**Model study on extraction of fermentable sugars and nonstructural carbohydrate from
sweet sorghum using diffusion process**

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ABSTRACT

Sweet sorghum stores a high concentration of soluble sugars in its stalk and produces grain in the panicle. This grain represents a significant amount of starch. The ethanol industry currently uses sugarcane processing methods for sweet sorghum; however, sweet sorghum differs from sugarcane in that sweet sorghum produces significant quantities of grain which is predominantly starch. The objective of this research was to increase ethanol production from sweet sorghum by fully utilizing all fermentable sugars which include starch in the grain and nonstructural carbohydrates in the stalk. The diffusion process was utilized to extract fermentable sugars and nonstructural carbohydrates from chopped sweet sorghum biomass and grains. Response surface methodology (RSM) was applied in order to optimize diffusion conditions and to explore effects of diffusion time, diffusion temperature, ratio of sweet sorghum grain to total biomass on starch-to-sugar efficiency, and total sugar recovery from sweet sorghum. RSM results showed that starch conversion efficiency and sugar recovery efficiency of 96% and 98.5%, respectively, were achieved at an optimized time of 114.9 minutes, temperature of 95 °C, and 22% grain loading.

Keywords: sweet sorghum, grain, diffusion process, sugar yield

1. Introduction

Sweet sorghum (*Sorghum bicolor*(L) Moench), a C₄ plant, is a unique and versatile sugar crop that can be separated into starchy grains, soluble sugar in juice extracted from the stalk, and lignocellulose biomass (Rao et al., 2013; Blummel et al., 2009). All these components can be processed into ethanol (starch-based and cellulosic), syrup, animal feed, and electricity, as well as used as substrate for hydrogen and methane production (Antonopoulou et al., 2008; Gnansounou et al., 2005, 2013; Li et al., 2013). The juice extracted from sweet sorghum stalks contains water-soluble nonstructural carbohydrates (sucrose, glucose, and fructose) and structural carbohydrates (cellulose and hemicellulose) (Li et al., 2013; Serna-Saldivar et al., 2012a). The juice from the stalk may contain 20 to 50% of dry matter of the entire plant (Whitfield, 2012). Using modelling, Rainey and O'Hara (2013) reported that a total ethanol yield of 8130 L/ha could be achieved from sweet sorghum, assuming a total productivity of 60 t/ha of sweet sorghum consisting of 3 t/ha of grain (73% starch), 50 t/ha of stalk (15% total sugars and 15% dry fiber), and 7 t/ha of leaves (40% dry fiber). The grain yield is typically 3-7 t/ha (Rainey and O'Hara, 2013, Rao et al., 2013), and stalk yield per hectare is 45-65 tons (Rao et al., 2013). Recent studies have shown that sweet sorghum juice could be incorporated into the current starch-based ethanol process in order to conserve water and achieve yields of 28% higher than the conventional process (Appiah-Nkansah et al., 2015). Ethanol fermentation efficiencies of sweet sorghum from juice could range from 85 to 93% (Appiah-Nkansah et al., 2015, Serna-Saldivar et al., 2012b).

Fermentable sugar composition of sweet sorghum feedstock range from 16 to 22% as compared to sugarcane juice (12 to 17.6%), sugar beet juice (16%), and watermelon juice (7 to 10%) (Zabed, et al., 2014). In addition, sugar yields from sweet sorghum have been reported to

be 4 to 10 t/ha as compared to sugarcane (5 to 12 t/ha) and sugar beet (11.25 to 18 t/ha) (Rao et al., 2013, Regassa and Wartmann 2014). Sweet sorghum grain, which consists of 60 – 70% starch, can be hydrolyzed and saccharified into glucose and subsequently fermented to produce biofuels (O’Hara, 2013; Albertson et al., 2013; Rainey and O’Hara, 2013). Based on these numbers, sweet sorghum is a competitive bioethanol feedstock that could be integrated into existing sugarcane ethanol-processing plants. However, only three sugarcane plants are known to incorporate sweet sorghum crop into their facilities – Mossman Central Factory in Australia, the Triangular Sugar Mills in Zimbabwe, and U.S. Department of Agriculture pilot plant in Texas (Rainey and O’Hara, 2013, Smith et al., Woods, 2000).

Sweet sorghum has a similar physiological structure to sugarcane, thereby allowing use of the same mechanical harvesting approach. Sweet sorghum can also be manually harvested and the stalk can be expressed in the field. In the manual harvesting process, the crop is topped and the leaves are stripped before crushing the stalk for juice extraction (Regassa and Wartmann, 2014). The topped panicle is composed of grain that is left in the field. Consequently, a significant amount of starch (60-70%) that could be hydrolyzed is left in the field.

Traditionally, juice extraction is achieved by pressing the stalk of the crop through a roller mill, but this process is slow, labor intensive, and less efficient, with juice recovery below 50% (Regassa and Wartmann, 2014; Whitfield et al., 2012). Low juice extraction yield could be attributed to the relatively high fiber content of sweet sorghum stalk compared to sugarcane (Gnansounou et al., 2005). Another drawback associated with the milling process is sugar loss due to microbial activities (Wu et al., 2010; Whitfield, 2012). Wu et al. (2010) reported that up to 50% of total fermentable sugars in sweet sorghum is lost if the expressed juice is stored for one week at room temperature. This loss is a result of microorganisms that metabolize the sugars at

room temperature (≈ 25 °C), under the low pH (≈ 4.7) and anaerobic into organic acids (lactic acid, formic acid, acetic acid), carbon dioxide, and ethanol.

Diffusion is an alternative to juice extraction from the stalk. In this process, biomass is hammer-milled to uniform particle sizes and then passed through a series of continuous hot water flushes in which the concentration of solute is continuously reduced (Rein, 1995). Thus, liquid extraction recovers the sugars from cane tissues, while the conventional milling process employs mechanical juice expression. The diffusion method is the more effective of the two methods because it can achieve very high sucrose extraction (pol/sucrose ration of 0.988) (Rein, 1995). The diffusion system is also energy efficient and requires lower maintenance and capital costs because of lack of excessive pressure and shear forces of the roller mills (Cotlear, 2004). Typically diffusion plants include dewatering mills which utilizes approximately half of the power required in energy-intensive hammer mill (Rein 2007).

The objective of this research was to enhance the economic attractiveness of ethanol production from sweet sorghum using technological developments in order to fully utilize fermentable sugars, starch in the panicle, and nonstructural carbohydrates in the stalk for high efficiency and low-cost ethanol production. In this work, response surface methodology (RSM) was applied in order to study the interactive effect of diffusion time, diffusion temperature, and grain loading on sugar extraction from sweet sorghum feedstock.

2. Material and methods

2.1. Materials

Sweet sorghum grain and dried bagasse were obtained from Texas A&M University, College Station, Texas, for this research. The sweet sorghum was harvested just after physiological maturity of the grain in the panicle. At this stage of growth the grain is fully

developed, has approximately 30% moisture content and stalk sugar content has peaked and will start to reduce with increased maturity. The bagasse was carefully screened to remove grain kernels using a Seedburo seed blower (Seedburo Equipment Co., Des Plaines, IL). The grain and bagasse, separately milled through a 3.99 mm screen in a Schutte Buffalo hummer mill (Schutte-Buffalo Hammermill, LLC, Buffalo, NY), were used for the diffusion test and analysis. Moisture content of the materials was determined as bagasse (4.88%) and grain flour (11.68%) using standard American Association of Cereal Chemists (AACC) and National Renewable Energy Laboratory (NREL) methods (AOAC, 2000; Sluiter et al., 2008.) The bagasse and grain flour were stored in sealed plastic bags at room temperature, and starch content of the sorghum grain was analyzed using a total starch kit (Megazyme International) in adherence to the AACC standard method (AACC, 2000).

2.2. Sugar extraction by starch hydrolyses and diffusion process

To determine starch-to-sugar conversion efficiency and sugar recovery efficiency, initial fermentable sugars in the sweet sorghum bagasse were used as the control. For sugar extraction, 40 g of biomass (grain + bagasse) was weighed in a 500 mL beaker. Approximately 750 mL of distilled water was preheated in a microwave for 5 min, and 500 mL of the preheated water was measured. Approximately 400 mL of the preheated water was poured into the 1 L reaction vessel of a Parr pressure reactor (Parr Instrument Co., Moline, IL), and the biomass sample were added. The reactor is equipped with impeller mixers and a controlled heating system. The remaining ~100 mL was used to thoroughly rinse the beaker into the reaction vessel. Thirty microliters of Liquozyme® SC DS (α – amylase 267 KNU/g, 1.266 g/mL; Novozyme Inc., Franklinton, NC) was added to the content. The reaction vessel was then coupled to the Parr reactor assembly and set to run for 120 min at a set temperature of 85 °C and an impeller speed of 100 revolution per

minutes (rpm). The heating source was removed and the reactor was cooled to a temperature below 50 °C. The vessel was disengaged and 150 µL of Spirizyme® Achieve (glucoamylase >900 AGU/g, 1.161 g/mL; Novozyme, Inc) was added to the mixture. The vessel was fixed again to the Parr reactor system and set to run for 40 min at a temperature of 60 °C; then the slurry was recovered. The described process was carried out with bagasse added together with the grain before starch hydrolysis and under varying conditions according to the experimental design presented in Table 1. The diffusion process was based on hot water extraction of residual sugars from the bagasse. All removed slurry was centrifuged in order to separate out the solid portions, which was filtered through a 0.2 µm membrane for sugar analysis.

2.3 Sugar analysis

A high pressure liquid chromatograph (HPLC) with a Rezex RCM Monosaccharide (300 X 7.80 mm) and a Refractive Index Detector RID – G1362A (Agilent Technologies, Santa Clara, CA) were used to analyze sugars at 40 °C and HPLC water as mobile phase at a flow rate of 0.6 mL/min. Chromatograph temperature was maintained at 80 °C.

2.4 Experimental design and statistics analysis

RSM was applied in order to optimize diffusion conditions and explore the effect of diffusion time, diffusion temperature, ratio of sweet sorghum grain to biomass on starch-to-sugar efficiency, and total sugar recovery from sweet sorghum. The ratio of grain to biomass, also known as the harvest index, represent the biomass yield or carbohydrate that could be considered for processing (Field et al., 2008). Optimum diffusion conditions for sugar extraction were analyzed using the Box-Behnken design. Three coded levels were used for design factors: time, temperature, and grain loading. The experimental design is described by the following equation:

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X_i^2 \quad [1]$$

where Y_i is the predicted response by the response surface model (Myers et al., 2009), β_0 is the intercept; β_i , β_{ij} , and β_{ii} represents partial regression coefficients for each linear term, interaction term, and quadratic term, respectively; and X_i represents design factors or condition parameters – time (minutes) (X_1), temperature ($^{\circ}\text{C}$) (X_2), and grain loading (%) (X_3). Design factors were in the range of 60 to 120 min, 75 to 95 $^{\circ}\text{C}$, and 15 to 25% (g grain per gram total biomass) for time, temperature, and grain loading, respectively. Table 1 shows the experiment design and corresponding values for starch conversion efficiency (%) and sugar recovery efficiency (%). Starch-to-sugar conversion efficiency, Y_{SE} , and sugar recovery efficiency, Y_{SR} , are defined in the following calculations:

$$Y_{SE} = \frac{M_G}{M_{GS} \times 0.69 \times 1.11} \times 100\% \quad [2]$$

$$Y_{SR} = \frac{S_T}{M_B + M_{GS} \times 0.69 \times 1.11} \times 100\% \quad [3]$$

where S_T is total sugars obtained after diffusion (g), M_B is mass of sugar from biomass (g), M_B used was determined based on the results of a preliminary experiment conducted using only the bagasse at 85 $^{\circ}\text{C}$ over 120 minutes) M_G is mass of sugar from grain (g), M_{GS} is mass of grain solid (g), 0.69 is the starch content, and 1.11 is the mass coefficient of starch to sugar (g/g). Considering a total biomass content (grain + bagasse) of 40 g used in the experiment, the mass of grain stated also indicated the ratio of bagasse used. Hence the mass of bagasse was not included in [3].

Data obtained from the experiments (Table 1) were analyzed using analysis of variance (ANOVA), and RSM results were analyzed using Design-Expert 9.0.3.1 (Stat-Ease, Inc., Minneapolis, MN). Mean values from the experiments were reported. Global F-Test was used to determine statistical significance of the developed models. Adjusted multiple coefficient of

determination, R^2_{adj} , was computed in order to determine how well the regression model fits the data (Mendenhall and Sincich, 2012). Statistical diagnostics in Design-Expert software was used to verify model assumptions of the residuals. Independent variables were coded according to the following equation (Zhang et al., 2012):

$$x_i = \frac{(X_i - X_i^*)}{\Delta X_i} \quad [4]$$

where x_i is the coded value of the i^{th} independent variable, X_i is the actual value of the i^{th} independent variable, X_i^* is the actual i^{th} independent variable at the center point, and ΔX_i is the step change value.

3. Results and discussion

3.1. RSM optimization of starch conversion efficiency

RSM linear and quadratic regression models were developed to investigate the effects of diffusion time, diffusion temperature, and grain loading and their interactive effect on starch-to-sugar conversion efficiency. Because the quadratic regression model fit the data better than the linear model, only the quadratic model is reported. ANOVA results for the second-order response model are shown in Table 2. ANOVA of the full quadratic regression model showed that the fitted model was highly significant, with Fishers' test (F -value) of 62.75 and the corresponding p -value of 0.0001 at 95% level of confidence ($P < 0.05$). Because the computed F -statistic value (62.75) exceeded the F -critical value (3.48), we rejected the null hypothesis (H_0) in favor of the alternative (H_1) based on a type I error rate (the error of rejecting a null hypothesis when it is actually true) of 5%; therefore, we concluded that a significant relationship exists between starch conversion efficiency and diffusion condition parameters. The coefficient of determination of the regression analysis, R^2 value, was 0.9912, meaning that approximately 99% of the variability in starch conversion efficiency was explained by the model. As an alternative

measure of model adequacy, the adjusted multiple coefficient of determination (adjusted R^2) was 0.9754 meaning that after adjusting the sample size and number of parameters, approximately 98% of total variation in starch conversion efficiency was explained by the model which suggests a suitable fitness of the model.

RSM developed a corresponding quadratic regression model for starch-to-sugar conversion efficiency, as shown in the following equation obtained for the coded factors:

$$Y_{SE} = 85.67 + 3.79X_1 + 8.53X_2 + 1.65X_3 + 0.43X_1X_2 + 0.66X_1X_3 + 0.31X_2X_3 - 1.92X_1^2 - 0.74X_2^2 - 1.55X_3^2. [5]$$

Table 3 shows regression coefficients of processing variables in the model for starch-to-sugar conversion efficiency. As shown in Table 3, linear effects of time (minutes) (X_1), temperature ($^{\circ}\text{C}$) (X_2), and grain loading (%) (X_3), as well as quadratic effects of time (X_1^2) and grain loading (X_3^2) significantly impacted starch conversion efficiency ($P < 0.05$). The temperature term (X_2) had the most significant effect on starch conversion efficiency, with $P < 0.0001$, indicating a stronger linear effect on the response variable (Y_{SE}). Based on F -values and p -values, the interactive terms of time and temperature (X_1X_2), time and grain loading (X_1X_3), and temperature and grain loading (X_2X_3) had a negligible interactive effect on the response ($P > 0.1$). Additionally, the quadratic term of temperature (X_2^2) had no significant effect on starch conversion efficiency ($P > 0.1$). Because interactive factors and the quadratic term of temperature were found to be insignificant, the full quadratic model was simplified and reduced to include only significant linear terms and second-order terms, as shown in Equation (6). The p -value (<0.0001) and corresponding F -value of 115.68 for the reduced model (Table 2) shows that the model is highly significant. The "Lack of Fit F -value" of 0.89 implies that "Lack of Fit" is not significant relative to the pure error.

$$Y_{SE} = 85.21 + 3.79 X_1 + 8.53 X_2 + 1.65 X_3 - 1.86X_1^2 - 1.49 X_3^2$$

[6]

Equation (6) is based on the coded factors and it is useful for identifying the relative impact of factors by comparing regression coefficients of individual parameters. Consequently, temperature had the highest impact on starch conversion efficiency, followed by time and grain loading, similar to full model analysis. Fig. 1a-c and 2a-c represent 3D response graphs and corresponding 2D contour plots based on the fitted model for the interaction between grain loading (%) and temperature (°C), temperature (°C) and time (minutes), and time (minutes) and grain loading (%) on sugar extraction from sweet sorghum bagasse and grain. The graphs were obtained by holding three variables constant while the two variables of interest varied within their experimental range. The response surface graph was used to determine optimum levels of diffusion parameters for maximum observed response at the highest point of the surface. In the 2D contour plots, color intensity represents the observed response (y); intense color indicates the region of maximum response that was restricted in the smallest ellipse. The 3D response graph and contour plot of starch conversion efficiency as a function of grain loading (%) and temperature (°C), and temperature (°C) and time (minutes) is shown in Fig. 2a-c, indicating that starch hydrolysis efficiency increased as time and temperature increased. High conversion efficiency of 95% was observed at 95 °C with grain loading of approximately 22 - 23% at 115 min. Heating at temperatures above 90 °C caused moisture absorption and swelling of starch granules, and shear-induced disruption with stirring caused rupture, thereby exposing granules to enzymatic hydrolysis. Amylopectin crystallites may have melted, causing amylose to leach out at 95 °C (BeMiller, 2007; Sun, 2005a) and enhancing increased enzymatic access to the granules. The 3D response surface graph and contour plot indicating the effect of grain loading and time

are represented in Fig. 1c, in which the starch conversion efficiency was shown to increase from 71.65% to 95.23% as temperature and grain loading increased. However, high starch conversion was not observed by increasing the amount of grain loading, possibly due to increased viscosity at high starch loading. Intensity of the interactive effect was reflected by the shape of the contour lines. High temperature cooking and stirring increases starch hydrolysis (Wu et al., 2006); therefore, starch efficiency increases with increasing temperature, time, and grain loading.

Diagnostic tests were conducted to validate model assumptions and check model accuracy. Diagnostic plots (not reported) established by Design-Expert software revealed no gross violation in model assumptions. A linear trend on the normal probability plot suggested that the normality assumption of the model was most likely satisfied. Studentized residual plots also showed no presence of an outlier since observed residuals lie within the range of ± 3 standard deviations of their mean of zero. Therefore, the assumption that residuals have a mean of zero was most likely satisfied. Residuals were evenly dispersed around the mean zero line in the residual plot, suggesting a lack of heterogeneity of residuals and indicating that the assumption of constant variances was most likely satisfied. Therefore, the model can be used to make predictions.

3.2 RSM optimization of sugar recovery efficiency

ANOVA results for second-order response surface models for sugar recovery efficiency are presented in Table 4. ANOVA of the quadratic regression model also showed that the fitted model was highly significant, with F -value of 55.20 and corresponding p -value of 0.0002 at 95% level of confidence ($P < 0.05$). The computed F -statistic value (55.20) exceeded the F -critical value (3.48) so the null hypothesis (H_0) was rejected in favor of the alternative (H_1) at a type I error rate of 5%. Based on this evidence, a combined effect of the independent variables (X_i)

significantly contributed to sugar recovery efficiency. The coefficient of determination of the regression analysis, R^2 value, was 0.9900, indicating that approximately 99% of sugar recovery efficiency variability could be accounted for by the model, suggesting model adequacy. The adjusted multiple coefficient of determination (adjusted R^2) was 0.9721. As mentioned, after adjusting the sample size and number of parameters, the model explained approximately 97% of total variation in sugar recovery efficiency.

Regression coefficients of processing variable in the model for sugar recovery efficiency are shown in Table 5. The corresponding second-order regression model for sugar recovery efficiency established by RSM is shown in Equation (7).

$$Y_{SRE} = 95.4 + 1.23 X_1 + 2.74 X_2 - 0.65X_3 + 0.41X_1X_2 + 0.48X_1X_3 + 0.71X_2X_3 - 0.65X_1^2 - 0.20X_2^2 - 0.34X_3^2$$

[7]

Results of experimental analysis by RSM indicated that linear terms of time (X_1), temperature (X_2), and grain loading (X_3), as well as the interactive term of temperature and grain loading (X_2X_3), the quadratic term of time (X_1^2), and grain loading significantly affected sugar recovery efficiency ($P<0.05$), as shown in Table 5. Equation (7) is also useful for identifying relative impact of the terms by comparing factor coefficients. Again, the linear term of temperature (X_2) had the most significant effect on the response, with $P<0.0001$, followed by time ($P = 0.0003$) and percentage grain loading ($P = 0.006$). Results also showed that interactive terms of time and temperature (X_1X_2) and time and grain loading (X_1X_3), as well as quadratic terms of temperature (X_2^2), grain loading (X_3^2), and temperature had no significant effect on the response ($P\geq 0.1$). The full quadratic model was simplified and reduced in order to include only significant linear terms, interactive terms, and quadratic terms shown in Equation (8). The

equation shows that temperature had the highest impact on starch conversion efficiency, followed by time. The negative coefficient for the single factor, grain (X_3) variable, denotes the effect of decreased grain loading on sugar recovery efficiency. In addition, the equation demonstrates that the interaction between temperature and grain significantly impacted starch efficiency. The p -value (<0.0001) and corresponding F -value of 60.82 (Table 4) shows that the reduced model is highly significant. A "Lack of Fit F -value" of 2.04 implies that the "Lack of Fit" was not significant relative to the pure error which is good.

$$Y_{SRE} = 95.09 + 1.23 X_1 + 2.74 X_2 - 0.65X_3 + 0.71X_2X_3 - 0.61X_1^2 \quad [8]$$

In addition, diagnostic plots (not reported) did not indicate any gross violation in model assumptions.

Fig. 2 represents the 3D response of a maximum sugar recovery efficiency of 98.47% and a minimum efficiency of 90.85% in relation to temperature and grain loading (at a constant time of 120 min), temperature and time (at constant grain loading of 25%), and time and grain loading (at a constant temperature of 95 °C).

3.3. Effect of time, temperature, and grain on sugar extraction

This study investigated sugar extraction from sweet sorghum bagasse and sweet sorghum ground grain with average particle size of approximately 4 mm. Sugar recovery from bagasse was achieved through the process of diffusion in which dissolved sugar molecules in solution were separated by the difference in concentration in the biomass and the solvent due to concentration gradient. Cane resident time is a significant design parameter in sugarcane extraction (Rein, 2007). Temperature also stimulates the rate of mass transfer (Jia et al., 2013). Rein (1974) estimated that a 5 °C increase of temperature from 75 °C to 80 °C could yield a 2%

increase in sugar extraction from sugar cane. In this work, high sugar recovery efficiency greater than 98% was achieved at an increased temperature of 90 °C and a diffusion time of 114 min (Fig. 2b). Therefore, the longer the diffusion time at an increased temperature, the higher the sugars extracted from the bagasse. High temperature increased molecule movement rate, thereby speeding up the rate of diffusion, as asserted in previous studies. Cotlear (2004) reported that sugarcane extraction was influenced by higher concentration differences between the interior cell and the extracting solvent. In addition, increased temperatures stimulated an increased rate of mass transfer of sugar molecules from feedstock cells by increasing molecular mobility. Because plant protein denatures at increased temperatures (Sun, 2005b; Harrison et al., 2003), the permeability of sugar molecules from the sweet sorghum cell may have been enhanced as well due to denaturing of the protein lining at the stalk cell walls. Protein denaturing of cell lining (Rein 2007) causes the release of sugar-containing cells, consequently promoting sugar extraction from these cells. Organic acid-producing organisms are active at room temperatures up to 70 °C (Wu et al., 2010; Rein, 1995; Rein, 2007); therefore, maintaining a diffusion temperature well over 90 °C may have prevented considerable sugar losses because microbial activities were controlled. Because sugar extraction is a mass transfer process, it can be assumed that the agitation by stirring might have caused increased molecular velocity, potentially facilitating the release and transfer of sugar molecules in ruptured cells into extraction solvent (Rein, 2007).

3.5. Optimization conditions

Optimum diffusion conditions obtained by Box-Behnken design in RSM are summarized in Table 6. The table also includes responses at grain loading of 10, 15, 20, 25, and 30%. According to our designed model, at a 95% confidence interval, the highest starch efficiency and

sugar recovery efficiency were obtained at 96.03% and 98.45%, respectively. Optimized diffusion parameters were diffusion temperature of 95 °C, diffusion time of 114.93 min, and grain loading of 22.04%.

4. Conclusion

In this study, a regression model was developed for the extraction of fermentable sugars and starch from sweet sorghum using response surface models among diffusion time, diffusion temperature, and grain loading. The models predicted sugar extraction from sweet sorghum. The interaction of time, temperature, and grain loading significantly affected starch-to-sugar conversion efficiency and sugar recovery efficiency. At an optimized time of 114.9 min temperature of 95 °C, and 22% grain loading, starch efficiency and sugar recovery efficiency of 96% and 98.5%, were obtained, respectively.

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436 **Table Captions**

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438 Table 1. Experimental design with RSM.

439 Table 2. ANOVA of full model and reduced model for starch conversion efficiency.

440 Table 3. Regression coefficients and their significance in the quadratic model for starch
441 conversion efficiency.

442 Table 4. ANOVA of full and reduced regression equation model for sugar recovery efficiency.

443 Table 5. Regression coefficients and their significance for quadratic model for sugar recovery
444 efficiency.

445 Table 6. Optimized diffusion conditions.

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Table 1. Experimental design with RSM

Run	Coded variables			Actual experimental variables			Starch efficiency,	Sugar recovery
	Time	Temp	Grain	Time	Temp	^a Grain	^b Y _{SE} (%)	efficiency,
	(X ₁)	(X ₂)	(X ₂)	(min)	°C	(%)		^c Y _{SRE} (%)
				(X ₁)	(X ₂)	(X ₃)		
1	-1	0	-1	60	85	15	77.85	94.46
2	+1	0	-1	120	85	15	84.36	96.09
3	-1	0	+1	60	85	25	78.74	91.78
4	+1	0	+1	120	85	25	87.88	95.31
5	-1	-1	0	60	75	20	71.65	90.90
6	+1	-1	0	120	75	20	78.12	92.98
7	-1	+1	0	60	95	20	87.04	95.84
8	+1	+1	0	120	95	20	95.23	98.47
9	0	-1	-1	90	75	15	72.57	93.14
10	0	-1	+1	90	75	25	76.32	90.85
11	0	+1	-1	90	95	15	89.81	97.45
12	0	+1	+1	90	95	25	94.82	98.00
13	0	0	0	90	85	20	84.37	94.98
14	0	0	0	90	85	20	86.67	95.72
15	0	0	0	90	85	20	85.97	95.50

^a Grain (%) is percentage of grain loading.

^bY_{SE} is the starch conversion efficiency.

^cY_{SRE} is the sugar recovery efficiency.

453 Table 2. ANOVA of full model and reduced model for starch conversion efficiency.

Source	Sum of Squares	df	Mean Square	<i>F</i> Value	<i>p</i> -value
Model	743.22	9	82.58	62.75	0.0001
Residual	6.58	5	1.32		
Lack of Fit	3.80	3	1.27	0.91	0.5611
Pure Error	2.78	2	1.39		
Cor Total	749.80	14			
$R^2 = 0.9912$, adjusted $R^2 = 0.9754$					
<i><u>ANOVA for the regression of the reduced model</u></i>					
Model	738.31	5	147.66	115.68	<0.0001
Residual	11.49	9	1.28		
Lack of Fit	8.71	7	1.24	0.89	0.6208
Pure Error	2.78	2	1.39		
Cor Total	749.80	14			
$R^2 = 0.9847$, adjusted $R^2 = 0.9762$					

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Table 3. Regression coefficients and their significance in the quadratic model for starch conversion efficiency.

Term	Coefficient Estimate	Standard Error	Mean Square	<i>F</i> -Value	<i>P</i> -Value
Intercept	85.67	0.66	82.58	62.75	0.0001
X ₁	3.79	0.41	114.84	87.26	0.0002
X ₂	8.53	0.41	582.09	442.31	< 0.0001
X ₃	1.65	0.41	21.68	16.47	0.0097
X ₁ X ₂	0.43	0.57	0.74	0.56	0.4872
X ₁ X ₃	0.66	0.57	1.73	1.31	0.3036
X ₂ X ₃	0.31	0.57	0.40	0.30	0.6065
X ₁ ²	-1.92	0.60	13.56	10.30	0.0237
X ₂ ²	-0.74	0.60	2.04	1.55	0.2680
X ₃ ²	-1.55	0.60	8.83	6.71	0.0488

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460 Table 4. ANOVA of full and reduced regression equation model for sugar recovery efficiency.

Source	Sum of Squares	df	Mean Square	F-Value	p-value
Model	80.42	9	8.94	55.20	0.0002
Residual	0.81	5	0.16		
Lack of Fit	0.52	3	0.17	1.22	0.4806
Pure Error	0.29	2	0.14		
Cor Total	81.23	14			
$R^2 = 0.9900$, adjusted $R^2=0.9721$					

ANOVA for the regression of the reduced model

Model	78.89	5	15.78	60.82	<0.0001
Residual	2.34	9	0.26		
Lack of Fit	2.05	7	0.29	2.04	0.3675
Pure Error	0.29	2	0.14		
Cor Total	81.23	14			
$R^2 = 0.9713$, adjusted $R^2=0.9553$					

464 Table 5. Regression coefficients and their significance for quadratic model for sugar recovery
 465 efficiency.

Source	Coefficient	Standard	Mean	F-Value	p-value
	Estimate	Error	Square		
Intercept	95.40	0.23	8.94	55.20	0.0002
X ₁	1.23	0.14	12.17	75.19	0.0003
X ₂	2.74	0.14	59.94	370.24	< 0.0001
X ₃	-0.65	0.14	3.37	20.84	0.0060
X ₁ X ₂	0.14	0.20	0.076	0.47	0.5232
X ₁ X ₃	0.48	0.20	0.91	5.60	0.0642
X ₂ X ₃	0.71	0.20	2.01	12.45	0.0168
X ₁ ²	-0.65	0.21	1.56	9.66	0.0266
X ₂ ²	-0.20	0.21	0.15	0.94	0.3765
X ₃ ²	-0.34	0.21	0.43	2.63	0.1657

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472 Table 6. Optimized diffusion conditions.

Response	Time	Temp	Grain	flour	Starch	Sugar recovery
	(min)	(°C)	loading (%)		efficiency	efficiency (%)
					(%)	
1	120.00	95.00		10.00	86.42	98.33
2	115.75	95.00		15.00	92.49	98.38
3	114.82	95.00		20.00	95.6	98.43
4	115.52	95.00		25.00	95.78	98.49
5	120.00	95.00		30.00	93.01	98.56
Optimized	114.936	95.000		22.04	96.03	98.45
factors						

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Figure captions

Fig. 1. 3D response surface of starch conversion efficiency in relation to (a) temperature and grain loading with constant time; (b) temperature and time with constant grain loading; (c) time and grain and their corresponding 2D contour plot.

Fig. 2. 3D response surface of sugar recovery efficiency in relation to (a) temperature and grain loading; (b) temperature and time; (c) time and grain and their corresponding 2D contour plots.

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487 **a**

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Fig. 2. 3D response surface of sugar recovery efficiency in relation to (a) temperature and grain loading; (b) temperature and time; (c) time and grain and their corresponding 2D contour plots.

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