Gluten-Free Bread from Sorghum: Quality Differences Among Hybrids

Tilman J. Schober,[1-3] Manuela Messerschmidt,[1,4] Scott R. Bean,[5] Seok-Ho Park,[5] and Elke K. Arendt[1]

Gluten-free breadmaking quality of 10 sorghum flours was compared using (relative basis) decorticated sorghum flour (70), corn starch (30), water (105), salt (1.75), sugar (1), and dried yeast (2). Batter consistency was standardized by varying water levels to achieve the same force during extrusion. Crumb properties were evaluated by digital image analysis and texture profile analysis (TPA). Significant differences ($P < 0.001$) in crumb grain were found among the hybrids with mean cell area ranging from 1.3 to 3.3 mm^2 and total number of cells ranging from 13.5 to 27.8/cm^2. TPA hardness values of the crumb also varied significantly ($P < 0.001$). Based on significant correlations ($P < 0.01$), starch damage, influenced by kernel hardness, was identified as a key element for these differences. Breads differed little in volume, height, bake loss, and water activity. Investigation of added ingredients on bread quality was conducted using response surface methodology (RSM) with two sorghum hybrids of opposite quality. Addition of xanthan gum (0.3–1.2% flour weight basis [fwb]) and skim milk powder (1.2–4.8% fwb) and varying water levels (100–115% fwb) were tested using a central composite design. Increasing water levels increased loaf specific volume, while increasing xanthan gum levels decreased the volume. As skim milk powder levels increased, loaf height decreased. Quality differences between the hybrids were maintained throughout the RSM.

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal grain with 60 million metric tons harvested from 44 million hectares in 2004 (FAO 2004). It is extremely important in the semiarid zones where, frequently, little else can be grown. It is thus an important staple in Africa (Nigeria, Sudan, Burkina Faso, Ethiopia), Asia (India, China), and the drier parts of Central and South America (Dendy 1995). It is estimated that ≈40% of the worldwide sorghum production is used for human food consumption (Rooney and Waniska 2000).

Celiac disease is a syndrome characterized by damage to the mucosa of the small intestine caused by ingestion of certain wheat proteins and related proteins in rye and barley (Fasano and Catassi 2001). The gliadins of wheat gluten contain protein sequences toxic to persons with celiac disease (Kagnoff et al 1982). Recent work has also shown that glutenins of wheat contain toxic sequences (van de Wal et al 1999; Wieser et al 2004).

Modern screening studies show that celiac disease is much more prevalent than previously thought; the average worldwide prevalence is estimated as high as 1:266 (Fasano and Catassi 2001). The cornerstone treatment for celiac disease remains the total lifelong avoidance of gluten ingestion, which means that wheat, rye, and barley have to be avoided, including durum wheat, spelt wheat, kamut, einkorn, and triticale (Kasarda and D'Ovidio 1999; Kasarda 2001).

Sorghum is often recommended as a safe food for celiac patients because it is more closely related to maize than to wheat, rye, and barley (Kasarda 2001). Sorghum might therefore provide a good basis for gluten-free bread. However, the bulk of studies dealing with leavened breads containing sorghum have focused on composite breads from wheat and sorghum, in which a maximum of only 30% low-tannin sorghum are regarded as acceptable (Munck 1995). While such breads have been found acceptable by consumers (Carson et al 2000), they are inappropriate for celiac patients.

Breads made from sorghum without added wheat, as all gluten-free breads, require a different technology. Gluten-free doughs are more fluid than wheat doughs and closer in viscosity to cake batters (Cauvain 1998) due to the lack of a gluten network. These batter-type doughs have to be handled similarly to cake batters rather than typical wheat doughs. Furthermore, gas holding is more difficult and the use of gums, stabilizers, and pregelatinized starch have been suggested as a means to provide gas occlusion and stabilizing mechanisms (Cauvain 1998; Satin 1988). Milk powder has also been described to have positive effects on gluten-free breads (Gallagher et al 2003, 2004) and has been suggested for use in gluten-free sorghum bread (Cauvain 1998).

Only a limited number of studies have addressed wheat-free sorghum breads, and most have used extra ingredients like methylcellulose (Hart et al 1970), xanthan gum (Satin 1988), carboxy methyl cellulose and skimmed milk powder (Cauvain 1998), egg (Keregero and Mtebe 1994; Cauvain 1998), or rye pentosans (Casier et al 1977). Bread based on simply 70% sorghum and 30% cassava starch has been developed by Olatunji et al (1992). Preliminary experiments in our laboratory with sorghum and ≤30% corn starch confirmed that it is possible to produce good sorghum bread without any of these extra ingredients. A simple recipe may help reduce costs, especially with regard to developing countries, and provide a healthy cereal-based bread without added ingredients (egg, milk powder), which might cause new problems for people with allergies.

One problem in evaluating nonwheat breads is the lack of standardized baking tests. In wheat baking tests such as ICC Standard No. 131 (ICC 2000), standardization of the water level to achieve constant dough consistency is a widely accepted technique. Dough consistency is then measured using standardized physical dough testing equipment such as the Brabender farinograph. For gluten-free batters, no standard methods for consistency measurements exist. Sanchez et al (2002) used a cone penetrometer to adjust water so that gluten-free batters reached a fixed consistency. In the present study, batter consistency is measured through extrusion force using a computerized texture analyzer. This method enables quick, reliable measurements as well as easy data handling.

Although previous research has been conducted on producing bread from sorghum flour, virtually no work has been done to examine the differences in sorghum bread quality among sorghum hybrids. Such information is necessary to determine whether certain hybrids yield better quality bread than others, to identify the responsible physicochemical factors, and to begin to select sorghum

[1] Department of Food and Nutritional Sciences and National Food Biotechnology Centre, University College Cork, College Road, Cork, Ireland.

[2] Present address: Department of Biological and Agricultural Engineering, Seaton Hall, Kansas State University, Manhattan, KS 66506.

[3] Corresponding author. Phone: 785-776-2708. Fax: 785-537-5534. E-mail: tilman.schober@gmprc.ksu.edu

[4] Department of Food Technology, University of Applied Sciences, Fulda, Germany.

[5] USDA-ARS, GMPRC, Manhattan, KS 66502. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

hybrids specifically for bread production. The major aim of the present study was to compare the breadmaking potential of a number of sorghum hybrids, applying a simple, wheat-free recipe. A secondary goal was to determine whether additional ingredients would eliminate any differences between good and poor quality sorghum bread. At the same time, this allowed critical investigation of the effect and necessity of xanthan gum and skim milk powder in such gluten-free breads.

MATERIALS AND METHODS

Sorghum Samples and Baking Ingredients

Nine white or red grain sorghum hybrids (Table I) were obtained from commercial seed companies and Kansas State University. For the preparation of flour, samples were first decorticated until 20% of the initial weight was removed using a tangential abrasive dehulling device (TADD) (Venables Machine Works, Saskatoon, Canada) equipped with an 80-grit abrasive pad. Afterwards, the samples were milled (Udy Co., Fort Collins, CO, USA) with a 0.25-mm screen. A commercial grain sorghum flour (Twin Valley Mills, Ruskin, NE, USA) was used as a baking standard. For batter extrusion and baking experiments, flour moisture was determined at 130°C with an infrared dryer (Mettler-Toledo, Greifensee, Switzerland) until the weight loss was <2 mg/30 sec and all calculations were done on a 14% moisture basis of the sorghum flour.

Other ingredients used were unmodified native corn starch (BCT 715, National Starch and Chemical, Bridgewater, NJ, USA), tap water, salt (microgranulated table salt, Hibernian salt, Cork, Ireland), sugar (granulated saccharose, Irish Sugar, Carlow, Ireland), instant active dried yeast (Mauripan, Burns Philp Food, Lasalle, Quebec, Canada), skim milk powder (Golden Vale, Charleville, Ireland), and xanthan gum (Quest International, Naarden, The Netherlands).

Breadmaking

Breadmaking experiments used the following basic formulation: 70 parts (relative mass) sorghum flour (14% moisture), 30 parts corn starch, 105 parts water, 1.75 parts salt, 1 part sugar, 2 parts dried yeast. The sum of sorghum flour and corn starch (100) was interpreted as flour weight basis (fwb). This basic recipe was modified for the different parts of the study. When comparing the breadmaking potential of the sorghum hybrids, the amount of water was standardized to obtain constant consistency in extrusion. When testing additional ingredients using response surface methodology, the amount of water was varied, and xanthan gum and skim milk powder were added according to the central composite design (Table II). In all cases, 520 g of batter was prepared for one replicate, resulting in one bread loaf. Dried yeast was reactivated by 10 min of prefermentation at 30°C in the water and sugar. The remaining ingredients were sieved and mixed together with the prefermented yeast for 2 min in a Kenwood Major mixer (Kenwood, Hampshire, UK) equipped with a paddle tool (K beater) at slow-medium speed (level 2 out of 6). After 30 sec of mixing, the mixer was stopped and the mixer bowl was scraped. Mixing then continued for the remaining time. Batter (500 g) was weighed into a baking tin (875 mL volume; 7 cm height; 15 × 9 cm top; 13 × 8 cm bottom) and proofed at 30°C and 85% rh (retarder Koma Popular, Koma, Roermond, The Netherlands). Proofing to height was done until the batter reached the edge of the tin (corresponding to an increase in height of ≈3 cm, typical proof times were ≈35 min). Baking was done in a deck oven (MIWE, Arnstein, Germany) at 190°C top and bottom heat for 45 min. The oven was steamed before loading (0.3 L of water) and, on loading, was steamed by injecting 0.7 L of water. After baking, the loaves were depanned and cooled for 1.5 hr on cooling racks at room temperature.

Bread Evaluation

After cooling, loaves were weighed and loaf volume was measured by rapeseed displacement. Loaf specific volume (loaf volume [mL]/loaf weight [g]) and bake loss ({500 g – loaf weight [g]}/500 g) were calculated. Color of crust and crumb were determined with a chromameter (Minolta CR-300, Osaka, Japan) applying the $L\ a\ b$ system. Crust color was measured at six positions on top of the bread. The bread was then sliced transversely using a slice regulator and a bread knife to obtain three uniform slices of 25 mm thickness. Crumb color (six values) was measured in the center on both sides of each slice. Loaf height was measured in the middle of the central slice. Digital image analysis of the crumb grain was performed as previously described (Crowley et al 2000). The crumb grain features evaluated in the present study were mean cell area (mm^2) and total number of cells ($1/cm^2$). Texture profile analysis (TPA) (Bourne 1978) of the crumb was made with the three slices using a texture analyzer (TA-XT2i, Stable Micro Systems, Godalming, UK) equipped with

TABLE II
Coded and Actual Levels of the Factors Water, Xanthan Gum, and Skim Milk Powder in the Central Composite Design[a]

	Actual Levels (% fwb)		
Coded Levels	Water	Xanthan Gum	Skim Milk Powder
–1.682	95.0	0.00	0.00
–1	100.1	0.30	1.22
0	107.5	0.75	3.00
1	114.9	1.20	4.78
1.682	120.0	1.50	6.00

[a] Design comprised 20 runs in random order; all points in coded factor levels in the order water, xanthan gum, skim milk powder: the center point (0, 0, 0) was replicated six times, factorial points were not repeated and comprised all combinations of –1 and 1 levels of the factors (–1, –1, –1), (–1, –1, 1), (–1, 1, –1), (1, –1, –1), (–1, 1, 1), (1, –1, 1), (1, 1, –1), (1, 1, 1). Star points were not repeated and comprised the following points: (–1.682, 0, 0), (1.682, 0, 0), (0, –1.682, 0), (0, 1.682, 0), (0, 0, –1.682), (0, 0, 1.682).

TABLE I
Kernel Properties and Flour Analytical Data of Sorghum Samples[a]

Sample No.	Hybrid Name	Pericarp Color	Protein (N × 6.25) (% db)	Starch (% db)	Starch Damage (% db)	α-Amylase (U/(g of dry flour)	Total Pentosans (% db)	Soluble Pentosans (% db)	SKCS Hardness	Flour Particles (% > 100 μm)
1	MNO2 INC 5	Red	12.5b	79.8bc	14.8bc	0.33a	1.07bc	0.19b	87.1bc	19.8cd
2	MNO2 INC 6	White	12.0c	80.6ab	13.2c	0.13e	0.80ef	0.13c	81.7cd	22.7b
3	MNO2 INC 7	White	11.2d	80.7ab	13.5c	0.11f	0.94c–e	0.15c	87.3b	18.6cd
4	MNO2 INC 9	Red	12.1c	79.6bc	11.1d	0.16d	1.03b–d	0.19b	81.7cd	18.5d
5	GS 2799	White	12.9a	79.2bc	14.2c	0.26b	0.87d–f	0.20b	80.7de	18.4de
6	GS 2801	White	10.0f	82.1a	16.5a	0.09g	0.85d–f	0.19b	93.3a	20.4c
7	GS 2802	White	9.5g	82.1a	16.0ab	0.11fg	0.75f	0.20b	95.1a	20.1cd
8	1486	White	11.0e	78.8cd	11.3d	0.14d	1.59a	0.28a	76.1e	14.8f
9	NK 8828	White	10.9e	79.6bc	14.3c	0.03h	1.15b	0.23b	91.0ab	16.7e
10	Commercial sorghum	White	7.8h	77.3d	···	0.22c	···	···	···	39.0a

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.05$); $n = 2$ (except SKCS hardness, $n = 3$).

a 25-kg load cell and a 35-mm aluminum cylindrical probe. Test speed, pretest speed, and posttest speed were 2 mm/sec, trigger force was 20 g, distance was 10 mm (40% compression), and wait time between first and second compression cycle was 5 sec. TPA crumb hardness (N) (a measure of firmness of the crumb) and cohesiveness (-) were extracted from the curves. Water activity (a_w) was determined with material taken from the center of the crumb using a water activity meter (AquaLab CX-2, Decagon Devices, Pullman, WA, USA). In all tests applied for bread evaluation, repeated values obtained with the same loaf were averaged into one replicate.
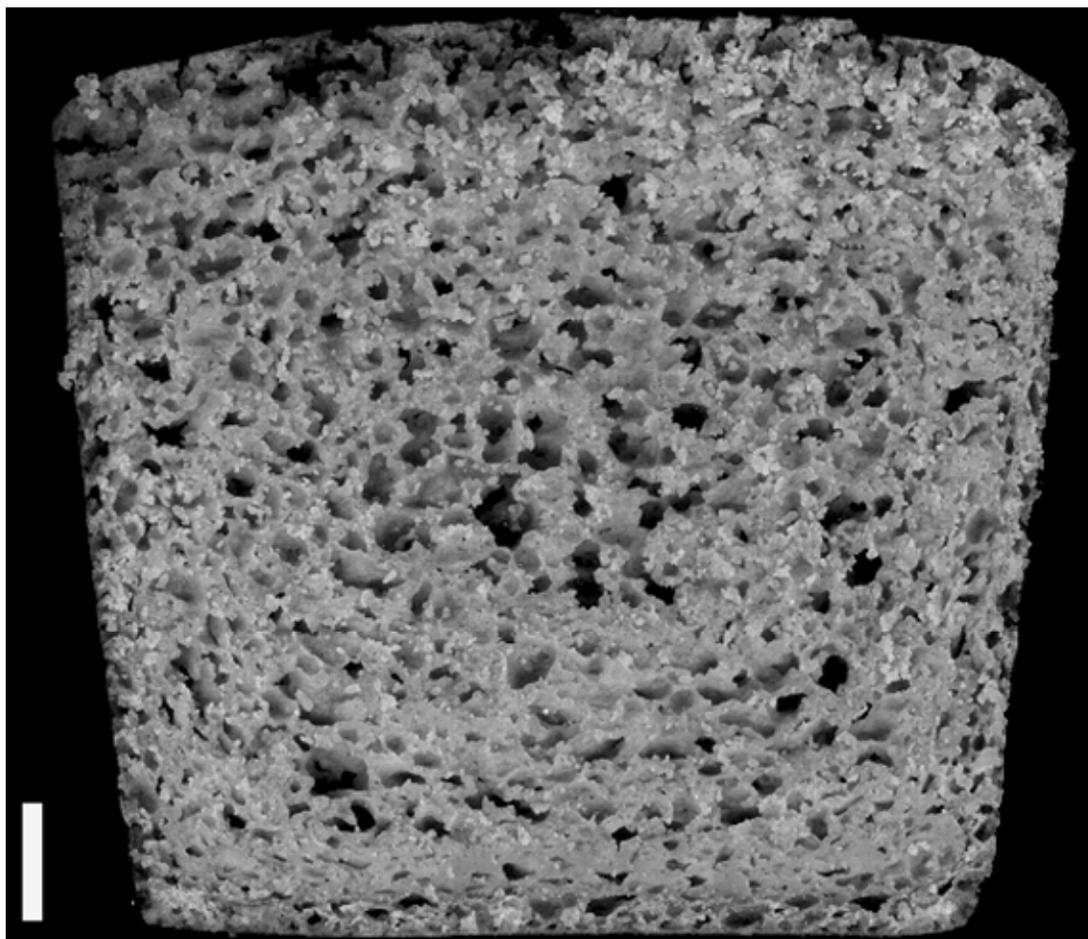
Batter Extrusion

Batter extrusion was performed with a texture analyzer equipped with a 5-kg load cell (TA-XT2i, Stable Micro Systems, Godalming, UK) using the forward extrusion cell HDP/FE with the 10-mm nozzle. For the extrusion tests, 220 g of batter was prepared as described for the baking experiments, except that no yeast was used and prefermentation was omitted. Batters were extruded using three different water levels: 105% flour weight basis (fwb) (standard), 105% fwb × 1.05 (5% more) and 105% fwb × 0.95 (5% less). Five minutes after mixing, the batter was stirred briefly to guarantee a homogenous system and loaded into the extrusion cell until 1 cm from the top. Random air pockets in the batter were removed with a spoon, and the batter surface was smoothed. To achieve reproducible starting conditions, the sample was precompressed until the piston was one-third down the cell. Then the extrusion force was measured at a test speed of 1.0 mm/sec over a distance of 20 mm. The averaged force after reach-ing a plateau (at 8–18 mm) was used as an indicator of batter firmness. Pretest and posttest speeds were 1 mm/sec and 10 mm/sec, respectively; trigger force was 50 g. The measurement was repeated three times with the same batter and averaged into one replicate. At least three batters from each sorghum sample were tested according to a blocked design.

Flour Analyses

Crude protein (N × 6.25) was determined by combustion (Approved Method 46-30, AACC International 2000) using a Leco FP-528 nitrogen determinator (St. Joseph, MI). Total starch (amyloglucosidase/α-amylase method, Approved Method 76-13), starch damage (Approved Method 76-31), and α-amylase activity (Ceralpha method, Standard No. 303, ICC 2000) were determined using commercially available test kits (Megazyme, Bray, Ireland). For total starch, method B was used, which involves pretreatment with dimethyl sulfoxide at 100°C. Calculation of starch damage and α-amylase activity was modified by calculating the results on a dry basis to account for a large range in flour moisture ($\approx$7–10%) within the samples. Moisture for the flour analyses was determined using the air-oven method (Approved Method 44-15A, AACC International 2000).

Total and soluble pentosans were determined following the colorimetric method of Hashimoto et al (1987) with modifications: 1) the difference of the absorptions at 670 and 580 nm was used for calibration, total, and soluble pentosans as suggested by Delcour et al (1989); 2) for total pentosans, after yeast fermentation and centrifugation, 3 mL of supernatant were used for the reaction with the chromogen reagents instead of 2 mL of super-

Fig. 1. Crumb structure of leavened sorghum bread baked according to the basic formulation and procedure with 105.0% fwb water, 70% commercial sorghum flour (No. 10), and 30% corn starch. Scale bar 1 cm.

natant plus 1 mL of water; 3) for soluble pentosans, the initial extraction was done with 100 mg of sample plus 5 mL of water (instead of 10 mL of water); for hydrolyzation, 2 mL of supernatant and 2 mL of $4N$ HCl were used (instead of 1 mL, respectively), and 3 mL of this hydrolyzed sample were used for the reaction with the chromogen reagents (instead of 1 mL plus 2 mL of water); 4) calibration was done with 6.7, 13.3, 20.0, 26.7, and 33.3 mg/L of xylose. The three latter modifications were required to account for the low pentosan content in decorticated sorghum samples.

Kernel hardness of the sorghum seeds was measured with a single kernel characterization system (SKCS) (4100, Perten Instruments, Springfield, IL, USA) based on the study of Pedersen et al (1996). Particle size of the sorghum flours was determined with an air jet sieve (200 LS-N, Hosokawa Alpine, Augsburg, Germany) at 3,000 Pa for 5 min with 20 g of flour, using 50- and 100-μm sieves.

STATISTICS

Design and Analysis of Variance for Comparing Hybrids

Experiments were conducted according to randomized block designs. Three blocks (3 replicates) were used in baking experiments and SKCS analyses, two blocks were used in all other flour analyses. Analysis of variance (ANOVA) included the calculation of F-ratios for block effects and sample effects (effects of the hybrids and commercial flour). Where the latter F-ratio was significant ($P < 0.05$, 0.01, or 0.001), all pairwise differences between the samples were tested using the Tukey test at a family error rate of $P < 0.05$. The general linear model procedure (v. 12.21, Minitab Inc., State College, PA, USA) was used for these calculations.

Response Surface Methodology (RSM)

Experiments were conducted according to a central composite design (Table II). The actual levels of the three factors water, xanthan gum, and skim milk powder were selected by conducting preliminary tests in a way that a large range of different results was achieved and that all combinations of the factors resulted in manageable batters and breads. Responses were those described under bread evaluation. Model selection (linear or quadratic) for each response was made on the basis of the sequential model sum of squares: the highest degree model was selected where F-tests showed significant ($P < 0.05$, 0.01, or 0.001) effects. The suitability of the selected model was then verified based on lack-of-fit tests and the multiple correlation coefficient (R^2). The coefficients for all terms in the model (all linear terms in linear model, all linear and squared terms and two-way interactions in quadratic model) were tested for significance (H_0: coefficient = 0 vs. H_A: coefficient $\neq 0$). Numerical optimization was based on the desirability function:

$$D = (d_1^{r_1} \times d_2^{r_2} \times ... \times d_n^{r_n})^{\frac{1}{\sum r_i}}$$

where d_i values are the desirability indices for each response ($d_i = 0$ least desirable; $d_i = 1$ most desirable) and r_i is the relative importance of each response. The greatest overall desirability was searched, allowing coded factor levels between –1 and 1. The responses loaf specific volume and TPA crumb hardness were selected for optimization. The goal for volume was a maximum with the relative importance of five, while the goal for crumb hardness was a minimum with the relative importance three. The selection of the two responses and their relative importance was based on the fact that volume is the primary quality parameter of bread, whereas softness of the crumb is especially desirable in gluten-free bread, which tends to be quite firm. The RSM was performed with Design-Expert 5 (Stat-Ease Corporation, Minneapolis, MN, USA).

Physicochemical Characterization of Sorghum Flours

Table I shows some kernel properties and analytical data of the sorghum samples. Significant ($P < 0.05$) differences between the samples were found for all variables listed. Excluding the commercial sorghum flour, protein (N × 6.25) was 9.5–12.9% db; starch was 78.8–82.1% db; starch damage was 11.1–16.5% db; α-amylase-activity was 0.03–0.33 U/(g of dry flour); total pentosan was 0.75–1.59% db; soluble pentosan was 0.13–0.28% db; SKCS hardness was 76.1–95.1; and flour particles at >100 μm were 14.8–22.7%. Flour particles at >50 μm were not significantly different between the nine hybrids. The average particle size distribution of the flours from the nine hybrids was 48% <50 μm, 33% 50–100 μm, and 19% >100 μm.

Development of Basic Recipe

Before breadmaking potential could be evaluated, a basic bread recipe and baking procedure had to be developed. The commercial sorghum flour (No. 10) was used for these experiments. The basic ingredients were water (variable amount, depending on consistency), salt (1.75% fwb), sugar (1.0% fwb), and dried yeast (2.0% fwb). Addition of xanthan gum, soya flour, and corn starch were tested. Best results were found with soft batters and with the addition of corn starch only, with increasing levels resulting in better bread (data not shown). The highest level of corn starch tested was 30%. At this level, satisfactory bread was produced and sorghum flour was still the major component. Figure 1 shows the crumb structure of bread prepared with 105% fwb water, 70% commercial sorghum flour, and 30% corn starch according to the basic formulation and breadmaking procedure.

Influence of Hybrid and Water Level on Batter Consistency

Batters were prepared according to the basic recipe with no added yeast and with the standard water level (105% fwb). The batter consistencies measured by extrusion showed considerable differences between the samples (Fig. 2, standard). The extrusion forces were 3.5–10.1N ($n \geq 3$). To standardize the consistencies of the batters from each hybrid, the value of the commercial flour (4.9N) was taken as a reference. Bread prepared from such batter was of good quality (see previous section). Depending on whether the batters from the individual hybrids were too soft or too firm,
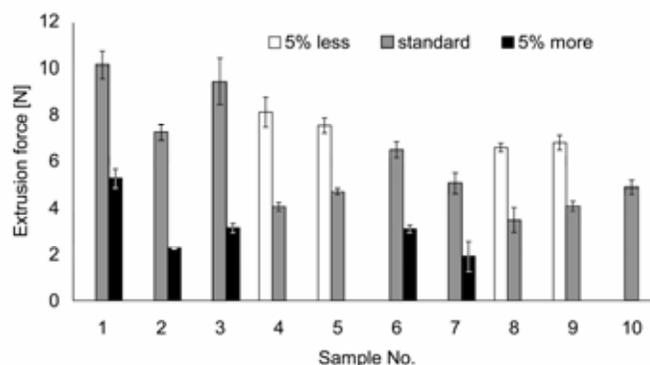
Fig. 2. Batter consistencies measured by extrusion through a 10-mm nozzle at a test speed of 1.0 mm/sec with the HDP/FE extrusion cell of the texture analyzer TA-XT2i (average ± standard error of the mean, $n \geq 3$). Batters were prepared from the samples listed in Table I according to the basic recipe, but without yeast. In addition to the standard water level (105% fwb), 5% less or 5% more water were used to cover a range including the consistency of the commercial sorghum flour (No. 10, reference). Water levels required to obtain the consistency of the reference were calculated by linear interpolation and for hybrids No. 1–9 were: 110.7, 107.5, 108.8, 103.9, 104.7, 107.5, 105.3, 102.6, 103.5, and for the reference 105.0 (% fwb).

5% less or 5% more water was added, respectively. The consistencies obtained with the standard water level and 5% more or less water included the consistency of the reference in all cases except No. 1, where the value was marginally too high. In a preliminary experiment with the commercial flour (No. 10), five different water levels were added (standard, 5% less and more,

10% less and more), and consistencies were measured by extrusion. The correlation coefficient between water and extrusion force was –0.991. Linear interpolation or extrapolation in this range was therefore regarded as sufficiently accurate. The resulting calculated water levels required to obtain the consistency of the reference are given in Fig. 2. The baking experiments for the comparison of all 10 samples were conducted with these water levels.

Baking Experiments with Hybrids

The variables and their ranges measured for the breads produced from the sorghum hybrids and commercial flour are listed in Table III. No significant differences were found for loaf specific volume, loaf height, bake loss, and water activity. All crust and crumb color values, however, showed significant differences. Crust colors were medium light (*L*), between slightly greenish and clearly reddish (*a*), and clearly yellowish (*b*). Crumb colors were medium light (*L*), between greenish and slightly reddish (*a*), and clearly yellowish (*b*). In crust colors, it was the two red hybrids (No. 1 and 4) that had the highest (positive) *a* values, whereas in crumb colors, only the *a* value of No. 1 was not negative.

The most characteristic differences in the breads were found in the crumb properties as measured by digital image analysis and TPA. Mean cell area and total number of cells/cm^{2}, and TPA crumb hardness varied significantly (Table III, Fig. 3). Hybrids No. 3 and 7 represented the extremes of these differences with No. 3 having a high number of small pores and a firm crumb and No. 7 having a low number of large pores and a soft crumb texture. Crumb cohesiveness showed minor differences; only the commercial flour (No. 10) had a clearly lower value (0.46) than the others (0.62–0.71).

Proof time was affected by sample and block effects according to the *F*-test, although both effects were significant only at *P* < 0.05. Tukey test, however, showed no significant pairwise differ-

TABLE III
Variables Characterizing Sorghum Bread and Respective Ranges for Samples in Table I

Variable	Range Values[a]		F-Test
	Smallest	**Largest**	
Loaf specific volume (mL/g)	1.77	1.84	ns[b]
Loaf height (cm)	7.0	7.5	ns
Bake loss (%)	13.4	14.7	ns
Crust color[c]			
L	53.8	63.1	P < 0.01
a	–0.5	6.5	P < 0.001
b	25.1	32.5	P < 0.001
Crumb			
L	54.8	62.9	P < 0.001
a	–5.7	0.3	P < 0.001
b	15.2	19.3	P < 0.001 (be[d])
Mean cell area (mm[2])	1.3	3.3	P < 0.001
Total number of cells (1/cm^{2})	13.5	27.8	P < 0.001
TPA crumb hardness (N)	7.5	21.6	P < 0.001
Crumb cohesiveness (–)	0.46	0.71	P < 0.001
Water activity (–)	0.986	0.988	ns (be[d])
Proof time[e] (min)	24.5	42.0	P < 0.05 (be[d])

[a] Smallest and largest value of the 10 samples listed in Table I.
[b] ns = not significant (*P* > 0.05).
[c] *L* = lightness, *a* = green/red, *b* = blue/yellow.
[d] be = block effects (*P* < 0.05).
[e] Minimum and maximum out of all samples and individual replicates (30 values); average of all 30 values: 35.2 min.

TABLE IV
Models for Selected Responses (fit summaries and *t*-tests for model coefficients)[a]

		Loaf Specific Volume (mL/g)		Loaf Height (cm)		Bake Loss (%)		Mean Cell Area (mm[2])		TPA Crumb Hardness (N)		Crumb Cohesiveness (–)	
		No. 3	**No. 7**	**No. 3**	**No. 7**	**No. 3**	**No. 7**	**No. 3**	**No. 7**	**No. 3**	**No. 7**	**No. 3**	**No. 7**
Sequential sum of squares	Lin[b]	***	***	**	**	***	**	***	***	**	***	*	***
	Qua[c]	**	ns	**	*	**	ns	***	ns	**	**	ns	ns
Lack-of-fit[d]	Lin	*	ns	**	*	*	ns	***	ns	**	***	ns	ns
	Qua	ns	ns	*	ns	ns	ns	*	ns	*	**	ns	ns
R[2]	Lin	0.74	0.80	0.60	0.53	0.73	0.60	0.73	0.87	0.61	0.73	0.43	0.70
	Qua	0.95	0.90	0.91	0.87	0.94	0.74	0.96	0.92	0.93	0.94	0.69	0.77
Selected model		Qua	Lin	Qua	Qua	Qua	Lin	Qua	Lin	Qua	Qua	Lin	Lin
t-Tests[e,f]													
W		***	***	ns	**	**	*	***	***	***	***	ns	ns
S		**	ns	***	**	*	ns	***	ns	ns	ns	**	***
X		***	***	**	*	***	**	***	***	***	***	ns	ns
W[2]		ns	–	ns	ns	ns	–	ns	–	ns	**	–	–
S[2]		***	–	***	ns	**	–	***	–	***	*	–	–
X[2]		ns	–	ns	**	*	–	**	–	ns	*	–	–
W×S		ns	–	ns	ns	ns	–	ns	–	ns	ns	–	–
W×X		ns	–	ns	*	ns	–	ns	–	ns	**	–	–
S×X		ns	–	ns	ns	*	–	ns	–	ns	ns	–	–
Remarks		–	Slight lack of fit		–		Low *R*[2]	Slight lack of fit		Slight lack of fit	Lack of fit	Low *R*[2]	

[a] *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ns, not significant; Lin, linear; Qua, quadratic.
[b] *F*-test for significance of adding the linear terms to the intercept.
[c] *F*-test for significance of adding the quadratic terms to the linear model.
[d] Comparison of residual error to pure error from the replicated centerpoints.
[e] *t*-Test, whether the respective coefficient is different from 0 (H$_0$: coefficient = 0 vs. H$_A$: coefficient ≠ 0).
[f] W, water; S, skim milk powder; X, xanthan gum.

ences between any samples. Block effects indicate that there were global differences between the three blocks within which the three replicates were conducted (e.g., due to drifts or differences in room temperature or yeast activity affecting the proof time). The importance of proofing to height rather than for a constant time was thus confirmed.

Correlations Between Analytical Data and Baking Data

Significant (*, **, ***, $P \leq 0.05$, 0.01, or 0.001) correlations were found between protein content and starch content, α-amylase activity, crumb hardness, and SKCS hardness (–0.703*, 0.705*, 0.670*, and –0.666*, respectively); starch content and total pentosan, starch damage, and SKCS hardness (–0.707*, 0.723*, and 0.785*); total pentosan and soluble pentosan, and flour particles >100 μm (0.796**, –0.842**); soluble pentosan and water level, and flour particles >100 μm (–0.674*, –0.842**); starch damage and mean cell area, total number of cells, crumb hardness, and SKCS hardness (0.825**, –0.843**, –0.669*, and 0.844**); mean cell area and total number of cells, and crumb hardness (–0.959***, –0.727*); total number of cells and crumb hardness (0.712*); crumb hardness and crumb cohesiveness, and SKCS hardness (–0.940***, –0.682*); and crumb cohesiveness and SKCS hardness (0.673*). The correlations between starch damage and water level (0.433), and between total pentosan and water level (–0.442) were not significant. All correlation coefficients are based on the data for the nine hybrids without the commercial flour.

## Response Surface Methodology (RSM)

Two sorghum hybrids opposite in quality (No. 3 and 7) were selected to study the effects of adding xanthan gum and skim milk powder together with water level on bread quality using a central composite design (Table II). For several important responses, Table IV shows the fit summaries that were used to select the best models, and the significance of model coefficients. Figures 4–6 illustrate the results for loaf specific volume, loaf height, and mean cell area, respectively, by using three-dimensional (3D) response surface plots. For both hybrids and for each of these responses, three individual plots are presented, each showing the effects of a combination of two factors, with the third kept constant at the medium level (corresponding to a coded factor level of 0).

Within the selected range of the factor levels, loaf specific volume was clearly increased by increasing water levels and clearly decreased by increasing levels of xanthan gum (Fig. 4). Both factors had significant ($P < 0.001$) linear effects for both hybrids. The effects of skim milk powder on loaf specific volume were less obvious. In No. 3, a minimum was reached with medium to high levels of skim milk powder, in agreement with significant ($P < 0.001$) quadratic effects. In No. 7, skim milk powder had no significant effects.

Loaf height generally decreased with increasing levels of skim milk powder (Fig. 5), in agreement with significant linear, and in No. 3, also quadratic effects (Table IV). The remaining factors exerted different effects on the two hybrids: water had little influence on loaf height of No. 3 (Table IV, Fig. 5), in clear contrast to its effect on volume. Loaf height of No. 7, however, significantly increased with increasing amounts of water. Xanthan gum showed a slight trend only to decrease height of breads prepared from No. 3 (Fig. 5), while a clear optimum was found for No. 7. The position of this optimum depended on the levels of the other factors. Lower xanthan levels produced the best loaf height at low water levels, whereas medium xanthan levels resulted in maximum loaf height at high water levels. These findings are in agreement with significant ($P < 0.01$) quadratic effects of xanthan gum and significant ($P < 0.05$) water by xanthan gum interactions for loaf height of No. 7 (Table IV). Differences between volume and height may result from the fact that the top of the breads varied between

round, flat, or even collapsed. This outer shape of the breads was most clearly affected by skim milk powder, where high levels tended to cause a collapsed top and low levels tended to cause a round top.

Bake loss was significantly affected by both water and xanthan gum (Table IV). As expected, water increased bake loss and xanthan gum decreased it (data not shown). Additionally, in No. 3, skim milk powder had a significant effect (linear and quadratic), and skim milk powder also interacted significantly ($P < 0.05$) with xanthan gum. Skim milk powder tended to increase bake loss of No. 3. The interactions with xanthan gum were reflected in the fact that increasing levels of skim milk powder increased bake loss more pronounced at lower xanthan levels and hardly at all at highest xanthan levels (data not shown). A low R^2 in No. 7 indicates that modeling in this case was not completely satisfactory, which might explain that only the major effects of water and xanthan gum were found for the bake loss of this hybrid.
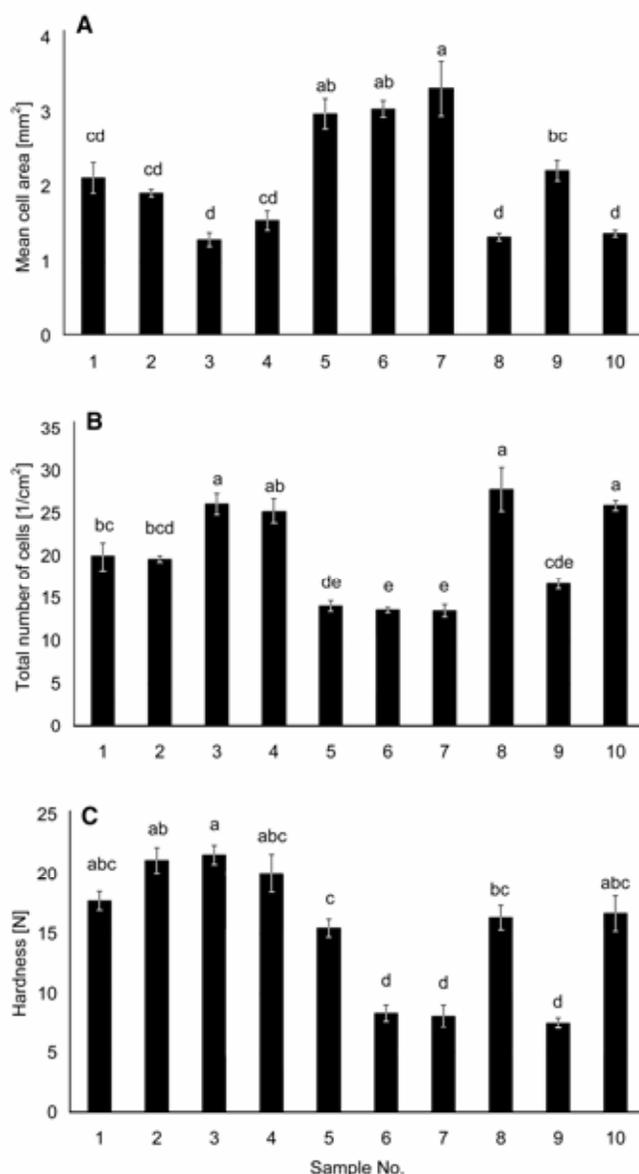

Fig. 3. Crumb properties of breads produced from the 10 sorghum samples (Table I) according to the basic recipe, with water levels modified to obtain constant consistency (Fig. 2). **A,** Average pore size (mean cell area). **B,** Total number of cells/cm². **C,** TPA crumb hardness (average ± standard error of mean, $n = 3$). Values sharing the same letter in the same graph are not significantly different ($P < 0.05$).

Mean cell area, characterizing the average pore size of breads, was significantly ($P < 0.001$) influenced by both water and xanthan gum in both hybrids, and by skim milk powder in No. 3 (Table IV). Additional significant quadratic effects were found for the latter hybrid. Overall, the graphs for mean cell area (Fig. 6) and loaf specific volume (Fig. 4) were very similar. This suggests that larger pores in the RSM part of the study went along with a higher volume, in contrast to what was found when comparing the 10 samples. In agreement with the comparison of the 10 samples is, however, that No. 3 had a smaller mean cell area (a finer crumb structure) than No. 7. This was the case for all levels of water, xanthan gum, and skim milk powder (Fig. 6)

TPA crumb hardness showed almost the exact opposite trends to loaf specific volume for all factors (data not shown). This means that a smaller volume (a denser crumb) generally went along with higher crumb hardness. Despite a remaining lack of fit, very high R^2 values confirmed a good modeling for this response in both hybrids and various coefficients were significant (Table IV). This is in distinct contrast to crumb cohesiveness, where R^2 was low, especially in No. 3 (Table IV). The results are nevertheless inter-
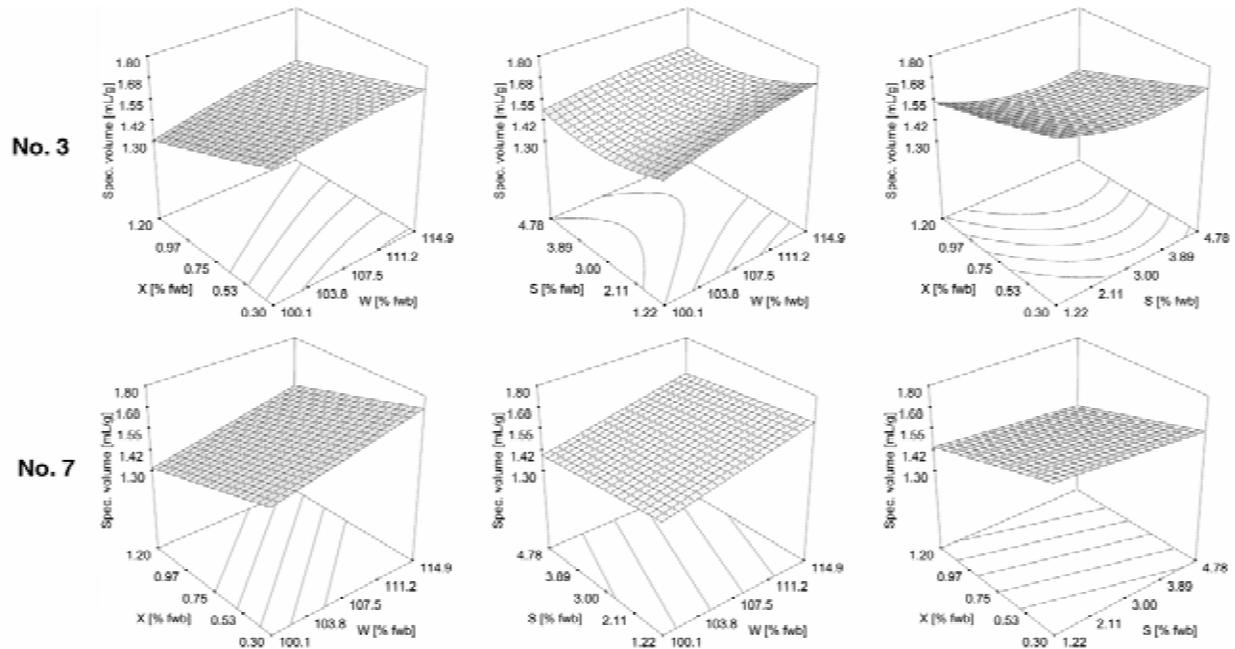
Fig. 4. Three-dimensional response surface plots illustrating the effects of water (W), xanthan gum (X), and skim milk powder (S) on loaf specific volume, for hybrids No. 3 (top) and No. 7 (bottom). For each hybrid, three individual plots are presented, each showing the effects of a combination of two factors, whereas the third is kept constant on the medium level (0 in coded terms).
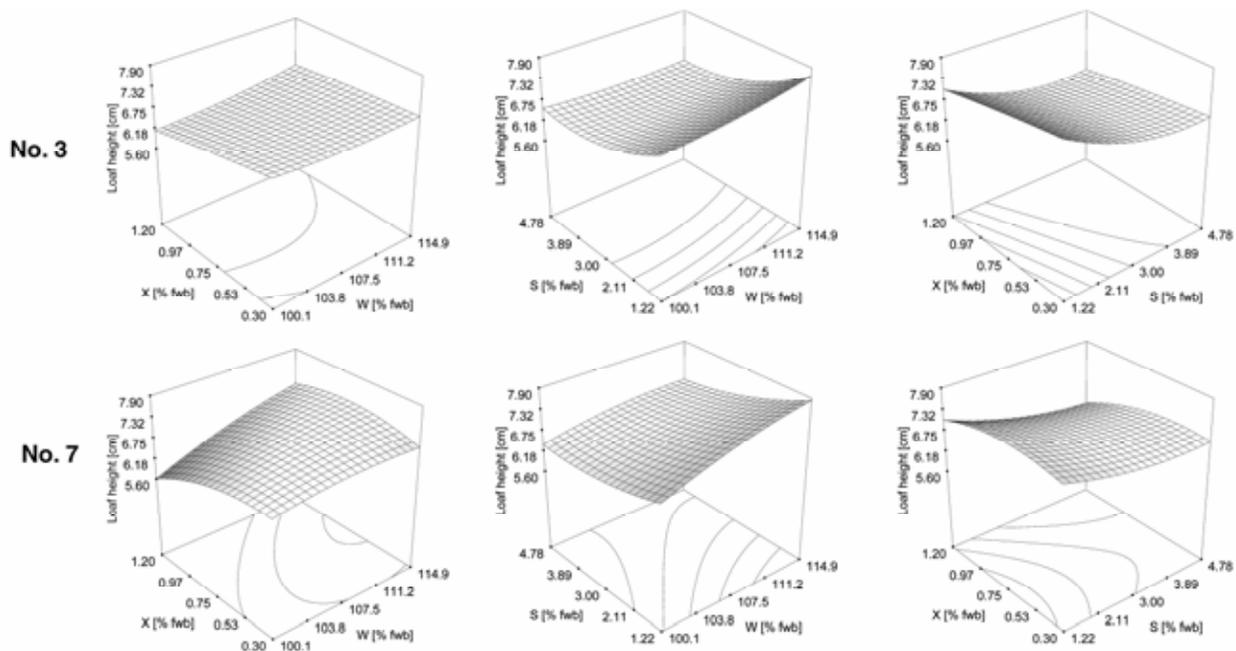
Fig. 5. Three-dimensional response surface plots illustrating the effects of water (W), xanthan gum (X), and skim milk powder (S) on loaf height for hybrids No. 3 (top) and No. 7 (bottom). For each hybrid, three individual plots are presented, each showing the effects of a combination of two factors, whereas the third is kept constant on the medium level (0 in coded terms).

esting. Significant effects of skim milk powder only were found for both hybrids. Increasing levels of skim milk powder reduced cohesiveness (data not shown).

In addition to the above effects, xanthan gum and skim milk powder influenced the appearance of the bread crust. As expected, browning of the crust was improved when skim milk powder was added, with the crust becoming darker with increasing levels (data not shown). Addition of xanthan gum made the surface smoother. Without xanthan gum, the crust was covered with small cracks. Figure 7 shows the top of two breads from No. 3 prepared without and with xanthan gum.

With regard to bread optimized by the RSM procedure, loaf specific volume and crumb hardness were improved by increasing water levels; however, xanthan gum had negative effects on these attributes. Not surprisingly, for both hybrids numerical optimization resulted in maximum water levels (114.9% fwb) and minimal levels of xanthan gum in addition to minimum levels of skim milk powder (0.30 and 1.22% fwb, respectively). To verify that the models predicted the responses correctly, breads were prepared from both hybrids according to the standard procedure, applying these optimized levels of the three factors. Table V compares the results of these experiments with the values of the responses predicted from the models for loaf specific volume, loaf height, bake loss, mean cell area, TPA crumb hardness, and crumb cohesiveness. Although the predicted specific volume could not completely be reached, especially in No. 3, the predicted values generally agreed well with the experimental values. The optimum reflects the finer crumb (smaller mean cell area) of No. 3.

DISCUSSION

The largest difference in bread quality among the sorghum hybrids was the visual appearance of the crumb. Using digital image analysis, fine crumb structure was characterized by a high number of relatively small pores per area unit, while coarse crumb structure was characterized by the opposite properties. Fine crumb structure tended to go along with higher crumb firmness detected with TPA. However, for white wheat pan bread, soft bread with a fine cell structure would be considered best (Hoseney

1998). In the present study, it was assumed that a relatively coarse, open crumb structure might provide less resistance to the probe during TPA due to large voids, but that it would not be perceived as a more desirable bread. Therefore, good quality was defined as finer crumb structure. Differences in pore size between the two most opposite hybrids (No. 3 and 7) were maintained throughout the study. Differences between these hybrids were found when initially comparing breads from all 10 samples produced with identical batter consistencies with no added xanthan gum and skim milk powder, and also in the RSM part of the study when these additional ingredients were included and water was widely varied. These findings suggest that physicochemical differences between the samples are responsible for quality differences.

Considering the correlations between analytical data and baking data, it appears that starch damage is a key element in the

TABLE V
Predicted and Experimental Values for Selected Responses of Optimized Breads from Two Sorghum Hybrids[a,b]

	No. 3		No. 7	
	Predicted[c]	Experimental	Predicted[c]	Experimental
Loaf specific volume (mL/g)	1.80 ± 0.04	1.71	1.76 ± 0.04	1.71
Loaf height (cm)	7.8 ± 0.2	7.8	7.4 ± 0.3	7.5
Bake loss (%)	12.7 ± 0.3	12.5	13.0 ± 0.3	12.2
Mean cell area (mm²)	2.7 ± 0.1	2.8	3.9 ± 0.2	3.6
TPA crumb hardness (N)	8.8 ± 2.2	8.1	5.5 ± 1.5	4.7
Crumb cohesiveness (–)	0.66 ± 0.01	0.67	0.72 ± 0.01	0.73

[a] Optima in both hybrids: water 114.9% fwb, xanthan gum 0.30% fwb, skim milk powder 1.22% fwb; (+1, –1, –1) in coded factor levels.
[b] Predicted values calculated with models in Table IV.
[c] ± Standard error of the mean.
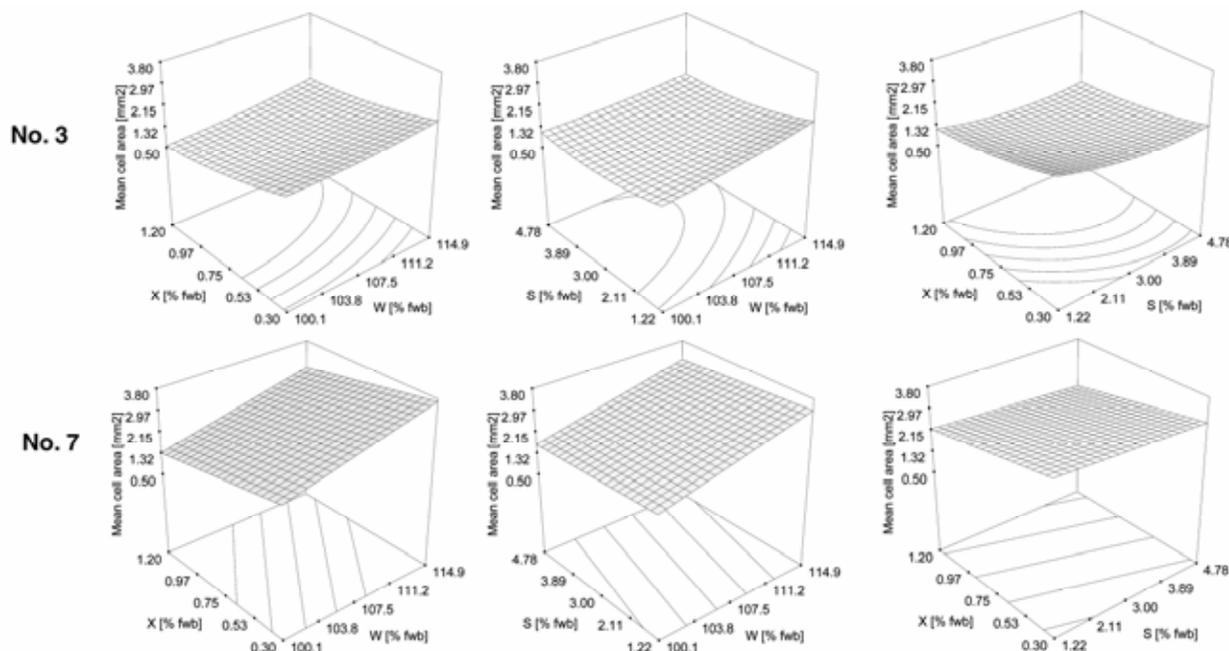
Fig. 6. Three-dimensional response surface plots illustrating the effects of water (W), xanthan gum (X), and skim milk powder (S) on average pore size (mean cell area) for hybrids No. 3 (top) and No. 7 (bottom). For each hybrid, three individual plots are presented, each showing the effects of a combination of two factors, whereas the third is kept constant on the medium level (0 in coded terms).

bread quality: higher starch damage goes along with a larger mean cell area, a smaller number of cells, and a softer crumb. On the other hand, a higher starch damage is obtained if the SKCS hardness (kernel hardness) is higher. Damaged starch is more easily degraded by amylases, resulting in a larger amount of sugars for yeast fermentation and thus more gas produced by yeast. At the same time, a higher starch degradation most likely weakens the starch gel that plays a central role in the bread architecture in the absence of a gluten network. Starch damage and kernel hardness differed significantly between No. 3 and 7.

Starch damage in the flours from the sorghum hybrids was overall very high. Aboubacar and Hamaker (1999) reported much lower values for flours from decorticated grain of eight sorghum cultivars (3.2–7.2%), comparable to the range found by Ranhotra et al (1993) in commercial flours from hard and soft wheats (2.9–10.1%).

Differences between the methods for the determination of starch damage between the studies were considered and the regression equations suggested by Gibson et al (1993) were applied. The ranges found by Aboubacar and Hamaker (1999) and Ranhotra et al (1993) would correspond to 1.8–4.5% and 2.1–7.3%, respectively, when corrected to the same method as applied in the present study. Damaged starch granules hydrate rapidly and contribute significantly to water absorption in dough (Tipples 1969; Evers and Stevens 1985; Gibson et al 1993). This explains why the sorghum flours could bind much water without any added gums in clear contradiction to the widespread opinion that bread without wheat requires additives (gums, stabilizers, pregelatinized starch) to substitute for the gluten (e.g., Nishita et al 1976; Ács et al 1996; Cauvain 1998; Kadan et al 2001; Sanchez et al 2002). It is noteworthy though that starch damage and water levels required for identical consistency were not significantly correlated. This might indicate that other factors besides starch damage contribute to water binding. Small absorption by intact starch granules might be of importance due to the abundance of starch. Pentosans seem to play no decisive role in the binding of water in the batter as indicated by the negative correlations to water level required for identical consistency (the more pentosans, the softer the batter consistency and the less water required). Water binding in the batter could possibly be enhanced by a small flour particle size, which may help easy swelling of all components. The flours from the hybrids were very fine; the fraction of coarse particles (>100 μm) in all cases was much smaller than the percentage of particles at >125 μm in the study of Aboubacar and Hamaker (1999). This factor, however, was not decisive for water binding or baking quality; the commercial sorghum flour had distinctly coarser particles but its water binding was not exceptional and it also yielded good bread. Only reduced crumb cohesiveness pointed in the direction of a slightly weaker structure.

Besides starch damage and particle size, the analytical data of the sorghum samples agree well with the literature. Ranges for protein and starch content are comparable to those found by Abou-
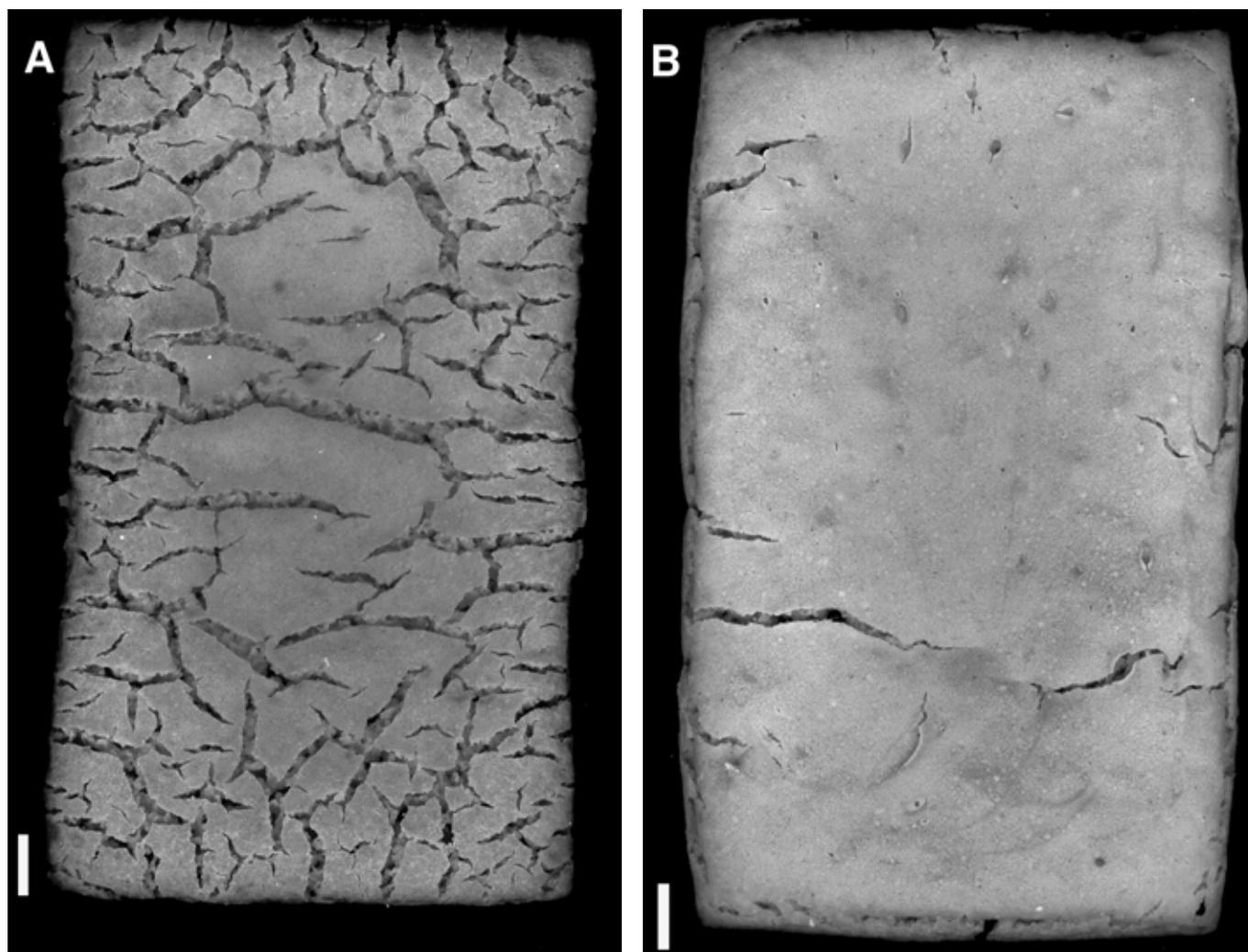
Fig. 7. Influence of xanthan gum on sorghum bread crust. **A,** Bread without xanthan gum. **B,** Bread with 0.75% fwb xanthan gum. Breads were from two of 20 runs for the central composite design (Table II) with hybrid No. 3. Levels of water (107.5% fwb) and skim milk powder (3.00% fwb) were identical for the two runs. Scale bar 1 cm.

bacar and Hamaker (1999). Total pentosan contents correspond to the value reported by Karim and Rooney (1972) for sorghum endosperm rather than for whole kernels, in agreement with the fact that decorticated sorghum was used in the present study where most pericarp was removed.

In addition to differences in crumb properties detected by TPA and digital image analysis, color differences of crust and crumb were found. These reflected the different kernel colors of the samples. Bread from the red hybrids No. 1 and less so No. 4 had an attractive pinkish-brown color which might be a selling point when promoted well. It is noteworthy that together with other hybrids, even the red hybrid No. 4 had a slightly negative crumb *a* value, indicating a green color component. Negative crumb *a* values were also reported by Abdel-Aal et al (1999) for bread from wheat and spelt wheat. This fact requires further research. It might be that shadows or reflections of light in the voids of the bread crumb reduce the accuracy of crumb color measurements.

Besides starch damage, another key variable to gluten-free bread quality appears to be a low viscosity of the batter system. This is clearly indicated by the fact that increasing water levels up to ≈115% fwb and decreasing levels of xanthan gum, both of which lead to reduced batter viscosity, resulted in a higher volume in the RSM part of the study. In accordance with that, once the consistency was standardized during the comparison of the 10 different samples, bread volume and height did not differ significantly between the samples. These findings are in principal agreement with Cauvain (1998), who described wheatless bread doughs as more fluid than wheat doughs and closer in viscosity to cake batters.

Some authors described the use of milk powder in wheat-free sorghum or other gluten-free breads (Cauvain 1998; Gallagher et al 2003, 2004). Except for improved crust browning, however, the present study showed only negative effects of skim milk powder on sorghum bread quality. These negative effects were reduced bread height, a collapsed top, increased bake loss, and reduced crumb cohesiveness, which all indicated a weakened bread structure. Structure building effects of milk proteins can thus be ruled out for this type of bread. More likely, milk proteins and lactose seem to interfere with the starch gel, e.g., by competition for water or by disrupting uniformity of the starch gel. With regard to bake loss, high levels of xanthan gum helped to mask the negative effects of skim milk powder and to retain water in the bread as shown by the interaction between skim milk powder and xanthan gum for No. 3.

Due to the nature of the central composite design, optimization was only allowed between the coded –1 and +1 levels of the factors. This excluded bread made with 0% xanthan and 0% skim milk powder. As such, calculated optima still suggested incorporation of small levels of skim milk powder and xanthan gum. The experimental reproduction of the calculated optima was important to confirm the adequacy of the models. However, for practical baking, nothing was gained by the use of xanthan gum or skim milk powder other than improved crust appearance. For most applications, this is of limited importance and we would avoid both.

The present study was not designed to develop the best possible sorghum bread but to compare hybrids under standardized conditions and to provide a system for understanding the basis for quality of wheat-free leavened bread. The present optimized bread might possibly be further improved by additional ingredients or technological steps. The overall quality was nevertheless rated as desirable with regard to softness and flavor in informal taste panel evaluations. The specific volume of the present sorghum bread (1.8 mL/g) was in the same range described by other researchers for gluten-free whole rice bread (1.8–1.9 mL/g, Kadan et al 2001), gluten-free corn starch, cassava starch, and rice flour breads (1.9–2.5 mL/g, López et al 2004), and sorghum bread (2.5 mL/g, Casier et al 1977), although these studies used extra additives (hydroxypropyl methylcellulose, egg plus xanthan gum, or rye

pentosans, respectively). Olatunji et al (1992) reached 2.2 mL/g with sorghum bread (70% sorghum, 30% cassava starch) without such additives. Substantially higher specific volumes were reported for gluten-free breads from wheat starch and soy protein isolate plus xanthan gum (4.6 mL/g, Ranhotra et al 1975), cornstarch, rice flour, and cassava starch plus a nonspecified gum (4.3 mL/g, Sanchez et al 2002), and white rice flour plus hydroxypropyl methylcellulose (≈5 mL/g, Nishita et al 1976; Hall 1996; Kadan et al 2001). The latter rice breads were described as crumbly and dry with a bland flavor (Kadan et al 2001). We found similar properties for gluten-free breads based predominantly on pure starches (*unpublished data*). Sorghum bread might therefore add a desirable, new flavor within gluten-free bread types for celiac patients and should be regarded as a speciality bread.

CONCLUSIONS

Clear differences were found between various sorghum hybrids in their potential to produce gluten-free leavened loaf bread. Crumb structure (pore size, number of pores, and TPA crumb hardness) differed most characteristically, whereas volume and height did not show significant differences among the samples evaluated. Kernel hardness and damaged starch seem to be key elements in these differences. With regard to the technology of sorghum bread, larger bread volumes may be reached when corn starch is added to the sorghum flour, and a soft (low viscosity) batter is produced, using high water levels. The use of xanthan gum and skim milk powder had negative effects on crumb structure. The complete control of starch damage to achieve desired bread quality seems possible, and research should focus on the effect of milling techniques on starch damage and gluten-free bread quality.

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