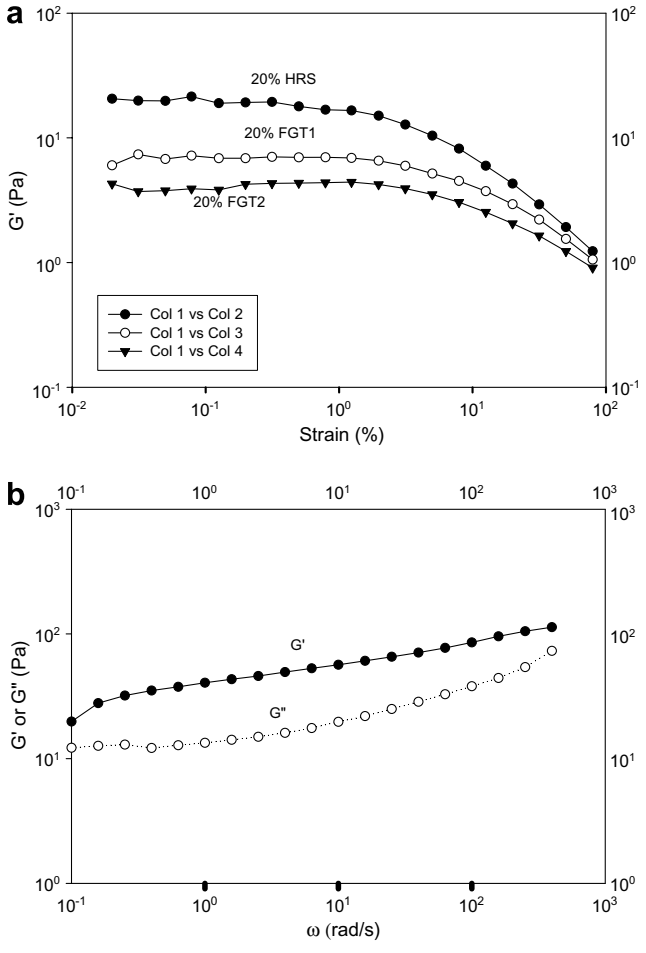

Fig. 1. (a) Strain sweep experiments for 20% (wt.%) suspensions of HRS wheat flour, FGT1, and FGT2. (b) Linear dynamic frequency-dependence storage or elastic moduli (G') and loss moduli (G'') for 20% suspension of HRS wheat flour, G', and G''.

or elastic moduli (G') and phase shifts (δ) for the FGT0 control sample were in the range of 20–113 Pa and 18–32°, respectively (Fig. 1b). The results indicated that the FGT0 flour suspension exhibited viscoelastic solid behavior as reported by Ferry (1980). The FGT1 and FGT2 blends however exhibited different physical characteristics compared with the FGT0 control (Fig. 1a). The FGT1 and FGT2 exhibited similar linear rheological properties with slope curves for both storage (G') and loss (G'') moduli, while the G'' were higher than G' over the measured frequencies indicating that the suspensions had viscoelastic fluid properties (Ferry, 1980). The G'' values of FGT2 were higher than G' values over most parts of the measured frequencies, and no plateau of the moduli was observed (Fig. 2a). The G' and phase shift values for FGT2 were in the range of 1.3–188 Pa and 40–63°, respectively. Similarly, G' and phase shift values for FGT1 were in the range of 4–219 Pa and 32–52°, respectively, indicating that FGT1 had slightly stronger viscoelastic properties than FGT2, and may be attributed to higher gluten content. The FGT1 suspension also had more data points of $G' > G''$ and short plateau of the moduli at low frequencies than FGT2 (Fig. 2a). This may also be attributed to higher gluten content in FGT1, which exhibited slightly stronger viscoelastic properties and better solid-like behavior than FGT2. From the linear rheological properties of the FGT0 control HRSW flour, FGT1 and FGT2 suspensions, we predicted that (1) the baking quality of the control flour would be better than that of FGT1 and FGT2 blends; (2) the baking quality of FGT1 should be slightly better than that of FGT2, but not significantly different from each other; (3) bread dough produced from FGT1 and FGT2 should be more sticky and chewy than the FGT0 control flour; and (4) the textural properties of breads made from FGT0 control flour should be stronger than breads produced from FGT1 and FGT2 C-TRIM blends. The non-linear shear behaviors of FGT0 control flour, FGT1 and FGT2 are presented in Fig. 2a. All the three blends showed shear-thinning behaviors. The FGT1 had higher viscosities than FGT2 at low shear rates, but had similar viscosities at high shear rates (2b). At low shear rates, the FGT0 control had higher viscosities than FGT1 and FGT2. At higher shear rates however, the FGT0 control suspension had lower viscosities than FGT1 and FGT2 (Fig. 2b). As a result of higher shear rates in the FGT0 control HRSW and industrial processing shear rates being within the range of 1–100 s^{-1} in the food industry (Bloksma, 1988), the blend would be easier to process into dough and may require lower processing energy than FGT1 and FGT2 C-TRIM blends.

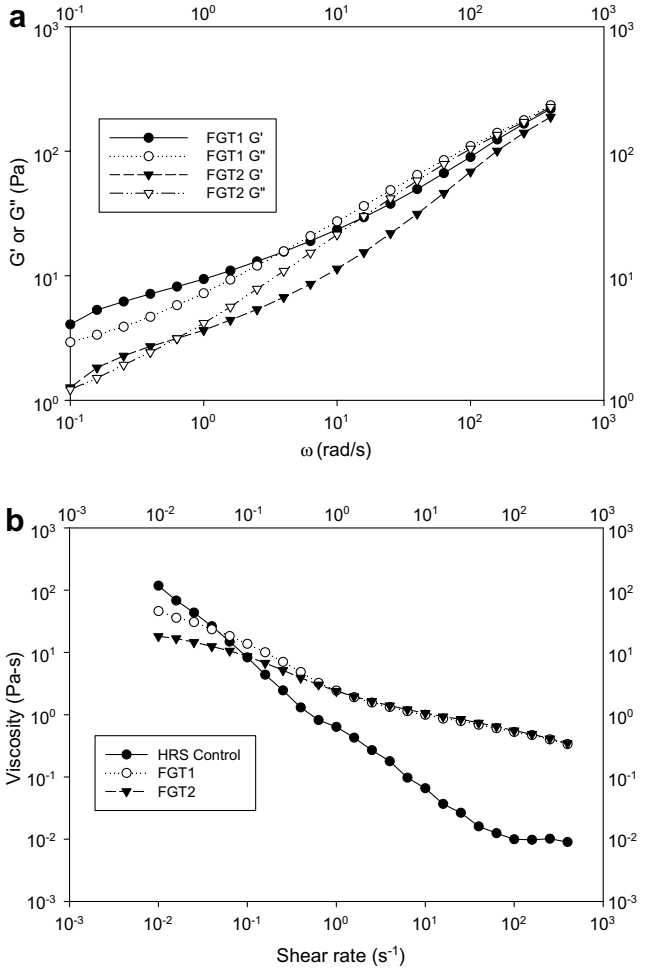

Fig. 2. (a) Linear dynamic frequency-dependence storage or elastic moduli (G') and loss moduli (G'') for 20% suspensions of FGT1 and FGT2. (b) Non-linear steady shear viscosities for 20% suspensions of HRS wheat flour (control), FGT1, and FGT2.

The loaf volume, which is a good measure of flour and bread quality, was not significantly affected by the presence of C-TRIM due to the amount of vital gluten included in the bread formula, which prevented the dilution of the gluten existed in the control flour. The loaf-volume of the control, FGT0-B was 515 ± 13.2 cc, and FGT2-B blend was 516.6 ± 15.2 cc, which is not significant different, while the loaf-volume of FGT1 blend was significantly higher (561 ± 20.1 cc) than the others. This is attributed the higher vital gluten added (25%) in FGT1 versus 22.5% for FGT2.

Bread firmness is one of the measures of the degree of staling along with other attributes, such as aroma. The time and temperature for testing bread firmness (2, 5, and 7 days, at 25, 4, and −20 °C) were selected to reflect average storage time and temperature at the supermarket. The FGT0-B control had higher firmness values than FGT1-B and FGT2-B breads (Table 1). This may be attributed to higher starch and amylose in the FGT0-B control than FGT1-B and FGT2-B breads with higher protein levels. Researchers have indicated that bread staling is caused by amylose and to a lesser extent by amylopectin retrogradation. Willhoft (1971) suggested that anti-staling effect of monoglycerides could result from interaction with gluten and was confirmed by other scientists. After the monoglycerides interact with gluten during dough mixing, surfactants migrates towards the starch gel in baking to

Table 1
Bread firmness, after storage at 25, −4.0, and −20 °C for 2, 5, and 7 days

Sample/days	2	5	7
25 °C			
Control	1018.56 ± 48 b	1232 ± 54 a	1287.53 ± 213 a
FGT1	124.83 ± 35 d	178.57 ± 47 cd	308.4 ± 88 c
FGT2	172.4 ± 29 cd	232 ± 38 cd	333.73 ± 80 c
4 °C			
Control	1050.3 ± 88 a	790.8 ± 74 b	804.1 ± 11 b
FGT1	185.3 ± 34 e	227.0 ± 30 de	238.9 ± 45 de
FGT2	228.4 ± 17 de	371.4 ± 68 c	324.5 ± 24 cd
−20 °C			
Control	336.17 ± 34 a	292.4 ± 19 ab	340.2 ± 74 a
FGT1	122.3 ± 7 d	255.6 ± 23 bc	195.4 ± 21 cd
FGT2	156.7 ± 37 d	195.8 ± 58 cd	197.8 ± 43 cd

Predicted mean values followed by the same letter within temperature are not significantly different based on overlap of the 95% confidence intervals.

interact with amylose and reduce amylose retrogradation in bread (Knightly, 1996, chap. 2). Since higher shortening and protein content was used in the formulation, we observed lower firmness values in FGT1-B and FGT2-B at all the storage temperatures studied (Table 1). The firmness of the FGT0-B control increased by 26% after 7 days storage at 25 °C as compared with 2 days storage. Whereas, FGT1-B, at the same storage time had firmness increased by 148%, and the firmness for FGT2-B increased by 93%. The high protein and C-TRIM in FGT1-B significantly lowered the bread firmness after storage for 2, 5, and 7 days at 25, 4, and −20 °C. High protein and C-TRIM may have altered the macromolecular content of the bread or water migration and the overall glass transition of the system. Since glass transition is the measure of molecular mobility, the change in the glass transition may be directly related to the molecular relaxation of the bread, which in turn affected the staling process (Parker & Ring, 2001). The viscous nature of β-glucan found in C-TRIM allowed the formation of a semi solid structure different from the protein network, which resulted in weakening protein network and producing softer bread. After 2 days of storage at 25 °C and 4 °C, the firmness of the FGT0-B control was 1018.6 g and 1050.3 g, respectively, whereas, the one stored at −20 °C had 336.2 g. The 5 and 7 days storage at −20 °C showed the same low firmness scores. At 4 °C storage temperature however, the bread firmness was lower than 25 °C storage temperature (Table 1). The presence of C-TRIM, low storage temperature and high gluten in FGT1-B and FGT2-B may have contributed in lowering the bread firmness.

The presence of a sizeable endothermic transition around 0 °C during DSC analysis of bread was attributed to ice melting (Vodovotz et al., 1996). The DSC profile of the bread samples showed the presence of an endothermic peak around −4 °C and 0 °C in the FGT0-B control and the two blends, respectively. The FGT0-B control with higher amylose content than the two blends, and the two blends with higher protein and β-glucan than the FGT0-B caused the different endothermic peak temperatures values. The FGT0-B control (−4 °C) endothermic transition was lower than the blends (−09 and −1.0 °C). The results indicated that the water present in the FGT1-B ($\Delta H = 126.6$ J/g) and FGT2-B ($\Delta H = 135.6$ J/g) breads may be more trapped or bound within the matrix as compared to the FGT0-B control ($\Delta H = 60.0$ J/g) as a result of high water holding capacity of β-glucan as indicated in the ΔH values. The DSC profile exhibited a glass transition (T_g) around 37 °C. The T_g temperature was influenced by the bread formula or composition, but the ΔCp (J/g/°C) of the FGT0-B control was (0.201 ± 0) higher than FGT1-B (0.119 ± 0) and FGT2-B (0.112 ± 0). The results is consistent with higher firmness values reported earlier in the FGT0-B control, indicating more molecular interactions (protein–starch), which require more energy to initiate molecular mobility.

The freezable water as calculated by the DSC from the peak enthalpy divided by the latent heat of fusion of ice and the total amount of water showed differences between FGT0-B and the two blends (FGT1-B and FGT2-B). With the FGT1-B (33.7 ± 1.8%) and FGT2-B (36.2 ± 1.4%) C-TRIM treated bread having higher freezable water than FGT0-B control (16.0 ± 0.6%). This may be attributed to more free or trapped water in C-TRIM treated bread than the FGT0-B control. It is worth mentioning that, the amount of water added to form the dough was 27%, 50.4%, and 49.0% for FGT0-D, FGT1-D, and FGT2-D, respectively. The freezable water of FGT1-B and FGT2-B were however not significantly different from each other. The un-freezable or bound water values in FGT0-B (10.7 ± 0.5%, FGT1-B 16.6 ± 1.3% and FGT2-B 12.9 ± 1.1% were not significantly different from each other. The linear regression plot of freezable water versus C-TRIM concentration in bread showed a good fit with $Y = 10.5X + 15.55$ and ($R^2 = 0.84$).

The storage moduli (G') obtained from dynamic mechanical analysis (DMA) measurements for the FGT0-B control, FGT1-B and FGT2-B were fitted with a model proposed by Peleg (1993), Peleg (1994). The model equation is presented as

$$R(T) = 1/\{1 + \exp[(T - T_c)/a]\} \quad (1)$$

where $R(T)$ is stiffness ratio, T is temperature, T_c is the temperature characteristic of the transition region and the inflection point of the stiffness, and is the indicator of the steepness of the curve of $R(T)$ versus T. In this model, $R(T)$ can be expressed as $R(T) = G'/G'(-30\ ^\circ\text{C})$ as presented in Fig. 3. The fitted results indicated an $R^2 = 0.99$ for the control and the two blends. The FGT0-B control (T_c) and (a) from Eq. (1) (−16.6 ± 0.1 °C), were slightly lower than the blends (FGT1-B (−15.6 ± 0.1 °C) and FGT2-B (−15.6 ± 0.1 °C), which indicated a little shift from the transition region to the lower temperature and a gradual drop in G' within the transition region. The higher (a) value for the FGT0-B (4.7 ± 0.1 °C) control versus

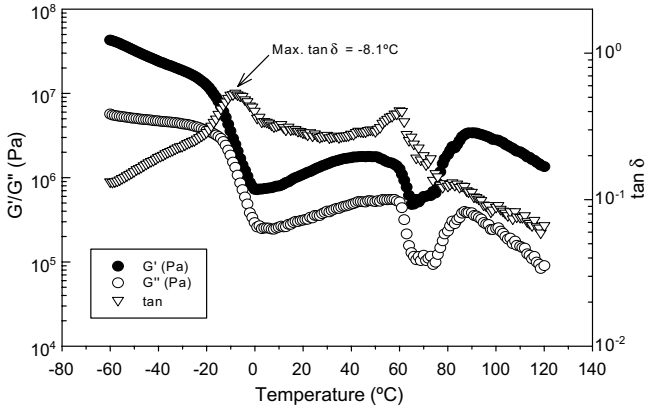

Fig. 3. Characteristic DMA profile of 100% wheat bread, showing G', G'', and tan δ.

Table 2
The color of FGT0-B (control), FGT1-B and FGT2-B bread

	L^*[a]	a^*[b]	b^*[c]
Control	55.67 ± 1.93 b[d]	1.99 ± 0.26 a	19.28 ± 0.21 a
FGT1	68.95 ± 2.49 a	0.21 ± 0.11 b	15.10 ± 0.43 b
FGT2	55.62 ± 0.69 b	2.05 ± 0.19 a	19.03 ± 0.58 a

[a] L = lightness, higher values indicate lighter color.
[b] a = redness.
[c] b = yellowness; higher color intensity is indicated by higher values.
[d] Predicted mean values followed by the same letter within a column are not significantly different based on overlap of the 95% confidence intervals.

3.9 ± 0.1 °C and 4.0 ± 0.1 °C for FGT1-B and FGT2-B, which was reflected in the curve steepness may be attributed to the heterogeneity of the control as compared to the two blends. The FGT1-B and FGT2-B (T_c) and (a) were very close or similar, and the properties of the two breads were the same around the glass transition temperatures as predicted in the rheological properties.

The color 'a' and 'b' values of FGT1-B was significantly higher than FGT0-B control and FGT2-B breads (Table 2). This may be attributed to higher protein in FGT1-B (25%) than FGT0-B (10.7%) and FGT2-B (22.5%) bread loaves. The high protein in FGT1-B may be involved in browning reactions (Millard and Caramelization) and generation of the desired aroma, flavor and brown tan color of bread. The color 'L' value of FGT1-B was also higher than FGT0-B and FGT2-B breads.

The extracted proteins from blends (FGT0, FGT1 and FGT2), dough (FGT0-D, FGT1-D and FGT2-D) and bread (FGT0-B, FGT1-B and FGT2-B) are presented in Fig. 4. The amount of extracted proteins from FGT0 control was lower than FGT1 and FGT2. After addition of water and other ingredients, the proteins of FGT0-D, FGT1-D and FGT2-D were low because of dilution effect of water and other ingredients. During bread baking, some of the water may have been dried by high temperatures, and the protein levels of FGT0-B, FGT1-B and FGT2-B breads may have increased (Fig. 4). Interactions of proteins with starch and other ingredients may also form complexes that may lower the protein levels in dough and bread. More proteins were extracted at pH 7.0 than with 0.1 M acetic acid in all the treatments investigated (Fig. 4). This may be attributed to positive charges on the proteins at low pH than at neutral pH, and interaction of protein with water and other ingredients.

The proteins were characterized using FZCE as presented in the electrophoregram of the blends, dough and bread (Fig. 5). The FZCE simplified the effect of pH on the extracted protein, with the most positively charged protein moiety eluting first from the FZCE column (Mohamed, Rayas-Duarte, Gordon, & Xu, 2003). The differences between the proteins in dough and bread were also shown by FZCE (Fig. 5). The dough displayed a set of sharp peaks as compared with the bread. A sharp peak around 5 min of retention time appeared on all samples, but in bread the peak was sharper and high in intensity (Fig. 5).

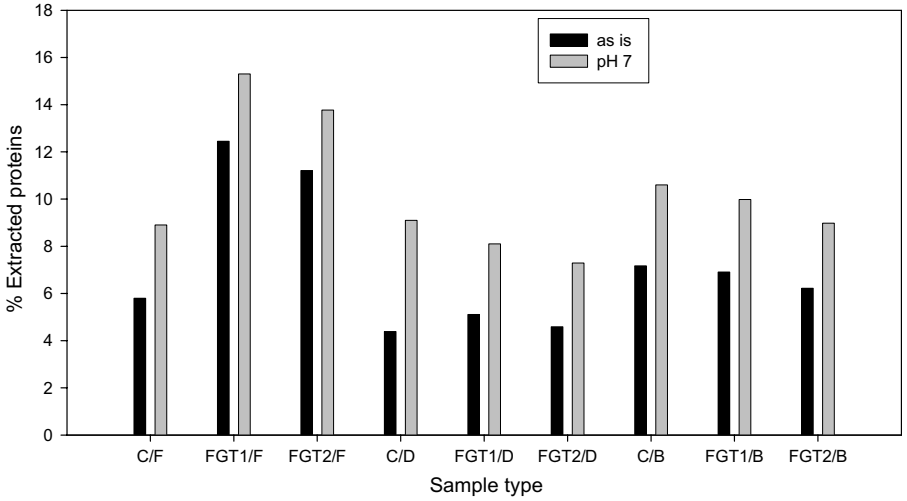

Fig. 4. Acetic acid percent protein extract as is or at pH 7.0 for control (flour) and FGT1 or FGT2, dough, and bread and their respective controls. The CF = control flour, CD = control dough, FGTD = blend dough, CB = control bread, and FGTB = blend bread.

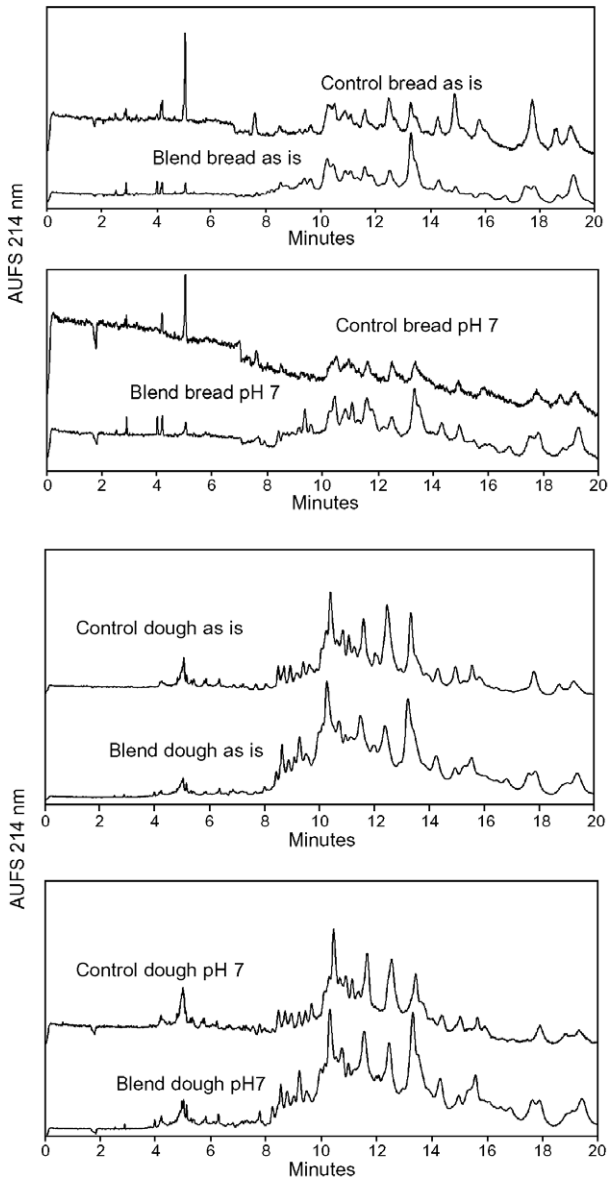

Fig. 5. Capillary zone electrophoresis profile of acetic acid extracted protein from the control and FTG1 as is and at pH 7. The profile includes flour and dough extracts.

Mohamed et al. (2003) reported similar wheat protein peaks eluting between 8 and 12 min after retention time in dough.

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