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Article

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Effect of sorghum flour composition and particle size on quality properties of gluten-free bread

Emily Frederick Trappey[1], Hanna Khouryieh[2], Fadi Aramouni[3] and Thomas Herald[4]

Abstract

White, food-grade sorghum was milled to flour of varying extraction rates (60%, 80%, and 100%) and pin-milled at different speeds (no pin-milling, low-speed, and high-speed) to create flours of both variable composition and particle size. Flours were characterized for flour composition, total starch content, particle size distribution, color, damaged starch, and water absorption. Bread was characterized for specific volume, crumb structure properties, and crumb firmness. Significant differences were found ($P < 0.05$) in the composition of sorghum flours of varying extraction rate, most notably for fiber and total starch contents. Flour particle size and starch damage were significantly impacted by extraction rate and speed of pin-milling. Water absorption increased significantly with increasing extraction rate and pin-milling speed. Breads produced from 60% extraction flour had significantly higher specific volumes, better crumb properties, and lower crumb firmness when compared with all other extractions and flour types. The specific volume of bread slices ranged from 2.01 mL/g (100% extraction, no pin-milling) to 2.54 mL/g (60% extraction, low-speed pin-milling), whereas the firmness ranged from 553.28 g (60% extraction, high-speed pin-milling) to 1096.26 g (commercial flour, no pin-milling). The bread characteristics were significantly impacted by flour properties, specifically particle size, starch damage, and fiber content ($P < 0.05$).

Keywords

Sorghum, flour characterization, composition, particle size, gluten-free bread

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INTRODUCTION

Celiac disease is an autoimmune disorder that affects genetically susceptible individuals. It is caused by the ingestion of wheat gluten, as well as proteins in related cereals, such as barley, rye, and possibly oats (Alaedini and Green, 2005). Portions of these proteins elicit an autoimmune response that causes inflammation of the upper small intestine, and thus causing a variety of undesirable symptoms (Alaedini and Green, 2005). The only effective and available treatment is the lifelong avoidance of gluten-containing foods. Celiac disease is common throughout the world and affects around 1 in 100 to 1 in 300 of the population (Rewers, 2005). According to Rubio-Tapia et al. (2012), the prevalence of celiac disease in the United States was 0.71% (1 in 141), similar to that found in several European countries. However, most cases are believed to be undiagnosed.

Grain sorghum is the third most important cereal crop grown in the United States and the fifth most important cereal crop grown in the world (U.S. Grains Council, 2008). Sorghum does not contain

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gluten proteins that have been harmful to individuals with gluten. Sorghum has been studied in many food products, including breads (Schober et al., 2005, 2007), tortilla chips (Rooney and Waniska, 2000), and noodles (Suhendro et al., 2000; Liu et al., 2012). The increased awareness and better diagnosis for celiac disease has caused a bigger demand for gluten-free products. In 2012, the U.S. market for gluten-free foods and beverages reached more than $4.2 billion with growth rate of 28% over the 2008–2012 period and the U.S. sales are expected to exceed $6.6 billion by 2017 (Packaged Facts 2013).

It is widely accepted that gluten proteins are responsible for the gas-holding matrix that sets the structure in wheat bread (Hoseney, 1994). Sorghum does not contain such structure-forming proteins. Without these structure-forming proteins, it is a challenge to produce high-quality gluten-free bread that is acceptable to consumers. Although there are a handful of commercially available gluten-free breads, these products have an undesirable firm texture, large crumb structure, bland taste, and poor shelf-life.

The effects of wheat flour composition and particle size on end-product quality have been widely studied (Chaudhary et al., 1981; Farrand 1972; Hatcher et al., 2009; Kurimoto and Shelton, 1988; Oh et al., 1985). It has been reported that flour particle size affects the overall baked product quality, but specifically loaf volume. Wheat flour forms a cohesive and visco-elastic dough due to the gluten proteins. These dough properties hold gas cells nucleated by yeast fermentation and/or chemical leavening, and upon baking, the dough sets to form a sponge-like structure. Hoseney (1994) noted that finer wheat flour particle size—with the correct water absorption—makes the dough more cohesive in most baking systems. Wheat flour particle size can be reduced by regrinding a sample. However, an additional reduction of particle size is typically associated with an increase in starch damage (Gaines et al., 1988). The importance of flour composition and flour particle size on sorghum bread quality has not been investigated. At present, it has been observed that commercially available sorghum flour is milled at a very high extraction rate with no particular quality specifications regarding particle size.

Although previous research has been conducted on producing bread from sorghum flour (Schober et al., 2005, 2007), no work has been done to examine effects of particle size and composition of sorghum flour in sorghum bread quality. It is hypothesized that composition and particle size of sorghum flour will affect its functionality in a gluten-free bread system. Therefore, the main objectives are to study the effects of sorghum flour composition and particle size on functionality in gluten-free bread and to provide information that will assist millers in producing a higher value sorghum flour that can be successfully used in a variety of baking applications, as well as to enable product developers to produce higher quality gluten-free bread for consumers that require them.

MATERIALS AND METHODS

Sorghum kernel characterization

Sorghum grain was analyzed for kernel characteristics using the single kernel characterization system SKCS 4100 (Perten Instruments, Inc., Springfield, IL) based upon AACC Method 55-31 (AACC International, 2000).

Laboratory milling methods

Fontanelle D-1000-7, white, food grade sorghum was selected because it does not contain high levels of bitter phenolic compounds often associated with traditional red sorghum (Awika and Rooney, 2004). The grain was cleaned on a dockage tester (Carter-Day Company, Minneapolis, MN). Sorghum grain was milled to flour of varying extraction rates (60%, 80%, and 100%). A Buhler Laboratory Mill (MLU-202, Uzwil, Switzerland) was used to produce flour extractions of 60% and 80% (AACC Method 26-22, AACC International, 2000). The sorghum grain milled to produce 60% and 80% extraction flours was tempered to 16% moisture and held for 24 hr prior to milling to facilitate the efficient separation of various sorghum kernel components. Some details regarding the extraction steps have been omitted due to their proprietary nature.

60% extraction flour. After milling, bran—the coarse byproduct from the break portion of the mill—was set aside and considered to be feed stock. The shorts fraction—the coarse byproduct from the reduction portion of the mill—was sieved for 2 min on a Great Western Laboratory Sifter (Great Western Manufacturing, Inc., Leavenworth, KS), running at a 4-in. diameter throw at 180 rpm. The sieve frame used was clothed with a 150-μm aperture screen opening and removed any flour which tailed over the coarse reduction sieves. A total of 7-flour streams (including three each from the break and reduction systems, respectively, and the sifted shorts flour) were combined into the first flour extraction. To generate additional flour, a series of additional grinding and separation procedures were conducted.

80% extraction flour. The 60% flour extraction rate procedure was used to obtain an 80% flour extraction, except that at this point, the three break, three reduction, and shorts flour streams were combined and set aside. To generate the additional flour necessary to reach 80% extraction, a series of additional grinding and separation procedures were conducted. Upon the final, the resulting flour was combined with all other flour streams in amounts necessary to achieve 80% extraction. The combined flour streams were blended for 30 min using a flour-blending drum and subjected to the appropriate pin milling treatments.

100% extraction flour. The sorghum was not tempered prior to milling, as bran removal was not necessary. A series of grinding and separation procedures were conducted to produce the 100% extraction flour. No by-products or co-products were manufactured from this milling process, and thus generating a 100% whole, white sorghum product. The flour was blended for 30 min using flour-blending drum and subjected to the appropriate pin milling treatments.

Pin milling. To obtain flours of varying particle size with consistent composition, each experimental flour unit was subjected to one of three pin-milling treatments that corresponded with the appropriate rotor speed: no pin-milling (No), low-speed pin-milling (Lo), and high-speed pin-milling (Hi). Pin-milling was performed on an Alpine Pin Mill (160 Z, Augsburg, Germany). The mill had both a static and dynamic set of rotor pins. The stationary pin set consists of four rows of 0.32 cm diameter pins set in a circular pattern with the inside set having a 8.57 cm diameter pattern and the outside set having a 14.6 cm diameter pattern. The rotating pin set had four rows of 0.32 cm diameter pins with an inside diameter of 7.62 cm and the outside set having a 15.88 cm diameter pattern. The motor speed was 3440 rpm, with the belt drive and internal speed up drive resulting in a low rotor speed of 7642 rpm and a high rotor speed of 14,620 rpm. The inside/outside tip speed for the low rotor speed is 30.5/63.5 m/s, and is 58.3/121.5 m/s for the high rotor speed setting. The resulting flours were stored in re-sealable plastic bags at 4 °C prior to analyses.

Commercial flour. Commercial sorghum flour (CF) was obtained directly from the vendor (Authentic Foods, Gardena, CA) and used as a control for comparing final bread quality. To ensure a homogenous mixture, the flour was blended for 15 min using a ribbon blender (Wenger Double Ribbon Stainless Steel Blender, Wenger Mfg., Sabetha, KS). After mixing, the flour samples were pinned milled as described earlier and then stored in a re-sealable plastic bag at 4 °C prior to analyses.

Sorghum flour characterization

Proximate analyses. Proximate analysis on the flour was performed according to the following AOAC standard methods (AOAC, 1995): moisture (method 930.15), ash (method 942.05), protein (method 990.03), and fat (method 920.39). Crude fiber contents were measured using AOAC method 962.09.

Flour analyses. Flour particle size distribution was determined with an LS 13 320 single wavelength laser diffraction particle size analyzer using the Tornado dry powder system (Beckman-Coulter, Inc., Miami, FL). The total starch content was determined using the Megazyme Total Starch Assay Kit, K-TSTA 05/2008 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland), based on the Amyloglucosidase/Alpha-Amylase method (AACC Method 996.11, AACC International 2000). Starch damage was determined using the Megazyme Starch Damage Assay Procedure, K-SDAM 05/2008 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland) following AACC Method 76.31. Color (L^*, a^*, and b^*) of the flour samples was determined using a HunterLab MiniScan (Model MS/S-4000S, Hunter Associates Laboratory Inc., Reston, VA). Water absorption characteristics of each flour were evaluated based upon AACC Method 56-11, Solvent Retention Capacity (SRC) Profile. SRC is defined as the weight of solvent held by flour after centrifugation, expressed as a percent of flour weight.

Sorghum bread production and characterization

Water optimization. Prior to baking, the water addition necessary for each flour treatment was optimized by standardizing the batter consistency (Table 1). For wheat bread, it is widely accepted that optimum water absorption may be determined using either a Brabender farinograph or mixograph. To date, there are no standard methods for water absorption optimization for

Table 1. Water addition (% flour basis) to bread formulation for each flour treatment

Flour extraction level (%)	Pin milling treatment		
	No	Low	High
60	104	107	109
80	113	116	118
100	125	129	135
CF	105	108	111

gluten-free breads. As a result, water optimization for this study was conducted by measuring the force necessary to extrude each batter using a texture analyzer, a method described by Schober et al. (2005) and pioneered by Sanchez et al. (2002). Based on preliminary results, a pre-determined value (5% more water and 5% less water) was used to interpolate the optimum amount of water for each of the flours. The batter was loaded into the texture analyzer (TA-XT2, Stable Micro Systems, Godalming, United Kingdom), which was equipped with a 30 kg load cell, the forward extrusion cell, and a 10-mm nozzle. The extrusion force was measured at a test speed of 1.0 mm/s over a distance of 20 mm, a pre-test and post-test speeds of 1.0 and 10.0 mm/s, respectively, and a trigger force of 50 g. The averaged force after reaching a plateau was used an indicator of batter firmness.

Breadmaking. Breads were prepared as described by Schober et al. (2007). Ingredients used were as follows: sorghum flour (70%), unmodified potato starch (30%) (Bob's Red Mill, Milwaukie, OR), iodized salt (1.75%) (Kroger, Cincinnati, OH), granulated sugar (1%) (Walmart Stores, Inc., Bentonville, AR), hydroxypropyl methylcellulose (2%) (Methocel K4M, E464, Dow Chemical Co., Midland, MI), and active dry yeast (2%) (Red Star Yeast, Milwaukee, WI). The sum of the sorghum flour and potato starch was interpreted as the flour weight basis. The addition of water to the formulation was modified for each flour to standardize the consistency of each batter, as previously described (Table 1).

The dried yeast was reactivated with 5 min of prehydration in the amount of water (30 °C) appropriate for each flour treatment. The remaining dry ingredients were mixed separately, breaking up any clumps, and added to the yeast and water mixture. The batter was mixed with a 300 W Kitchen Aid mixer (Ultra Power, St Joseph, MI) with a flat beater attachment for 30 s at the lowest speed, and then scraped. Mixing was continued for an additional 90 s at level 2 out of 10. 250 g of each batter was weighed into greased baking tins ($9 \times 15 \times 5.5$ cm) and proofed at 32 °C and 85% relative humidity in a proofing cabinet (National Manufacturing Co., Lincoln, NE). Each batter was proofed to height, corresponding to 1 cm below the edge of the tin. Approximate proof time was about 40 min. After proofing, the batters were baked for 30 min in an electrically powered reel-type test baking oven (National Manufacturing Co., Lincoln, NE) preheated to 232 °C. Upon entering the oven, each batter was "steamed" by injecting 0.7 L of water (by spraying with a spray bottle). After baking, the loaves were depanned and cooled for 1.5 hr at ambient temperature.

Specific volume. After cooling, loaves were weighed and loaf volume was measured by rapeseed displacement (AACC Method 10-05).

Crumb structure evaluation. The bread was sliced transversely using an in-house manufactured slice regulator and bread knife to obtain four slices of 25-mm thickness. The bread slices were assessed for crumb grain characteristics using a C-Cell Instrument (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). Images from the C-Cell Instrument were used to determine bread crumb attributes, including average cell diameter and volume, average cell wall thickness, average crumb fineness, and slice brightness.

Crumb firmness. Firmness of the crumb was performed on one slice from each loaf using a texture analyzer (TA-XT2, Stable Micro Systems, Godalming, United Kingdom) equipped with a 38 mm Perspex cylinder probe along with a 30 kg load cell. Texture profile analysis was carried out with a constant speed of 2.0 mm/s (applying to the pre-test speed, test speed, and post-test speed) for a distance of 10.0 mm, corresponding to 40% compression of the 25-mm slices. There was a 5s wait time between the first and second compression cycles; the trigger force was 20.0 g.

Statistical design and data analysis

A split-plot design with a completely randomized main plot was used. Three replications and at least 2 subsamples were performed. The main-plot effect was the extraction level, with three replications under each main-plot level. Subsamples produced from each main-plot replication were randomly assigned to the sub-plot main effect, or pin milling treatment. Proximate analyses and measurements of flour color were determined in triplicates, while total starch, starch damage, particle size, and water absorption were determined in duplicates in each replication. Replications of each flour treatment were baked in duplicate loaves, and 8 slice views were evaluated for crumb characteristics. All data were analyzed using SAS, Software Release 9.1.3. When treatment effects were found significantly different, the least square means with Tukey–Kramer groupings were used to differentiate treatment means. A level of significance was observed at $\alpha \leq 0.05$. Multiple linear regression was carried out to determine significance of interaction between variables. Results were expressed as mean values ± standard deviation of three replicates.

RESULTS AND DISCUSSION

Sorghum kernel characterization

Sorghum kernel hardness index (75.84), weight (29.62 mg), and diameter (2.37 mm) for Fontanelle variety D-1000-7 sorghum grain were determined using single kernel characterization system. In a study comparing gluten-free bread-making quality of different sorghum hybrids, Schober et al. (2005) found a range of kernel hardness indices ranging from 72.1 to 82.7. Kernel hardness in sorghum has been linked to milling quality (Rooney and Waniska, 2000), as well as end-product quality, including cooked grain texture of sorghum (Cagampang and Kirleis, 1984), porridge quality (Rooney et al., 1986), couscous quality (Aboubacar and Hamaker, 1999), and bread quality (Schober et al., 2005).

Sorghum flour characterization

Proximate analysis. Significant differences were found ($P < 0.05$) in composition of sorghum flours evaluated (Table 2). Farrand (1972) stated that controlled measures taken to alter levels of starch damage are unlikely to significantly affect any flour constituents other than the physical state of the starch. As such, results for compositions of all flours prior to pin-milling only are shown. Among laboratory-milled flour samples, protein content significantly increased with increasing extraction level. The protein content of CF fell between 80% and 100% extraction flours. For fat content, CF had the highest level, followed by 100% extraction. The 60% and 80% extraction flours had the lowest levels of fat and ash among all flours studied. Measurements of fiber content showed significant differences in each sample. The 100% extraction flour had the highest fiber content, followed by CF commercial flour; 60% extraction flour had the lowest fiber content. Increases in each of these four components are associated with increases in extraction rate. Higher extraction flours contain higher concentration of the aleurone layer and the peripheral endosperm in the flour, which are the components of the caryopsis that are the main sources of fat, ash, protein, and fiber (Rooney and Clark, 1968). Similar results were found in a study on the effect of extraction level of wheat flour on tortilla texture (Ramirez-Wong et al., 2007). Effects of these flour components on bread-making are discussed further where applicable.

Significant differences for moisture content were found ($P < 0.05$) among various flour extractions (Table 2). Differences in moisture among laboratory-milled samples may be due to milling processes necessary to achieve the specific and desired extraction rate. The mechanical force necessary to produce flours of greater extraction resulted in heat generation on the milling rolls, and thus caused a significant moisture loss in the flour. Another reason could be preconditioning before milling as the sorghum grain milled to produce 60% and 80% extraction flours was tempered to 16% moisture.

Significant differences were found ($P < 0.05$) for total starch content between flour treatments (Table 2). Among laboratory-milled sorghum flour samples, the total starch content decreased with an increasing flour extraction rate. The commercial flour samples were found to be not significantly different from the 80% extraction flour sample. The decrease in total starch content noted with increasing extraction rate can be attributed to dilution from other caryopsis components, including the pericarp, aleurone layer, germ, as well as the endosperm which also contains protein (Wall and Blessin, 1969).

Flour particle size. Significant differences in flour particle size were found ($P < 0.05$) among all flours studied, both within extraction level and pin-milling treatment (Table 3). Flour particle size for each flour treatment at the 90 volume percent (d90) was reported, which indicated that 90% of the flour particles were less than the stated size. Among the laboratory-milled sorghum flour samples, particle size was significantly different among flour samples within each pin-milling treatment ($P < 0.05$); particle size decreased with increasing pin mill speed. Within the no pin-milling and low-speed pin-milling treatments, particle size

Table 2. Compositions of laboratory- and commercially milled sorghum flours

Flour extraction slevel (%)	Protein (% db)	Fat (% db)	Ash (% db)	Fiber (% db)	Moisture (%)	Total starch (% db)
60	7.74 ± 0.09^{d}	1.97 ± 0.19^{b}	0.90 ± 0.11^{b}	0.78 ± 0.07^{d}	12.22 ± 0.62^{a}	85.71 ± 1.61^{a}
80	9.19 ± 0.25^{b}	2.22 ± 0.13^{b}	1.01 ± 0.05^{b}	1.00 ± 0.07^{c}	11.32 ± 0.59^{a}	81.54 ± 2.22^{b}
100	9.81 ± 0.33^{a}	3.03 ± 0.09^{a}	1.44 ± 0.03^{a}	2.14 ± 0.02^{a}	9.11 ± 1.23^{b}	71.16 ± 1.79^{c}
CF	8.47 ± 0.01^{c}	3.04 ± 0.03^{a}	1.34 ± 0.01^{a}	1.50 ± 0.03^{b}	8.49 ± 0.02^{b}	79.70 ± 2.05^{b}

Means with different superscripts in columns indicate are significant difference ($P < 0.05$).

Table 3. Particle size distributions, mean particle size distributions, and starch damage of sorghum flours milled to varying extractions and pin-milling treatments

Flour extraction level (%)	Particle size distribution (d90)			Mean particle size			Starch damage (%)		
	Pin milling treatment			Pin milling treatment			Pin milling treatment		
	No	Low	High	No	Low	High	No	Low	High
60	168.84 ± 2.16^{Ac}	140.26 ± 0.69^{Bc}	114.60 ± 2.78^{Ca}	98.76 ± 0.78^{Ac}	75.57 ± 1.96^{Bc}	54.99 ± 1.80^{Cb}	9.90 ± 0.14^{Cc}	11.10 ± 0.26^{Bc}	13.06 ± 0.41^{Ac}
80	211.51 ± 14.57^{Ab}	163.05 ± 9.00^{Bb}	119.53 ± 2.50^{Ca}	117.97 ± 3.11^{Ab}	88.36 ± 4.65^{Bb}	57.96 ± 0.99^{Cab}	13.92 ± 0.16^{Ca}	16.07 ± 0.38^{Ba}	18.07 ± 0.15^{Aa}
100	256.96 ± 22.55^{Aa}	197.29 ± 22.55^{Ba}	117.93 ± 7.17^{Ca}	129.14 ± 9.67^{Aa}	100.34 ± 9.32^{Ba}	55.21 ± 3.31^{Cb}	12.18 ± 0.61^{Bb}	11.86 ± 0.58^{Bb}	16.51 ± 3.43^{Ab}
CF	132.07 ± 1.22^{Ad}	132.18 ± 3.36^{Ac}	120.10 ± 6.20^{Ba}	67.61 ± 0.95^{Ad}	68.28 ± 2.89^{Ac}	71.68 ± 19.55^{Aa}	8.35 ± 0.08^{Cd}	8.96 ± 0.12^{Bd}	9.73 ± 0.07^{Ad}

For each column, mean values with the same lowercase superscript are not significantly different within each variable ($P > 0.05$).
For each row, mean values with the same uppercase superscript are not significantly different within each variable ($P > 0.05$).

significantly increased with increasing extraction rate. However, within the high-speed pin-milling treatment, flour particle size was not considered to be significantly different among varying extraction rates. The same trends in results and significant differences were found in the data for mean particle size.

It appears that the present pin-milling treatments were successful in reducing flour particle size of the flours studied. However, of particular interest are the particle size results for CF. There was not a significant decrease in particle size found between the no pin-milling and low-speed pin-milling treatments, and while the high-speed pin-milled CF flour was found to have a significantly lower particle size than the no or low-speed pin-milled flours, the decrease was to a lesser degree than seen for the laboratory-milled flours. These results may indicate that CF is a soft sorghum flour. Hard wheat produced larger flour particles than soft wheat (Farrand, 1972; Oh et al., 1985).

Starch damage. Significant differences in starch damage were found ($P < 0.05$) within each extraction level (Table 3); starch damage increased with increasing speed of pin-milling. Within each pin-milling treatment of the laboratory-milled samples, flours were found to be significantly different from one another. However, there was an unexpected relationship between extraction level and starch damage. For the no- and low-speed pin-milling treatments, the 80% extraction flours had the highest starch damage levels. Although it was thought that higher starch damage would result from a greater degree of regrinding (due to increasing the extraction rate, i.e. 100% extraction rate), the observed opposite effect can best be explained by a variation in milling method. As described previously, the 100% extraction flours were milled on Hal Ross roller mills, and the lower extraction flours were milled on Buhler Laboratory milling equipment. Although every effort was made to standardize the milling procedures for each treatment, roll pressure, roll surface, and roll speed differential have been found to be responsible for producing damaged starch.

Flour color. L^*, a^*, and b^* color values of sorghum flours milled to varying extractions and pin-milling treatments are shown in Table 4. Within flour samples subjected to no additional pin-milling, L^* values decreased significantly ($P < 0.05$) with increasing extraction rate. For flours milled to 60% and 80% extraction, the low- and high-speed pin-milled samples are not considered to be significantly different from one another in degree of whiteness. However, 100% extraction flours within the same pin-milling treatments were considered to be significantly lower in L^* values when compared with other extraction rates. 100% extraction

Table 4. L^*, a^* and b^* values of sorghum flours milled to varying extractions and pin-milling treatments

Flour extraction level (%)	L^*			a^*			b^*		
	Pin Milling Treatment			Pin Milling Treatment			Pin Milling Treatment		
	No	Low	High	No	Low	Hi	No	Low	High
60	87.79 ± 1.86^{Ba}	88.19 ± 1.50^{ABa}	89.46 ± 1.29^{Aa}	0.04 ± 0.22^{Ab}	-0.18 ± 0.15^{Bb}	-0.22 ± 0.11^{Bb}	11.82 ± 0.44^{Ac}	12.46 ± 1.00^{Abc}	11.56 ± 1.05^{Ac}
80	86.01 ± 2.09^{Bb}	87.49 ± 1.26^{ABa}	88.66 ± 0.95^{Aa}	-0.05 ± 0.09^{Ab}	-0.03 ± 0.15^{Ab}	-0.16 ± 0.07^{Ab}	12.62 ± 1.02^{Ac}	12.18 ± 0.47^{Ac}	12.51 ± 1.40^{Ab}
100	82.56 ± 0.51^{Bc}	83.58 ± 1.12^{Bb}	85.45 ± 0.69^{Ab}	0.56 ± 0.09^{Aa}	0.42 ± 0.13^{Aa}	0.52 ± 0.18^{Aa}	13.55 ± 0.42^{Ab}	13.26 ± 0.64^{Ab}	13.07 ± 0.64^{Ab}
CF	83.35 ± 2.25^{Bc}	83.80 ± 0.86^{ABb}	85.17 ± 0.73^{Ab}	0.39 ± 0.14^{Ba}	0.64 ± 0.26^{Aa}	0.68 ± 0.12^{Aa}	15.17 ± 0.83^{Aa}	14.26 ± 0.69^{Aa}	14.66 ± 0.73^{Aa}

For each column, mean values with the same lowercase superscript are not significantly different within each variable ($P > 0.05$).
For each row, mean values with the same uppercase superscript are not significantly different within each variable ($P > 0.05$).

flour and CF were not considered to be significantly different among all extraction rates in relation to L^* values. Ash, fiber, and particle size significantly contributed to L^* in this particular study ($P < 0.0001$). Kurimoto and Shelton (1988) reported that L^* values showed a significant increase with decreasing particle size ($P < 0.01$), suggesting that finer flour appears to be brighter or whiter.

The 60% and 80% extraction flours had significantly lower a^* values than 100% extraction and CF in each pin-milling category ($P < 0.05$). For flours milled to 80% and 100% extraction rates, degree of pin-milling had no significant effect on a^* values. However, for the 60% extraction flour treatments, flours pin-milled at low and high-speeds had significantly lower a^* values than flour that was not pin-milled. Similar results are seen for CF, but flours pin-milled at low and high-speeds had significantly higher a^* values than flour that was not pin-milled. Although results for the collection of samples exhibited both positive and negative values, overall, the values were close to zero, and indicate a gray appearance. Extraction rate also seems to have an impact on a^* values of flour samples. A correlation coefficient of 0.75 was observed in this study between fiber and a^* values. Ramirez-Wong et al. (2007) found significant differences in a^* values with variation in extraction rate. Specifically, as the rate of extraction increased, the a^* values decreased.

For each extraction rate, b^* values were not significantly affected by degree of pin-milling ($P < 0.05$). CF had the highest b^* values among all flour treatments, followed by 100% extraction flours. The 60% and 80% extraction flours were not significantly different with the exception of flour subjected to high-speed pin-milling; high-speed pin-milled 80% extraction flour had a significantly higher b^* value than 60% extraction flour of the same pin-milling treatment.

Water absorption. Significant differences were found ($P < 0.05$) for water absorption values among flours evaluated (Figure 1). Within each pin-milling treatment, 60% extraction samples had the lowest values for water absorption; 80% and 100% extraction samples were not considered to be statistically different from one another ($P < 0.05$). With the exception of the commercial flour samples, water absorption increased significantly with increasing pin-milling speed ($P < 0.05$). For CF, samples did not significantly increase in water absorption with increasing pin mill speed ($P < 0.05$). This can be attributed to the earlier-speculated soft nature of the grain used to produce CF, causing the flour to have non-significant decreases in particle size and only slight increases in starch damage with increasing pin-milling speed.

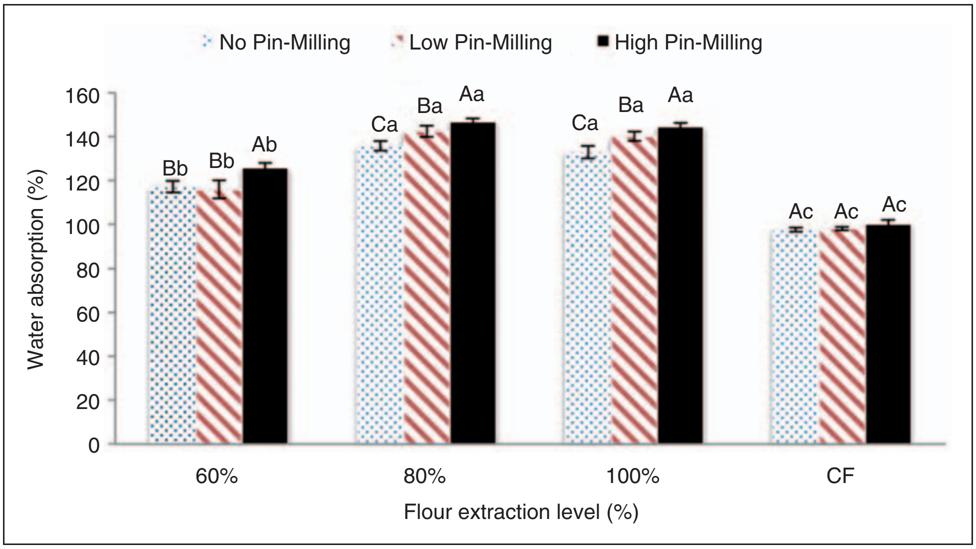

Figure 1. Water absorption of sorghum flours milled to varying extraction levels and pin-milling treatments. Mean values for a given pin-milling treatment with the same lowercase superscript are not significantly different ($P > 0.05$). Mean values with the same uppercase superscript within flour extraction level are not significantly different ($P > 0.05$).

Torres et al. (1994) found that particle size distribution of the sorghum flour was an important factor affecting water absorption of tortilla doughs. In this study, no statistically significant correlation was observed between particle size and water absorption for simple linear regression. However, analysis of multiple linear regression showed that flour particle size did significantly contribute to water absorption ($P < 0.0001$). The lack of a significant one way interaction between particle size and water absorption may suggest that several flour characteristics are responsible for sorghum flour water absorption. Because the decrease in flour particle size is often accompanied by an increase in starch damage, it is suggested that damaged starch may have greater effects on baked product quality than particle size alone (Kurimoto and Shelton, 1988; Shelton and D'Appolonia, 1985). A correlation coefficient of 0.87 ($P < 0.0001$) was found between water absorption and starch damage. This was higher than the correlation coefficient of 0.76 that was found between water absorption and starch damage of wheat flour by Kurimoto and Shelton (1988), indicating that perhaps there is a stronger relationship between the two variables in sorghum flour. Oh et al. (1985) observed an increase in water absorption in wheat flour samples with higher amounts of starch damage. Fiber was a significant predictor of water absorption ($P < 0.0001$). The increase in water absorption in flour due to the addition of dietary fiber has been widely reported (Gan et al., 1992; Gomez et al., 2003; Sabanis et al., 2009).

Sorghum bread characterization

Specific volume. Significant differences were noted ($P < 0.05$) for the specific volume of breads produced with all sorghum flours studied (Figure 2). Overall, specific volume was significantly affected by both extraction level and pin-milling treatment. Within all pin-milling treatments, breads produced from 60% extraction flour had significantly higher specific volumes when compared with all other extractions and flour types. Additionally, breads produced from 60% extraction flour subjected to low-speed pin-milling showed the highest specific volumes, followed by high-speed and no pin-milling, respectively. Specific volumes of breads produced from 100% extraction flour subjected to low- and high-speed pin-milling were significantly higher than non pin-milled flour of the same extraction. Breads produced from 80% extraction flour and CF were not significantly affected by pin-milling.

Specific volume has been shown to be affected by many factors, including dough composition, processing conditions, and dough rheology—all properties that impact gas retention capabilities (Gallagher, 2009). Although there is a nutritional benefit to the incorporation of dietary fiber into gluten-free products, this is met with the limitation of decreased volume (Chen et al., 1988; Sievert et al., 1990). In this study, fiber contributed significantly to specific volume ($P < 0.0001$). In general, flours with lower extraction rates produced breads with significantly higher loaf volumes. Research by Gan et al. (1989, 1992) stated that

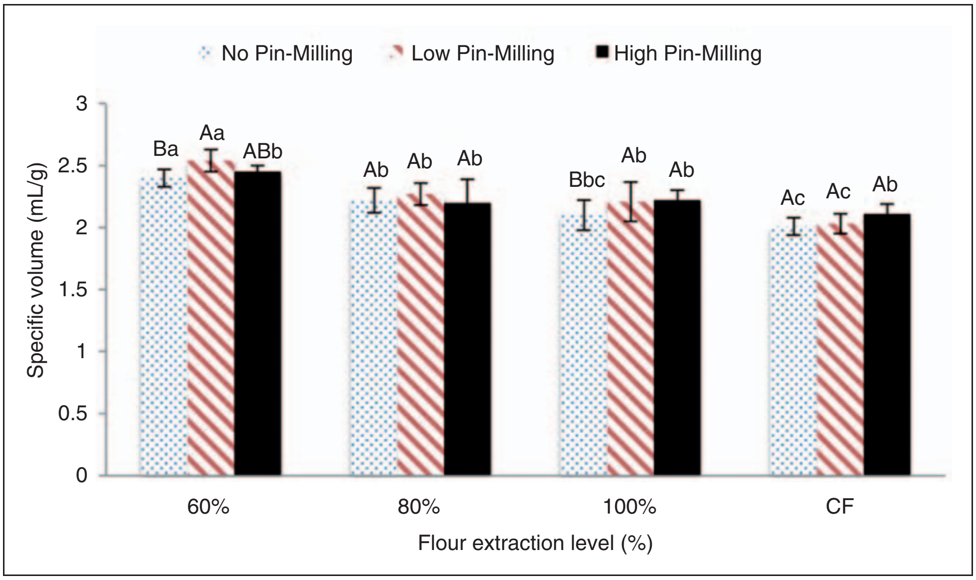

Figure 2. Specific volumes of bread produced from sorghum flours of varying extraction levels and pin-milling treatments. Mean values for a given pin-milling treatment with the same lowercase superscript are not significantly different ($P > 0.05$). Mean values with the same uppercase superscript within flour extraction level are not significantly different ($P > 0.05$).

the non-endosperm components of wheat (germ, bran, and epicarp hairs) were responsible for the depression of loaf volume. These results agree with the observations in this study that show a decrease in specific volume with an increase in extraction rate (i.e. an increase in fiber content).

Although increased fiber content is certainly a contributing factor of observed decreases in specific volume, a decrease in starch content (as a result of increased extraction rate) must also be considered responsible for alterations in loaf quality. This study showed that starch content significantly contributed to loaf volume ($P < 0.0001$). Olatunji et al. (1992) reported that favorable results were achieved for gluten-free bread by using a 70:20:10 blend of decorticated sorghum flour, gelatinized cassava starch, and raw cassava starch, respectively. Starch has several hypothesized roles in improving gluten-free sorghum bread. Some researchers have suggested a dilution effect. Endosperm and bran particles in sorghum flour become diluted by any added starch, disturbing the homogeneity of the starch gel and impeding gas cell formation (Gan et al., 1995). Schober et al. (2005) used similar reasoning to hypothesize that gluten-free breads produced from whole-grain flours (i.e. lower starch and higher fiber contents) would have lower loaf volume than pure starch breads.

Flour particle size has also been shown to affect overall baked product quality, but specifically loaf volume. Research on the effects of sorghum flour particle size on gluten-free bread systems was not found. As a result, studies on the effect of wheat flour particle size on breads and other grain based products were utilized to hypothesize as to how sorghum flour may perform. For this study, particle size was shown to be a significant predictor for specific volume ($P < 0.0001$). Miller et al. (1967) found that cake volume improved as cake flour particle size decreased as a result of pin milling to alter the flour properties. Such results may provide some rationale as to why significant increases in specific volume were noted for breads produced from 60% extraction and 80% extraction that had been subjected to some degree of pin-milling to reduce flour particle size. Flour particle size does appear to have an impact on loaf volume; the variable was significantly correlated with specific volume in this study ($P < 0.05$). However, flour particle size on its own is not the cause for alterations in specific volume (Chaudhary et al., 1981).

Because increased starch damage is a result decreasing flour particle size, its synergistic effects with particle size must not be ignored. Starch damage was a significant predictor for loaf volume ($P < 0.0001$). As mentioned earlier, specific volumes of the breads studied did not increase to the degree expected with increases in starch damage. In fact, for breads produced from 60% extraction flour, specific volume actually decreased between low- and high-speed pin-milling.

Table 5. Cell diameter and volume of bread produced from sorghum flours of varying extractions and pin-milling treatments

Flour extraction level (%)	Cell diameter (mm)			Cell volume (mm^3)		
	Pin milling treatment			Pin milling treatment		
	No	Low	High	No	Low	High
60	2.23 ± 0.12^{Ab}	2.30 ± 0.14^{Ab}	2.35 ± 0.22^{Aa}	8.00 ± 0.53^{Ab}	8.29 ± 0.67^{Abc}	8.61 ± 1.01^{Abc}
80	2.48 ± 0.14^{Aa}	2.43 ± 0.17^{Ab}	2.44 ± 0.18^{Aa}	9.53 ± 0.65^{Aa}	9.29 ± 0.90^{Ab}	9.31 ± 0.90^{Ab}
100	2.49 ± 0.15^{Aa}	2.68 ± 0.18^{Aa}	2.56 ± 0.21^{Aa}	10.28 ± 0.78^{Aa}	11.30 ± 1.07^{Aa}	10.63 ± 1.10^{Aa}
CF	2.10 ± 0.15^{Ab}	2.07 ± 0.11^{Ac}	2.13 ± 0.21^{Ab}	7.68 ± 0.77^{Ab}	7.67 ± 0.48^{Ac}	8.20 ± 1.16^{Ac}

For each column, mean values with the same lowercase superscript are not significantly different ($P > 0.05$).
For each row, mean values with the same uppercase superscript are not significantly different ($P > 0.05$).

Table 6. Cells per slice area and cell wall thickness of bread produced from sorghum flours of varying extractions and pin-milling treatments

Flour extraction level (%)	Cells per slice area (cells/cm^2)			Cell wall thickness (mm)		
	Pin milling treatment			Pin milling treatment		
	No	Low	High	No	Low	High
60	44.99 ± 1.48^{Ab}	42.28 ± 2.30^{Ab}	43.26 ± 3.10^{Ab}	0.532 ± 0.015^{Aa}	0.545 ± 0.017^{Aa}	0.545 ± 0.018^{Aa}
80	42.82 ± 1.91^{Ab}	42.53 ± 1.24^{Ab}	43.38 ± 3.10^{Ab}	0.555 ± 0.016^{Aa}	0.550 ± 0.014^{Aa}	0.547 ± 0.016^{Aa}
100	45.54 ± 3.95^{Ab}	42.32 ± 5.48^{Ab}	40.03 ± 2.57^{Bc}	0.541 ± 0.21^{ABa}	0.557 ± 0.024^{Aa}	0.562 ± 0.017^{Aa}
CF	55.17 ± 3.01^{Aa}	55.55 ± 2.35^{Aa}	52.99 ± 4.88^{Aa}	0.492 ± 0.017^{Ab}	0.489 ± 0.014^{Ab}	0.498 ± 0.020^{Ab}

For each column, mean values with the same lowercase superscript are not significantly different ($P > 0.05$).
For each row, mean values with the same uppercase superscript are not significantly different ($P > 0.05$).

Miller et al. (1967) reported that excessive pin-milling caused a considerable increase in starch damage which negatively affected cake quality.

Bread crumb cell diameter and volume. Within each flour extraction level, degree of pin-milling did not significantly affect bread cell diameter or volume (Table 5). Breads produced from 100% extraction flour tended to have significantly larger cell diameters and volumes than those made with lower extraction flours. Breads produced from CF had the lowest cell volumes among flours subjected to low-speed pin-milling. Breads produced from 60% extraction rate flour and commercial flour CF tended to have significantly lower cell diameters than other samples within the same pin-milling treatments.

In wheat bread, the extent to which cells are formed is a function of the protein-starch interactions (specifically from gluten) that provide viscoelastic properties to the dough (Lagrain et al., 2012). As gluten-free bread lacks the means necessary to produce such a network, another mechanism is utilized to form gas cells. Air cells, or alveoli, are created during mixing. Carbon dioxide, which is produced as a byproduct of yeast fermentation, diffuses into these air cells, causing them to expand (Gan et al., 1995). Overall, a smaller cell diameter is indicative of a smaller cell volume. In fact, in this study, the correlation coefficient between cell diameter and cell volume was 0.97. Smaller cells, whether defined by volume or diameter, are desirable in gluten-free bread products, as greater numbers of small gas cells have been found to produce loaves of higher specific volumes (Gallagher et al., 2003).

Cells per slice area and cell wall thickness. The cells per slice area and cell wall thickness are shown in Table 6. With the exception of breads produced with 100% extraction flour, pin-milling had no significant effect on either variable ($P < 0.05$); bread produced from 100% extraction flour with high-speed pin-milling had a significantly lower number of cells per slice area and significantly thicker cells walls than low-speed or non pin-milled samples of the same extraction rate. Among all laboratory-milled samples, extraction level had no significant effect on number of cells per slice area or cell wall thickness. Within each pin-milling treatment, bread produced from commercial flour exhibited the greatest number of cells per slice area and the thinnest cell walls compared with breads produced from laboratory-milled flours. This, again, may be an indication of the density of these bread samples.

The correlation coefficient between cells per slice area and cell wall thickness was −0.95; this could be interpreted to mean that breads with thicker cell walls were more likely to have a lesser amount of cells per standardized slice area, or vice versa. Thin cell walls predominate in fine-grained, fine-textured crumbs, and thicker cell walls are typically found in coarse-grained crumbs (Hayman et al., 1998).

The ratio of cells per slice area measurement attempts to provide standardization for variations in specific volume per loaf. However, this standardization effect has a tendency to diminish visible quality differences and should not be taken out of context. For example, the ratio of cells per slice area for bread produced from low-speed pin-milled 60% extraction flour is significantly lower than the ratio for CF with low-speed pin-milling, which numerically indicates a finer, more desirable crumb structure for the latter bread. However, as Figure 3 illustrates, bread produced from the 60% extraction flour has a distinct quality advantage and would be expected to be found more acceptable by consumers. As a second example, the ratios of cells per slice area for breads produced with 60% and 100% extraction flours with no pin-milling are not significantly different, indicating that the two breads do not differ in porosity. However, by examining Figure 4, it can again be seen that there are marked differences in crumb structure, the bread from 100% extraction bread being inferior. As such, it is this researcher's opinion that cells per slice area is not an accurate determinate of crumb quality for this particular study. It seems to be more appropriate for evaluating breads that are expected to have similar overall crumb characteristics, but slight differences in number of cells or slice area.

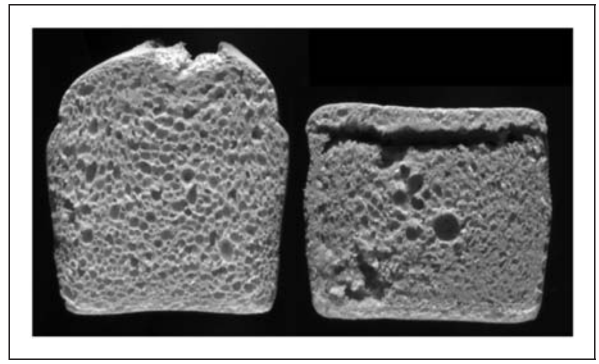

Figure 3. C-Cell images of sorghum bread slices showing crumb structure for 60% flour extraction with low-speed pin-milling (left) and commercial flour CF with low-speed pin-milling.

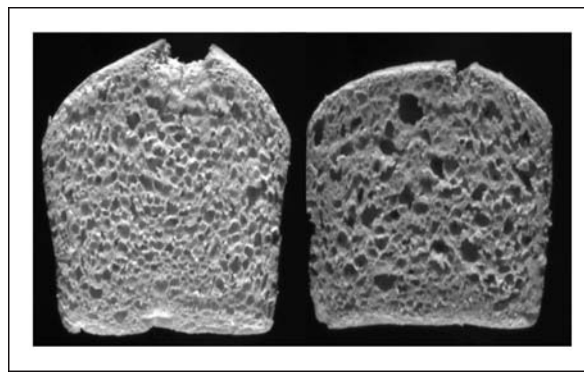

Figure 4. C-Cell images of bread slices showing crumb structure for 60% extraction flour with no pin-milling (left) and 100% extraction four with no pin-milling.

Slice brightness. Significant differences were found ($P < 0.05$) for slice brightness of breads produced from all flour treatments studied (Figure 5). For laboratory-milled flour samples, slice brightness significantly decreased with increased flour extraction ($P < 0.05$). Higher extraction flours have a higher fiber content; a correlation coefficient of −0.94 ($P < 0.0001$) was observed between slice brightness and fiber content. Higher extraction flours have a lower total starch content; a correlation coefficient of 0.82 ($P < 0.0001$) was observed between slice brightness and total starch content. Per the results for L^* and a^* values for each flour treatment, it was discussed that fiber and ash contents impacted flour color. Sabanis et al. (2009) hypothesized that crumb color is correlated to the color of the fiber. Additionally, Oh et al. (1985) found that an increase in extraction rate caused a significant decline in brightness of noodles made with such flour.

Two bread samples showed significant differences in slice brightness within pin-milling treatment: breads produced with the high-speed pin-milled flours for the 100% extraction and CF showed significantly lower slice brightness values compared with the lower speed or non pin-milled samples. Otherwise, no significant changes were observed for slice brightness due to pin-milling treatment. With that said, multiple linear regression did identify flour particle size as a significant predictor for slice brightness ($P < 0.0001$).

Crumb firmness. Significant differences were found ($P < 0.05$) for firmness of bread slices (Figure 6). Among laboratory-milled sorghum flours, there was an overall significant effect on bread texture from speed of pin-milling ($P < 0.05$); high-speed pin-milled flours produced breads with the softest crumb texture within each extraction rate. There was significant effect

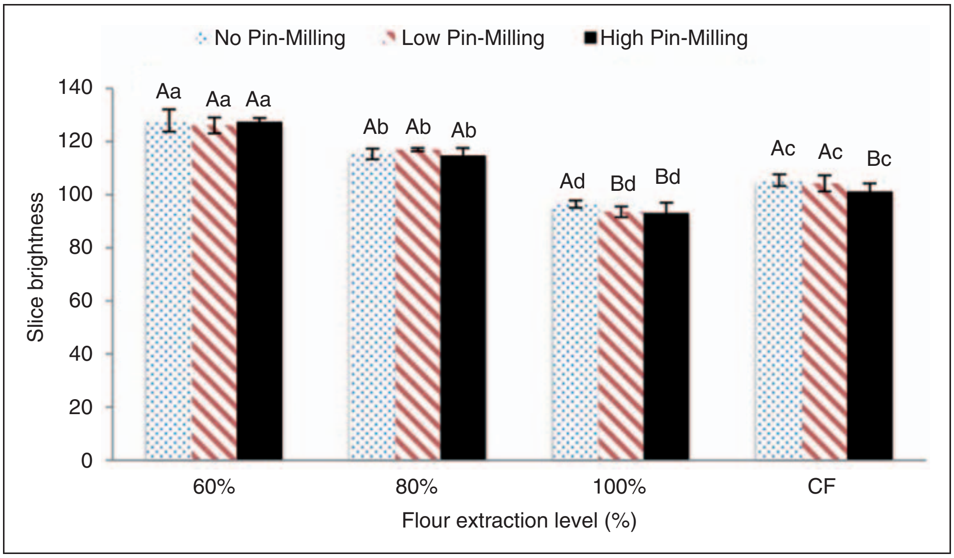

Figure 5. Slice brightness values of bread produced from sorghum flours of varying extraction levels and pin-milling treatments. Mean values for a given pin-milling treatment with the same lowercase superscript are not significantly different ($P > 0.05$). Mean values with the same uppercase superscript within flour extraction level are not significantly different ($P > 0.05$).

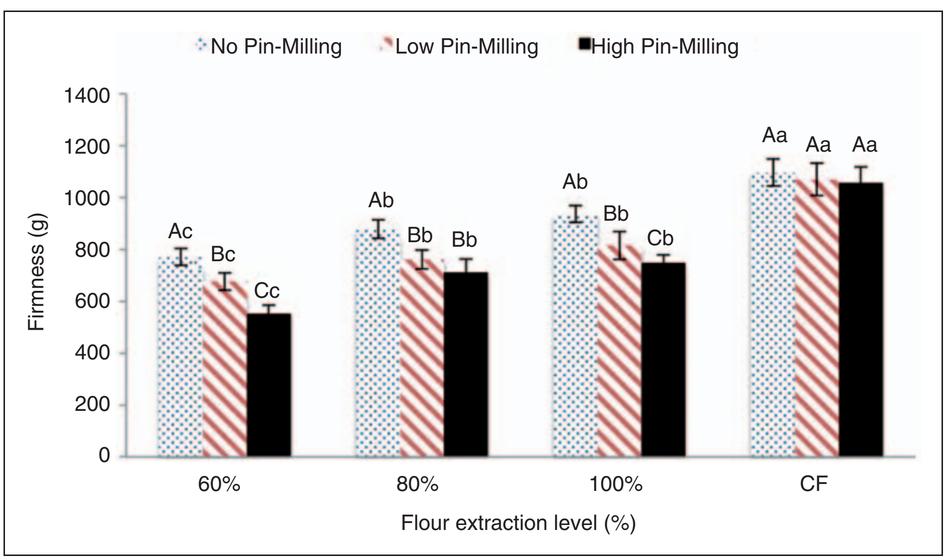

Figure 6. Firmness values of bread produced from sorghum flours of varying extraction levels and pin-milling treatments. Mean values for a given pin-milling treatment with the same lowercase superscript are not significantly different ($P > 0.05$). Mean values with the same uppercase superscript within flour extraction level are not significantly different ($P > 0.05$).

from extraction rate on bread texture; bread produced from 60% extraction flour had the softest crumb structure compared to 80% and 100% extraction flours, but the latter 2 samples were not considered significantly different from one another. Among all flour samples studied, bread produced from CF had the firmest bread texture, followed by 100% extraction which was significantly lower than CF, but significantly higher than either 60% extraction flour.

Crumb firmness is a key attribute in baked goods, as it is strongly associated with consumers' perception of bread freshness (Ahlborn et al., 2005). In white pan bread, most consumers prefer a soft, resilient, and short crumb (Pyler, 1988). As breads produced from 60% extraction flour had the softest crumb, these flours are recommended for production of gluten-free sorghum bread. Although it appears that a higher degree of pin-milling does improve crumb texture, the effect of particle size and starch damage on other previously discussed bread characteristics should be considered before deciding upon the ideal flour treatment.

Firmness of wheat bread crumb is influenced by numerous variables, including protein and fiber content, moisture, baking temperature, and loaf volume (Moore et al., 2004). Gluten-free bread has been shown to have much higher crumb firmness than other bread products. In a study by Ahlborn et al. (2005), crumb firmness values for gluten-free rice bread were four times higher than for standard wheat or low-protein starch breads. In this study, it appears that protein content had a somewhat significant effect on crumb firmness as it relates to extraction rate. In a study on the incorporation of protein powders into gluten-free bread, Gallagher et al. (2003) found that breads produced with more concentrated protein powders tended to have the firmest crumb compared with the control produced with no additional protein.

Increased fiber content is an outcome in flours with higher extraction rates. Ramirez-Wong et al. (2007) showed that an increase in firmness with an increase in extraction rate can partially be attributed to fiber content. This study showed that fiber content significantly contributed to firmness of bread ($P < 0.0001$). Sabanis et al. (2009) observed that fiber addition level significantly impacted crumb firmness of gluten-free bread at the $P < 0.0001$ level. Gomez et al. (2003) also reported an increase in crumb firmness upon the addition of wheat fiber into wheat bread. The researchers cited an explanation for increased firmness based upon the possible thickening of the cell wall due to fiber content.

Limited information exists on the effects of flour particle size and starch damage on the texture of bread, and especially gluten-free bread. However, in this study, bread firmness significantly decreased for all extractions and flour types, so the effects of starch damage and/or flour particle size cannot be ignored. Although statistical analysis showed that flour particle size did not significantly impact crumb texture, multiple linear regression revealed that starch damage was a significant predictor for crumb firmness ($P < 0.0001$). However, again, changes in starch damage were caused by changes in particle size through pin-milling. Hatcher et al. (2009) noted that both particle size and starch damage influenced white salted noodle quality. It was found that flours with fine particle size produced noodles with more acceptable textural attributes than noodles produced from coarser flour. Finer particle size flours with higher degrees of starch damage may have experienced increased swelling, and therefore softening of the cooked noodles.

CONCLUSION

Overall, this study demonstrates that sorghum flour composition and particle size affect the quality of gluten-free bread. To an extent, flours with lower amounts of fiber and a smaller particle size will produce breads with more acceptable characteristics, including volume, crumb structure, color, and texture. However, it is important to note that these flour characteristics do not exert their influences independently of one another. In fact, this research points to the importance in understanding the impact of starch damage on bread performance. This information may assist the milling industry in producing a more value-added sorghum flour, but will ultimately benefit consumers of gluten-free bread products. Further research is necessary to better understand the extent to which particle size, and therefore starch damage, can improve sorghum-based gluten-free breads.

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CONFLICT OF INTEREST

None declared.

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