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Abstract. *Influence of sulfur dioxide (SO_2) and lactic acid (LA) on grain sorghum steeping efficiency in terms of wet-milling characteristics was investigated using response surface analysis. Tested ranges of concentrations in steeping water were 0.050 to 0.278% and 0 to 0.282% for SO_2 and LA, respectively. Measured wet-milling variables were steeping solids yield, starch yield, protein yield, fiber/germ yield, starch recovery, protein recovery and protein content of starch. Starch and protein recovery values were highly correlated to each other ($r = 0.74$). Correlations of these attributes with fiber/germ yield were strongly negative ($r = -0.96$ and $r = -0.70$ for starch and protein recovery, respectively). A model comprised of the linear and quadratic terms of SO_2 and LA concentrations and the interaction between SO_2 and LA was fitted to data for each dependent variable. The model satisfactorily explained starch yield ($R^2 = 0.84$), starch recovery ($R^2 = 0.85$) and fiber/germ yield ($R^2 = 0.71$). Lactic acid was the factor that consistently influenced all significant surfaces ($P < 0.01$ for starch yield and starch recovery, and $P < 0.05$ for fiber/germ yield). Maximum stationary points for starch yield and recovery, and a minimum stationary point for fiber/germ yield were observed at similar combinations of SO_2 (0.191-0.209%) and LA (1.387-1.415%) concentrations.* **Keywords.** *Fiber, Germ, Protein, Starch.*

Grain sorghum (*Sorghum bicolor* L. Moench) is important for the economy of semiarid regions since it thrives and produces both grain and forage under conditions that cause other crops to fail (Zipf et al., 1950). It is a major crop in the Great Plains area of the U.S., particularly where rainfall becomes a limiting factor for corn production. Sorghum's drought resistance is well known (Watson, 1970).

The potential of sorghum for industrial starch production is similar to that of corn (Subramanian et al., 1994) since a sorghum wet-milling process is almost identical to the one used for corn starch manufacture (Watson, 1967). Successful wet-milling requires steeping of grain under controlled conditions of time, temperature, pH, and lactic acid (LA) and sulfur dioxide (SO_2) concentrations. These conditions soften and degrade the kernel structure to enhance milling recovery of relatively pure fractions of starch, protein, and germ (Shandera et al., 1995). According to Boundy et al. (1967), SO_2 weakens the protein matrix by breaking disulfide bonds and forming soluble S-sulfoproteins which prevent reformation of disulfide bonds. Lactic acid, the main fermentation product from soluble sugars in the steepwater solution, lowers pH, restricts growth of organisms other than *Lactobacillus*, causes some softening of kernel components, and produces favorable conditions for separation of kernel components, especially protein, after milling (Eckhoff and Tso, 1991).

Early work by Zipf et al. (1950) on sorghum wet-milling analyzed optimum steeping and milling conditions without considering LA addition, or even recognizing that little LA, if any, may be formed during laboratory steeping. They reported highest values of starch recovery with 0.25% SO_2 at 44 to 50°C during 24 h of steeping. Although the importance of LA in steeping has never been fully established (Eckhoff and Tso, 1991), and in spite of the confusion that seems to exist as to the merits of LA as a laboratory steeping agent (Shandera et al., 1995), most researchers after Zipf et al. (1950) have utilized LA for steeping sorghum grain. Watson and Hirata (1955) steeped sorghum for 48 h at 49°C using a solution of 0.5% LA and 0.15% SO_2 adjusted to pH 3.0 with potassium hydroxide. Norris and Rooney (1970) and Norris (1971) applied a two-phase steeping procedure originally described by Watson et al. (1951). Phase I consisted of steeping for 40 h at 52°C in a solution containing 0.05% SO_2 and 1.5% LA. Phase I solution was then replaced by phase II solution which contained 0.10% SO_2 and 0.5% LA, and the grain was steeped for 8 h more. Freeman and Watson (1969) used a solution of 0.1% SO_2—1% LA (pH 4.0) at 49°C for 48 h. However, Blessin et al. (1971), following the procedure by Anderson (1963), steeped sorghum with dilute SO_2 solutions of unspecified concentrations without LA addition.

All these different combinations of SO_2 and LA have been applied without actual experimental evidence to support their use in terms of wet-milling efficiency and the rationale behind them. The objective of this study was to determine the optimum combination of SO_2 and LA that maximizes wet-milling efficiency for one hybrid of grain

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sorghum. "Efficiency" was perceived as maximization of starch and protein yields with simultaneous minimization of fiber yield.

Materials and Methods

Grain Sorghum Sample

Dekalb 48, a typical red grain sorghum hybrid in the midwestern U.S., from the 1993 crop year was obtained from a farm in Milford, Nebraska. To prevent any effect of hot-air drying and mechanical shelling on grain properties and wet-milling potential, sorghum was harvested by hand, air-dried (ambient, 20-25°C) to 13 to 14% (w.b.) M.C., and then threshed in a laboratory thresher. Threshed grain was cleaned over a 6.35 mm screen in a Carter-Day dockage tester (SEA, Minneapolis, Minn.) and stored at 5°C until needed for analysis or milling.

Steeping Conditions

Since this study was limited to analysis of SO_2-LA interactions on wet-milling efficiency, all other parameters influencing steeping were fixed at constant values: pH 3.7, time 40 h, temperature 51°C, and hybrid Dekalb 48. Ranges of 0.050 to 0.278% SO_2 and 0 to 2.282% LA were tested using response surface methodology (RSM). These ranges comprehensively covered levels of SO_2 and LA used in grain sorghum steeping by previous researchers.

Laboratory Wet-milling

The laboratory wet-milling procedure was adapted from Zipf et al. (1950), Watson et al. (1951), Weller et al. (1988), Eckhoff et al. (1993), Shandera and Jackson (1993), and Shandera et al. (1995). Each treatment combination was replicated once except for the central design point that was run five times. Clean sorghum (600 g) was batch-steeped in 2.0 L of aqueous solution containing the corresponding combination of SO_2 and LA (table 1). In each case, sufficient sodium bisulfite (Fisher Scientific, Pittsburgh, Pa.) was added to steeping water to obtain the proper SO_2 concentration. Potassium hydroxide (Fisher Scientific) was used to adjust pH to 3.7. Lactic acid polymers present in the 85% concentrated racemic reagent (Fisher Scientific) were broken down by diluting to 10% (w/w) and then heating at 95°C for at least 24 h (Shandera and Jackson, 1993). The grain was steeped for 40 h at 51 ± 0.5°C. Steep water was pumped continuously from the steeping tank through a screened opening to a water bath (Blue M Electric Company, Model MW-1120-A1, Blue Island, Ill.) from which it was returned to the tank. Pumping rate was 525 mL/min using a peristaltic pump (Masterflex Model No. 7520-00, Cole Parmer Instruments Co., Chicago, Ill.). After the operation was completed, the steep water was drained and measured by using a 2000 mL graduated cylinder. A representative sample was retained for solids determination using AOAC method 33.042 (AOAC, 1984).

Table 1. Central composite design treatment combinations for concentrations of sulfur dioxide (SO_2) and lactic acid (LA) in steeping water of grain sorghum

Treat-ment	Number of Replications	Coded Values	SO_2 (% w/v)	LA (% w/v)
1	1	(-1.414,0)	0.0500	1.141
2	1	(-1,+1)	0.0641	2.141
3	1	(0,+1.414)	0.1641	2.282
4	1	(+1,+1)	0.2641	2.141
5	1	(+1.414,0)	0.2782	1.141
6	1	(+1,-1)	0.2641	0.141
7	1	(0,-1.414)	0.1641	0.000
8	1	(-1,-1)	0.0641	0.141
9	5	(0,0)	0.1641	1.141

The first step in the milling operation was degermination which was carried out in a commercial blender (Waring Model 5011, Waring Products Division, New Hartford, Conn.) set at 35% of the 120 V line voltage using a variable transformer. The bowl was charged with 200 mL of distilled water for each 150 g sorghum sample and blending lasted for 3 min. Germs were then available for skimming. However, this operation was not attempted as it was time-consuming and unnecessary for the main purpose of the study. Instead, germs were retained with the fiber fraction and thereafter noted as a combined fraction.

Fiber/germ separation was initiated by pouring the slurry onto a U.S. standard sieve no. 230 (63 μm square mesh spacing) fitted onto an empty plastic container while rinsing with 500 mL distilled water. Coarse material collected on the sieve was washed with 500 mL distilled water, re-wet with another 500 mL distilled water and transferred to a grinding mill (Model 4E, The Straub Company, Hatboro, Pa.) fitted with fine grinding plates. The mill ground the fraction of steeped sorghum retained on the sieve to physically break down pieces of vitreous endosperm that were softened during steeping but not broken during the degermination grind. Plates of the grinding mill were set to consistently just touch each other and resist movement by hand. Material in the mill was ground with 500 mL distilled water added slowly during the grinding procedure. Discharge from the mill was returned to the sieve and washed with 1000 mL distilled water. Material remaining on the sieve was considered the fiber/germ fraction. It was removed and dried at 50°C for 24 h before weighing and determining moisture content (M.C.) as described below. Filtrate through the sieve, which was considered the mill starch, was placed in a plastic bucket and held overnight at 4°C. During storage, starch and protein settled to the bottom of the bucket.

The final milling step (i.e., separation of mill starch into starch and protein fractions) began by adjusting the specific gravity of mill starch to 7 to 8° Baume (1.051-1.058 sp.g.). This was achieved by decanting off approximately 2000 mL of supernatant water and re-suspending starch and protein in the remaining water to give the desired Baume solution. This suspension was fed onto the upper end of a starch table in a steady stream. The starch table was 6.1 m long and 12.7 cm wide and was made of galvanized steel guttering. One end of each gutter was closed and raised 5.72 cm higher than the open end so that a slope of 9.38 mm/m was maintained. This slope was considered optimum for starch-protein separation in grain sorghum wet-milling (Zipf et al., 1950).

Control of flow rate of mill starch (approximately 250 mL/min) allowed the starch fraction to settle and the protein suspension to flow off at the open end of the table. After starch was deposited on the table, adhering protein was flushed from the starch surface by passing supernatant at a rate twice as fast as the flow rate of the mill starch. Another gentle washing of starch on the table followed using 1000 mL distilled water. The wash tailings were

combined with the protein overflow. The starch fraction consisted of what remained on the table. It was allowed to air-dry for at least 1 h before being removed by scraping towards the effluent end. Remaining material on the table was re-suspended with distilled water, collected at the end of the table and recovered by vacuum filtration through Buchner funnels using qualitative filter paper (Whatman No. 2, Whatman Laboratory Division, Maidstone, England). All starch was dried at 50°C for 24 h in an air-circulating oven before weighing and determining moisture, starch and protein as described below. The protein fraction was dewatered by vacuum filtration through Buchner funnels using qualitative filter paper as done for the starch fraction. It was then air-dried (20-25°C) and ground in a coffee grinder (Model GC-5, Salton Inc., Mt. Prospect, Ill.) before weighing and determining moisture and protein contents as described below.

Moisture Content

ASAE M.C. measurement method S352.2 for unground grains and seeds (ASAE, 1992) was used to determine m.c. of whole grain. AACC air oven method 44-15A (AACC, 1983) was used to measure m.c. of starch, protein, and fiber/germ fractions. Percent total solids of initial whole grain and dry basis percentages of fractions' yields were computed based on these moisture contents.

Proximate Analysis

Starch contents of whole grain and starch fractions were determined in triplicate by the AACC rapid enzymatic method 76-12 (AACC, 1995). Starch was gelatinized in the presence of dimethyl sulfoxide (DMSO) and hydrolyzed to dextrins with thermostable-amylase at 95 to 100°C, followed by pullulanase and β-amylase at 50°C. Hydrolysis products (maltose and maltotriose) were then quantitatively cleaved to glucose by amyloglucosidase. Glucose was measured using glucose oxidase/peroxidase reagent.

Protein contents of whole grain, starch and protein fractions were determined in duplicate according to the Corn Refiners Association macro-Kjeldahl method A-18 (CRA, 1986) using the Tecator Kjeltec System (Tecator, Hoganas, Sweden). The nitrogen to protein conversion factor used was 6.25. Crude free fat and ash contents of whole grain were determined in duplicate according to the AOAC methods 922.05 and 923.03, respectively (AOAC, 1984).

Fraction Yields, Protein Recovery, and Starch Recovery

Fraction yields (% d.b.) were defined as dry weights of corresponding fractions divided by initial dry total solids weight in the whole kernel and multiplied by 100. Starch recovery (%) was defined as the ratio of total weight of starch recovered from wet-milling to total weight of starch initially present in the grain multiplied by 100. Total weight of starch recovered for each treatment was calculated by multiplying the starch fraction dry weight by the percent starch in the recovered starch fraction. Total weight of starch in whole grain was calculated as the product of the percentage of starch content and the total dry matter weight in the initial 600 g of sorghum sample. Protein recovery (%) was defined as the ratio of total weight of protein recovered by wet-milling (i.e., protein contained in protein fraction) to total weight of protein initially present in the grain multiplied by 100.

Experimental Design and Statistical Analysis

RSM is a statistical tool for obtaining and modeling the response resulting from the interaction of two or more independent variables over a range of treatment combinations. The power of RSM lies in treatments being strategically chosen so that all possible combinations of independent variables (treatments) do not have to be observed to find optimum treatment combinations. A central composite design was used to determine treatment levels. The estimated optimal response point on the surface (stationary point) may represent either a maximum, minimum, or saddle point (Montgomery, 1984). A central composite design of second-order for a two-factor model was chosen to study SO_2-LA interactions. Tested treatment combinations are shown in table 1. Response variables were starch yield, starch recovery, protein yield, protein recovery, fiber/germ yield, and steeping solids yield. Regression models were evaluated for each response variable. Models tested possible linear, quadratic and linear cross-product relationships, analyzed through the center point. A model was considered an adequate approximation of the true surface if the error due to lack-of-fit was not significant ($P > 0.05$) and the variation removed due to regression was significant ($P < 0.05$). Regression parameters were used to interpret which treatment effects were significant. Response surface graphs were plotted, and optimum responses were identified within feasible treatments for the different response dependent variables.

The Statistical Analysis System (SAS) was used to analyze collected and computed data (SAS, 1989). Means, standard deviations, ranges, and coefficients of variation (CVs) were determined. Correlation coefficients (r) were computed by the SAS correlation procedure. Response surface model analysis was performed separately for each variable using the RSREG procedure in SAS.

Results and Discussion

The proximate composition (d.b.) of the hybrid Dekalb 48 was 72.1% starch, 9.0% protein, 4.4% crude free fat, and 1.1% ash. Corresponding CVs were 2.8, 1.4, 3.1, and 6.0%, respectively. Statistics for yield and recovery values for the different combinations of SO_2 and LA (table 2) revealed wide ranges and high CVs providing initial evidence of the significant influence of SO_2 and LA.

Correlations

Simple correlation coefficients among wet-milling attributes (table 3) indicated significant ($P < 0.01$) high relationships between starch yield and starch recovery ($r = 0.99$), starch yield and fiber/germ yield ($r = –0.96$), and starch recovery and fiber/germ yield ($r = –0.96$). Starch yield and starch recovery were reported as highly positively correlated ($r = 0.98$) in a study by Norris and Rooney (1970) using several sorghum hybrids. The high negative correlations between starch yield and fiber/germ yield and between starch recovery and fiber/germ yield showed that insufficient steeping conditions to degrade kernels led to poor separation and presence of significant endosperm chunks in the fiber/germ fraction. This was

Table 2. Yield and recovery values in sorghum wet-milling (hybrid Dekalb 48) under different combinations of sulfur dioxide and lactic acid concentrations in steeping water

Treat-ment*	Steeping Solids†	Starch Yield†	Protein Yield†	Fiber/Germ Yield†	Starch Recovery‡	Protein Recovery‡	Protein In Starch§
1	8.9	57.9	7.0	24.8	78.1	27.9	0.4
2	12.8	54.4	6.7	28.4	73.9	26.6	0.3
3	13.9	58.0	5.8	27.2	78.9	28.5	0.4
4	12.3	61.5	7.0	23.8	83.6	30.8	0.3
5	8.8	57.3	6.5	29.3	78.1	31.3	0.4
6	4.6	57.1	7.8	26.1	78.6	36.7	0.4
7	4.3	53.8	7.0	31.9	72.3	27.9	0.3
8	4.1	48.9	7.0	35.8	67.0	23.4	0.4
9a	8.9	62.5	7.3	22.0	85.7	31.3	0.3
9b	9.9	61.3	7.3	23.1	83.9	33.3	0.3
9c	9.2	61.7	7.1	23.0	83.8	33.3	0.3
9d	9.6	61.4	7.1	23.6	83.4	33.3	0.3
9e	9.3	61.4	6.7	23.5	83.8	31.4	0.4
Mean	9.0	58.3	6.9	26.4	79.3	30.4	0.3
SD‖	3.1	4.0	0.5	4.1	5.5	3.5	0.1

* Numbers identify treatments as specified in table 1. Letters in treatment 9 refer to the five replications performed for the central point of the design.
† Expressed as percent dry basis (% db).
‡ As defined in the MATERIALS AND METHODS section.
§ Expressed as percent weight/weight (% w/w).
‖ Standard deviation.

Table 3. Correlation coefficients (r) among wet-milling attributes of grain sorghum (Dekalb 48) for different combinations of sulfur dioxide and lactic acid concentrations in steeping water†

	Ssol	StchY	ProtY	FGY	StRec	PtRec
StchY	0.48					
ProtY	−0.56*	0.12				
FGY	−0.48	−0.96**	−0.23			
StRec	0.46	0.99**	0.15	−0.96**		
PtRec	0.01	0.72**	0.46	−0.70**	0.74**	
PtStch	−0.20	−0.26	−0.22	0.24	−0.25	−0.12

† Ssol = steeping solids; StchY = starch yield; ProtY = protein yield; FGY = fiber/germ yield; StRec = starch recovery; PtRec = protein recovery; and PtStch = protein in starch.
* Significant at 0.05 level.
** Significant at 0.01 level.

observed in treatments for which the corresponding combination of SO_2 and LA resulted in inadequate breakdown of the protein matrix and, consequently, poor separation. A significant highly negative correlation ($r = -0.70$, $P < 0.01$) also was observed between protein recovery and fiber/germ fraction yield indicating that the poorer the wet-milling efficiency, the more protein was lost in the fiber fraction. Protein recovery was positively correlated with starch yield ($r = 0.72$, $P < 0.01$) and starch recovery ($r = 0.74$, $P < 0.01$), but it did not have a strong correlation with protein yield ($r = 0.46$, $P > 0.05$). A less important negative correlation existed between steeping solids and protein yield ($r = -0.56$, $P < 0.05$). This negative correlation suggested that as more protein became soluble in steeping water, protein yield decreased.

STARCH YIELD AND RECOVERY

Yield and recovery data were analyzed for relationships between treatment levels of SO_2 and LA and their ability to predict response in a second-order model. When means for yields of treatments differed significantly (at least $P < 0.10$) within the experiment, the relationship of treatment factor effects on a fraction's yield or recovery value was estimated in a mathematical form and a response surface was drawn for visual representation of that relationship. Relationships could be predicted when more variability could be assigned to treatment factors than to the experimental error (between treatments).

Response surface total regression was significant ($P < 0.01$, $R^2 = 0.8448$) for starch yield so a meaningful response surface could be created. The response was significantly ($P < 0.05$) affected, both linearly and quadratically, by SO_2 and LA. Cross-interaction was not significant ($P > 0.05$). Figure 1 presents a visual representation of the response surface and its equation. The stationary point was a maximum with a predicted starch yield of 62.3% (d.b.). A combination of 0.206% SO_2 and 1.415% LA was associated with that maximum.

Starch recovery statistical values closely resembled those found for starch yield as expected from the high correlation between starch yield and starch recovery. The model, based on a highly significant total regression ($R^2 = 0.8458$), was influenced by LA (linear and quadratic effects) and SO_2 (linear and quadratic) while cross-interaction was not significant. The stationary point also was a maximum with a predicted starch recovery value of 84.9% which was contained within the experimentally observed range. The associated steeping chemicals' concentrations were 0.209% SO_2 and 1.398% LA, very close to those corresponding to predicted maximum starch yield. Figure 2 depicts the model and gives the characterizing equation.

PROTEIN YIELD, PROTEIN RECOVERY AND PROTEIN CONTENT OF STARCH

Protein yield's total regression did not provide a significant reduction in variation indicating that the model did not fit the data and no linear and quadratic relationships existed between concentrations of steeping chemicals and resulting protein yields. Protein recovery's total regression ($R^2 = 0.8298$) was significant at the 0.05 level, so feasibility of the model was initially expected. However, the predicting steeping chemicals' combination was 0.409% SO_2, a value outside the range of the experiment, and an obviously unfeasible negative value of −0.130% LA. Therefore, it was meaningless to obtain a surface or an

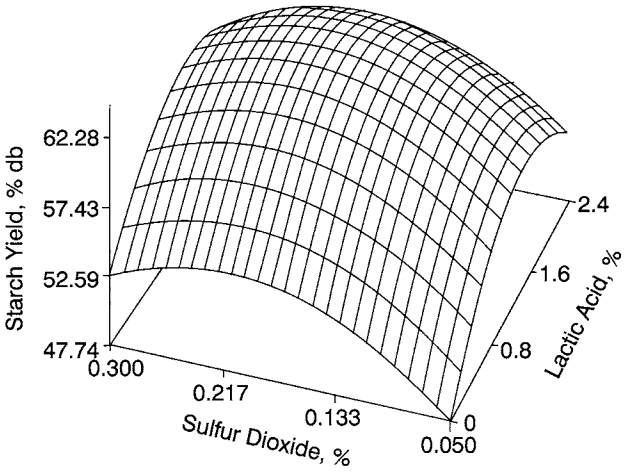

Figure 1–Starch yield (STY) response surface for wet-milled grain sorghum with lactic acid (L) and sulfur dioxide (S) concentrations in steeping water (% w/v), respectively, and starch yield (% d.b.). The surface is characterized by the following equation: $STY = 42.889 + 109.900S + 11.431L - 257.934S^2 - 3.847L^2 - 2.650SL$. Maximum starch yield of 62.3% d.b. occurred at 0.286% SO_2 and 1.415% LA.

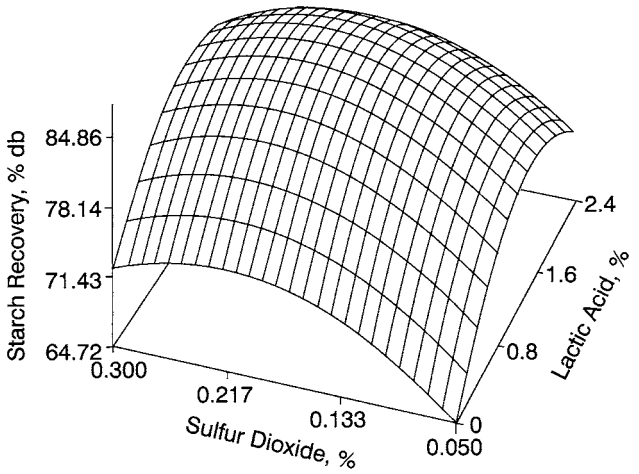

Figure 2–Starch recovery (STREC) response surface for wet-milled grain sorghum with lactic acid (L) and sulfur dioxide(S) concentrations in steeping water (% w/v), respectively, and starch recovery (%). The surface is characterized by the following equation: STREC = 58.054 + 150.531S + 15.862L – 344.633S^2 – 5.320L^2 – 4.725SL. Maximum starch recovery of 84.9% occurred at 0.209% SO_2 and 1.398% LA.

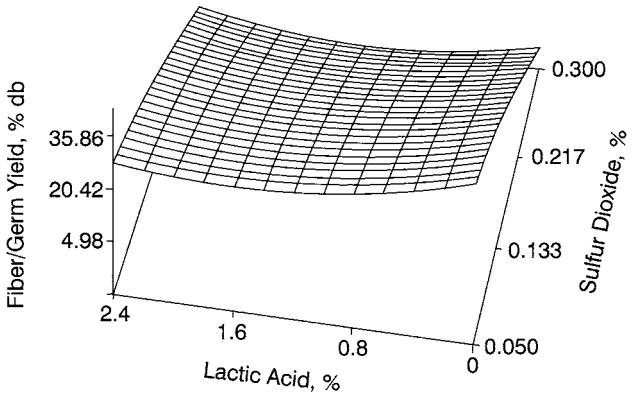

Figure 3–Fiber/germ yield (FGY) response surface for wet-milled grain sorghum with lactic acid (L) and sulfur dioxide (S) concentrations in steeping water (% w/v), respectively, and fiber/germ yield (% d.b.). The surface is characterized by the following equation: FGY = 41.015 – 93.296S – 13.261L + 197.559S^2 + 3.900L^2 + 12.775SL. Maximum fiber/germ yield of 22.9% d.b. occurred at 0.191% SO_2 and 1.387% LA.

overall model. The total regression for the variable "protein content of starch" was not significant ($P > 0.05$) indicating that the fitted model was unfeasible.

Steeping Solids

Steeping solids yields were fitted to the model by a significant ($P < 0.01$) total regression. The R^2 was 0.9947. Nevertheless, the model was of limited value due to an "optimum" combination of steeping chemicals (0.028% SO_2 and 7.469% LA) outside the tested treatment levels. Besides, the stationary point, a maximum on the surface, corresponded to an unfeasible yield of 22.5%.

Fiber/Germ Yield

The total regression ($R^2 = 0.7090$) for fiber/germ yield was significant at the 0.10 level. Prediction of a minimum at the stationary point, with an associated combination of 0.191% SO_2 and 1.387% LA, quite close to the ones found for starch yield and starch recovery, justified drawing the surface. The response surface is presented with its corresponding equation in figure 3. Only LA, both linearly and quadratically, had enough statistical significance to explain the model, which accounted for a predicted yield of 22.9% d.b. at the stationary point. It is noted that linear SO_2 was just above the 0.10 level ($P = 0.11$). The fact that fiber/germ yield was a minimum at a steeping chemicals' combination resembling those for starch yield and recovery, agreed with the strong negative correlations previously reported.

Implications

Despite the controversy on the merits of LA as a steeping agent, its importance on steeping effectiveness of one grain sorghum hybrid was clearly shown. LA may change structure of cell membranes (softening action) to allow for easier migration of sulfuric ions throughout the kernel into endosperm cells. Thus, increased cellular concentrations of SO_2 would enhance protein degradation and starch release (Shandera et al., 1995). Negative correlations between fiber/germ yield and both starch yield and starch recovery were satisfactorily matched with opposite stationary points in the corresponding constructed response surfaces at similar combinations of SO_2 and LA. The importance of optimum steeping conditions in appropriately breaking down grain sorghum kernels, thereby maximizing process efficiency, was stressed in this study. Results observed for the one hybrid of this study indicated optimal conditions occurred at approximately 0.2% SO_2 and 1.4% LA. Similar optimal conditions may apply to other sorghum hybrids however there is a need to confirm them.

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References

AACC. 1995. 9th Ed. Method 76-12. In *Approved Methods of the AACC*. St. Paul, Minn.: Am. Assoc. Cereal Chemists.

_____. 1983. 8th Ed. Method 44-15A. In *Approved Methods of the AACC*. St. Paul, Minn.: Am. Assoc. Cereal Chemists.

Anderson, R. A. 1963. Wet-milling properties of grains: Bench scale study. *Cereal Science Today* 8(6):190-192, 195, 221.

AOAC. 1984. 14th Ed. Methods 922.05 and 923.03. In *Official Methods of Analysis of the AOAC*. Washington, D.C.: Assoc. Official Analytical Chemists.

ASAE Standards , 39th Ed. 1992. S352.2. Moisture measurement —Unground grain and seeds. St. Joseph, Mich.: ASAE.

Boundy, J. A., J. E. Turner, J. S. Wall and R. J. Dimler. 1967. Influence of commercial processing on composition and properties of corn zein. *Cereal Chem.* 44(3):281-287.

Blessin, C. W., R. A. Anderson, W. L. Deatherage and G. E. Inglett. 1971. Effect of alkali dehulling on composition and wet-milling characteristics of sorghum grain. *Cereal Chem.* 48(5):528-532.

CRA. 1986. Method A-18. In *Standard Analytical Methods of the Member Companies of Corn Industry Research Foundation*. Washington, D.C.: Corn Refiners Assoc.

Eckhoff, S. R., K. D. Rausch, E. J. Fox, C. C. Tso, X. Wu, Z. Pan and P. Buriak. 1993. A laboratory wet-milling procedure to increase reproducibility and accuracy of product yields. *Cereal Chem.* 70(6):723-727.

Eckhoff, S. R. and C. C. Tso. 1991. Wet milling of corn using gaseous SO_2 addition before steeping and the effect of lactic acid on steeping. *Cereal Chem.* 68(3):248-251.

Freeman, J. E. and S. A. Watson. 1969. Peeling sorghum grain for wet milling. *Cereal Sci. Today* 14(2):10-12, 14-15.

Montgomery, D. C. 1984. *Design and Analysis of Experiments*. New York, N.Y.: John Wiley & Sons.

Norris, J. R. 1971. Chemical, physical and histological characteristics of sorghum grain as related to wet-milling properties. Ph.D. diss. College Station, Tex.: Texas A&M University.

Norris, J. R. and L. W. Rooney. 1970. Wet-milling properties of four sorghum parents and their hybrids. *Cereal Chem.* 47(1):64-69.

SAS. 1989. *Proprietary Software*, Release Ver. 6.08. Cary, N.C.: SAS Institute, Inc.

Shandera Jr., D. L. and D. S. Jackson. 1993. A simplified method for measurement of lactic acid and sulfur dioxide in corn wet milling steep water. *Starch-Staerke* 45(10):351-354.

Shandera Jr., D. L., A. M. Parkhurst and D. S. Jackson. 1995. Interactions of sulfur dioxide, lactic acid, and temperature during simulated corn wet milling. *Cereal Chem.* 72(4):371-378.

Subramanian, V., R. C. Hoseney and P. Bramel-Cox. 1994. Factors affecting the color and appearance of sorghum starch. *Cereal Chem.* 71(3):275-278.

Watson, S. A. 1967. Manufacture of corn and milo starches. In *Starch: Chemistry and Technology*, Vol. 2, eds. R. L. Whistler and E. F. Paschall, 1-51. New York, N.Y.: Academic Press.

Watson, S. A. 1970. Wet-milling: Process and products. In *Sorghum: Production and Utilization*, eds. J. S. Wall and W. M. Ross, 602-626. Westport, Conn.: AVI Publishing Co., Inc.

Watson, S. A., E. H. Sanders, R. D. Wakely and C. B. Williams. 1955. Peripheral cells of the endosperms of grain sorghum and corn and their influence on starch purification. *Cereal Chem.* 32(3):165-182.

Watson, S. A. and Y. Hirata. 1955. The wet-milling properties of grain sorghum. *Agron. J.* 47(1):11-15.

Watson, S. A., C. B. Williams and R. D. Wakely. 1951. Laboratory steeping procedures used in a wet milling research program. *Cereal Chem.* 28(2):105-118.

Weller, C. L., M. R. Paulsen and M. P. Steinberg. 1988. Correlation of starch recovery with assorted quality factors of four corn hybrids. *Cereal Chem.* 65(5):392-397.

Zipf, R. L., R. A. Anderson and R. L. Slotter. 1950. Wet-milling of grain sorghum. *Cereal Chem.* 27(6):463-476.