Comparative Study of Limit Dextrinase Potential of Three Sorghum Cultivars (Safrari, Madjeru, and S.35)

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ABSTRACT

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Limit dextrinase extracted from three sorghum cultivar malts (Safrari, S.35, and Madjeru) used for the production of *bili-bili*, a traditional beer in the northern part of Cameroon, was subjected to comparison using dextrin as a substrate. A four-factor Doehlert experimental design was used to build a model in order to optimize the impact of factors (temperature, pH, buffer concentration, and incubation time) involved in the extraction of free limit dextrinase from sorghum. The response surface methodology revealed that the action of those factors was different for each sorghum cultivar, with closer behavior between Safrari and S.35. Madjeru was revealed to be completely different. Optimizing the concerted actions of the factors for limit dextrinase specific activity gave the following combinations: for Safrari, 43°C, pH 4, 135 mM, and 300 min, with maximal specific activity of 3.10 U/μg; for Madjeru, 35°C, pH 5.77, 50 mM, and 60 min, with maximal specific activity of 1.51 U/μg; and for S.35, 50°C, pH 4, 191 mM, and 205 min, with maximal specific activity of 3.05 U/μg.

Keywords: Sorghum, Limit dextrinase, Modeling, Optimization

Sorghum is a native African cereal, very well adapted to the semiarid and soudano-Sahelian conditions of Africa. Like rice and barley, sorghum belongs to the family of Gramineae (2,31). The disponibility of sorghum and its high content in starch and protein should encourage local use. Its nourishing wort allows it to be one of best choices as a raw material in the brewing process (31). Although sorghum was used for centuries to brew traditional (opaque) beer in Africa, in recent years, the brewing of sorghum beer has been developed in breweries. These types of beers are different from European beers because they contain large quantities of materials that are not respectively solubilized and fermented during mashing and fermentation (2). Several works were published on sorghum, sorghum malt, and sorghum mashing technology (1,2,20,30,33,36). These publications focused on remarkable problems in mashing that are peculiar to the characteristics of sorghum grains and malt. Among the generally highlighted problems are insufficient enzymes, especially those hydrolyzing starch (1). Essays on the improvement of the brewing properties of sorghum were written for the malting and mashing processes with the aim of enabling the development of starch hydrolyzing enzymes (10,11,14,21,32) such as α-amylase, β-amylase, and limit dextrinase. The main role played by limit dextrinase on the α-1,6-glycosidic bond cleavage of starch has been recognized for years. Many studies were conducted to isolate and purify that enzyme from cereals and also to characterize its action on amylopectin and its degradation products. This was mainly done on barley (19,22,26,35) and other cereals such as maize (7), rice (41), and sorghum (18). The results of the study conducted on properties of three sorghum cultivars used for the production of *bili-bili* beverage in northern Cameroon revealed a high enzymatic potential of local *bili-bili* malt compared with the one produced in laboratory (30). The aim of the present work was therefore to extract limit dextrinase of malt of three sorghum cultivars (Madjeru, Safrari, and S.35) produced in Cameroon and compare its specific activity by modeling and optimization.

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EXPERIMENTAL

Biological Material

Sorghum cultivars (Safrari, Madjeru, and S.35) were obtained from IRAD, Maroua, Cameroon.

Chemicals

Dextrin from potato starch and Coomassie blue 98% were obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany). Sodium acetate buffer solution was obtained from SERVA Electrophoresis GmbH (Heidelberg, Germany). Potassium iodide, hydrochloric acid solution, ethanol 95%, and phosphoric acid 85% were from Fisher Scientific UK (Loughborough, U.K). Iodine was obtained from Thermo Fisher Scientific (Geel, Belgium). The grade filter papers Whatman No. 1 (Ø 90 mm) were obtained from Whatman, GE Healthcare UK Ltd.

Malting

Sorghum grains (200 g) (Safrari, Madjeru, and S.35 cultivars) were washed three times using 600 mL of distilled water to remove dirt and foreign bodies. The grains were steeped in 600 mL of distilled water for 48 h at 31°C in a Heraeus-type incubator (D-63450, Hanau, Germany) with two changes of water at intervals of 12 h before steep out. Germination was carried out for 72 h in the same Heraeus-type incubator at a temperature of 28.5°C with water (18 mL) sprinkled on the grains on a daily basis. The malt was then air dried at 28°C for 24 h using a ventilated Memmert-type incubator. The malt was rubbed off of its rootlets and afterward underwent grinding using Phinx Brilliant G250's manual grindstone. Finally, the obtained flour was sieved (Ø ≤ 0.5 mm) using manual sieving equipment (CSC Scientific, Fairfax, VA, U.S.A.) and stored in plastics sachets at 4°C until further use.

Enzymatic Extraction

The enzymatic extraction was made by modification of the technique of McCleary (29) (Megazyme International, Ireland). The modifications brought here concerned the buffer (sodium acetate) and the malt flour/volume of buffer ratio (1/10); no reducing agent was used during the extraction. After extraction, centrifugation was made at 6,000 × *g* for 30 min at 4°C.

Experimental Design, Modeling, Validation of the Model, and Optimization

Response surface methodology with a Doehlert design was used to carry out the experiments in order to model and optimize

the limit dextrinase extraction. The independent variables (factors) were the temperature (x_1), pH (x_2), buffer concentration (x_3), and incubation time (x_4). The intervals of these factors were: X_1, 30–50°C; X_2, 4–7; X_3, 50–200 mM, and X_4, 60–300 min. The response was the specific activity of limit dextrinase.

From the coded variables, many equations were used to transform them into real values to realize experiments in the laboratory. Those equations were as follows:

$$X_i = X_{0i} + x_i \times \Delta X_i \quad (1)$$

$$N = k^2 + k + k_0 \quad (2)$$

The four-factor Doehlert design gave a total of 25 experiments (with five replicates at the central point) as shown in Table I.

Mathematical models describing the relationships among the process-dependent variable and the independent variables in a second-order equation were developed (17). Design-based experimental data were matched according to the following second-order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum\sum_{i<j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

where Y was the response, x_i and x_j were the variables, β_0 was the constant, β_i was the coefficient of the linear terms, β_{ii} was the coefficient of the quadratic terms, and β_{ij} is the coefficient of the interaction terms.

The coefficients of the models were obtained using Minitab version 16 software (Minitab, Coventry, U.K.). This software had also given a statistical analysis (ANOVA) on the models, and the curves were plotted using Sigmaplot version 12.5 (Systat Software, San Jose, CA, U.S.A.).

The validation of the models was obtained by calculating average absolute deviation (AAD), the bias factor (B_f), and the accuracy factor (A_f) (6,34), which were expressed as follows:

$$\mathrm{AAD} = \frac{\left[\sum_{i=1}^{N}\left(\frac{\left|Y_{i,\exp} - Y_{i,cal}\right|}{Y_{i,\exp}}\right)\right]}{N} \quad (4)$$

$$B_f = 10^{\frac{1}{N}\sum_{i=1}^{N}\log\left(\frac{Y_{i,cal}}{Y_{i,\exp}}\right)} \quad (5)$$

$$A_{f1} = 10^{\frac{1}{N}\sum_{i=1}^{N}\left|\log\left(\frac{Y_{i,cal}}{Y_{i,\exp}}\right)\right|} \quad (6)$$

where $Y_{i,\exp}$ and $Y_{i,cal}$ were respectively experimental and calculated responses and N was the number of experiments used in the calculation.

Lastly, optimization was done using Mathcad 15.0 software (build 15.0.0.436 Parametric Technology, Needham, MA, U.S.A.).

Analysis

Moisture content. Moisture content of sorghum was determined on a ground sample by oven drying at 105°C for 24 h (3).

Analysis of total protein content. Total protein content was determined by a standard method (12) after sulfuric acid mineralization of samples in the presence of selenium catalyst, with a coefficient of conversion of nitrogen into protein of 6.25 (5).

Germinative capacity. Three lots of 200 grains of sorghum were immersed in 200 mL of hydrogen peroxide solution at 7.5

TABLE I
Doehlert Experimental Design: Coded Variables, Real Variables, and Responses[a]

	Coded variables				Real variables				Responses (specific activity of limit dextrinase [U/µg])								
									Safrari			Madjeru			S.35		
Number	x_1	x_2	x_3	x_4	X_1	X_2	X_3	X_4	Exp.	Cal.	Res.	Exp.	Cal.	Res.	Exp.	Cal.	Res.
1	1	0	0	0	50	5.50	125	180	1.254	1.182	0.072	0.510	0.540	0.031	1.171	1.102	0.070
2	–1	0	0	0	30	5.50	125	180	0.987	1.059	–0.072	0.668	0.638	–0.031	0.689	0.759	–0.070
3	0.5	0.866	0	0	45	7.00	125	180	0.821	0.832	–0.012	0.305	0.274	–0.031	0.814	0.917	–0.102
4	–0.5	–0.866	0	0	35	4.00	125	180	1.971	1.959	0.012	0.145	0.176	0.031	1.607	1.505	0.102
5	0.5	–0.866	0	0	45	4.00	125	180	1.942	2.011	–0.069	0.189	0.313	0.124	1.929	2.040	–0.111
6	–0.5	0.866	0	0	35	7.00	125	180	0.830	0.762	0.069	0.632	0.508	–0.124	1.219	1.108	0.111
7	0.5	0.289	0.816	0	45	6.00	200	180	1.107	0.972	0.134	0.766	0.731	–0.035	1.268	1.180	0.088
8	–0.5	–0.289	–0.816	0	35	5.00	50	180	1.577	1.712	–0.134	0.862	0.897	0.035	0.947	1.034	–0.088
9	0.5	–0.289	–0.816	0	45	5.00	50	180	1.714	1.781	–0.067	0.956	0.864	–0.092	0.711	0.725	–0.014
10	0	0.577	–0.816	0	40	6.50	50	180	1.223	1.146	0.077	0.816	0.894	0.078	0.623	0.593	0.029
11	–0.5	0.289	0.816	0	35	6.00	200	180	0.985	0.919	0.067	0.705	0.796	0.092	0.542	0.528	0.014
12	0	–0.577	0.816	0	40	4.50	200	180	1.458	1.534	–0.077	0.708	0.631	–0.078	1.298	1.327	–0.029
13	0.5	0.289	0.204	0.791	45	6.00	143.75	300	1.338	1.532	–0.195	0.771	0.806	0.035	0.799	0.855	–0.055
14	–0.5	–0.289	–0.204	–0.791	35	5.00	106.25	60	2.042	1.848	0.195	1.008	0.973	–0.035	0.911	0.855	0.055
15	0.5	–0.289	–0.204	–0.791	45	5.00	106.25	60	1.902	1.839	0.063	0.937	0.874	–0.063	1.053	0.997	0.056
16	0	0.577	–0.204	–0.791	40	6.50	106.25	60	1.765	1.899	–0.134	0.765	0.843	0.077	0.629	0.667	–0.038
17	0	0	0.612	–0.791	40	5.50	181.25	60	1.458	1.582	–0.124	0.686	0.707	0.021	0.680	0.753	–0.073
18	–0.5	0.289	0.204	0.791	35	6.00	143.75	300	1.338	1.401	–0.063	0.741	0.805	0.063	0.599	0.654	–0.056
19	0	–0.577	0.204	0.791	40	4.50	143.75	300	2.844	2.710	0.134	0.655	0.578	–0.077	1.294	1.256	0.038
20	0	0	–0.612	0.791	40	5.50	68.75	300	2.130	2.006	0.124	0.726	0.706	–0.021	0.680	0.607	0.073
21	0	0	0	0	40	5.50	125	180	1.434	1.523	–0.089	0.802	0.773	–0.029	0.780	0.767	0.012
22	0	0	0	0	40	5.50	125	180	1.465	1.523	–0.058	0.780	0.773	–0.007	0.788	0.767	0.021
23	0	0	0	0	40	5.50	125	180	1.503	1.523	–0.020	0.788	0.773	–0.016	0.769	0.767	0.001
24	0	0	0	0	40	5.50	125	180	1.669	1.523	0.146	0.745	0.773	0.028	0.764	0.767	–0.003
25	0	0	0	0	40	5.50	125	180	1.543	1.523	0.021	0.749	0.773	0.024	0.736	0.767	–0.031

[a] Exp. = experimental results; Cal. = calculated (theoretical) results obtained from the models; and Res. = residue coming from the difference between experimental and calculated values.

g/L at room temperature (25°C) for 2 days. After this time, the hydrogen peroxide solution was replaced by a fresh solution (at the same concentration) for an additional 24 h, after which the solution was poured. The germinated grains were counted, and the result was expressed as a percentage (3).

Germinative energy. Germinative energy was determined by placing 100 kernels of sorghum on two layers of Whatman No. 1 filter paper, in a 9.0 cm diameter Petri dish, and adding 4.0 mL of purified water. Samples were controlled at 25°C and 90% relative humidity in a germination chamber. Germinated kernels were removed after 24 and 48 h, and a final count was made at 72 h (3).

Water sensitivity. Water sensitivity is determined exactly as described for germination energy, except that 8.0 mL of purified water is added to each Petri dish (3). The water sensitivity value is the numerical difference between the 4 and 8 mL tests.

Assay of limit dextrinase. The limit dextrinase assay was a modification of the literature technique (16) using for that purpose dextrin as a substrate. In a containing test tube, 0.5 mL of dextrin (1%), 0.3 mL of acetate buffer, and 0.2 mL of distilled water were introduced. The mixture was heated at 70°C for 10 min and cooled to approximately 40°C in a water bath (Memmert, type F-Nr 760). Enzymatic extract (0.1 mL) was added to this mixture and maintained at 40°C for 10 min (Memmert, type F-Nr 760). The reaction was stopped by addition of 1 mL of hydrochloric acid 1M and cooled quickly at the ambient temperature (25°C). A volume of 0.1 mL of this mixture was taken, to which 15 mL of distilled water and 1 mL of iodized solution (0.2% of iodine in 2% of KI) were added. A blank was prepared by adding 0.1 mL of enzymatic extract after the reaction was stopped by addition of the hydrochloric acid. Absorbance was measured at 620 nm. The total activity was then obtained in U/mL. The specific activity was obtained in U/μg by integrating the protein content.

RESULTS AND DISCUSSION

Characterization of Sorghum Grains

The results of the characterization of sorghum grains with the aim of estimating their viabilities are presented in Table II. According to that table, the viability of grains was at the standard level (3) and the water sensitivity was similar to the literature (30).

Modeling

The limit dextrinase was extracted using a four-factor Doehlert experimental design (25 experiments), and in each case the specific activity was determined (Table I). This permitted us to obtain models via matrix calculation using Minitab version 16 software. The models were as follows:

$$\begin{aligned} Y_{Saf}(x_1,x_2,x_3,x_4) = {} & 1.5228 + 0.0615x_1 - 0.6860x_2 \\ & - 0.2473x_3 + 0.0765x_4 - 0.402x_1^2 \\ & - 0.042x_2^2 - 0.157x_3^2 + 0.647x_4^2 \\ & + 0.012x_1x_2 - 0.013x_1x_3 + 0.088x_1x_4 \\ & + 0.135x_2x_3 - 0.851x_2x_4 + 0.154x_3x_4 \end{aligned} \quad (7)$$

$$\begin{aligned} Y_{Mad}(x_1,x_2,x_3,x_4) = {} & 0.7728 - 0.0485x_1 + 0.0846x_2 \\ & - 0.1015x_3 - 0.0795x_4 - 0.1838x_1^2 \\ & - 0.5455x_2^2 + 0.2267x_3^2 + 0.1217x_4^2 \\ & - 0.214x_1x_2 + 0.056x_1x_3 + 0.128x_1x_4 \\ & - 0.042x_2x_3 + 0.105x_2x_4 + 0.471x_3x_4 \end{aligned} \quad (8)$$

$$\begin{aligned} Y_{S.35}(x_1,x_2,x_3,x_4) = {} & 0.7674 + 0.1713x_1 - 0.4390x_2 \\ & + 0.1394x_3 + 0.0158x_4 + 0.1626x_1^2 \\ & + 0.7790x_2^2 - 0.0393x_3^2 - 0.0792x_4^2 \\ & - 0.420x_1x_2 + 0.738x_1x_3 - 0.000x_1x_4 \\ & + 0.085x_2x_3 + 0.026x_2x_4 + 0.048x_3x_4 \end{aligned} \quad (9)$$

where $Y_{Saf}(x_1,x_2,x_3,x_4)$ represents the mathematical model for Safrari, $Y_{Mad}(x_1,x_2,x_3,x_4)$ the mathematical model for Madjeru, and $Y_{S.35}(x_1,x_2,x_3,x_4)$ the mathematical model for S.35; x_1 is temperature (°C), x_2 is pH, x_3 is buffer concentration (mM), and x_4 is time (min).

The mathematical models were polynomials having four variables with R^2 equal to 0.947, 0.914, and 0.964, respectively, for Safrari, Madjeru, and S.35. These coefficients, coupled to AAD values of 0.06, 0.10, and 0.06, respectively, for Safrari, Madjeru, and S.35, allowed the validation of the models for specific activities of limit dextrinase. In addition, a bias factor of 1.00, 1.01, and 1.00 coupled to exactitude factors of 1.06, 1.10, and 1.06 for Safrari, Madjeru, and S.35, respectively, also allowed for validation of the models according to the method described (34). The factors of the models were of first degree (x_1, x_2, x_3, and x_4), of second degree (x_1^2, x_2^2, x_3^2, and x_4^2), and of interaction (x_1x_2, x_1x_3, x_1x_4, x_2x_3, x_2x_4, and x_3x_4) form. They were statistically significant or not if the probability (P) was ≤0.05 or ≥0.05, respectively (Table III).

The factor x_1, corresponding to temperature as the sole factor, had no significant impact on the limit dextrinase activity of Safrari and Madjeru cultivars, with a respective probability of 0.414 and 0.283. However, it had significant impact on the limit dextrinase activity of S.35 with a probability of 0.003. Its contribution was 1.71, 2.00, and 5.45% (Table III), respectively, for Safrari, Madjeru, and S.35. Concerning S.35, that factor contributed to increase the limit dextrinase activity. In fact, the observation was similar to the literature, where an increase of limit dextrinase activity with temperature was noted (24,27,35). The temperature factor in the second degree (x_1^2) had significant impact on the reduction of limit dextrinase activity of Safrari and Madjeru, with respective probabilities of 0.014 corresponding to 11.25% contribution and 0.044 corresponding to 7.62% contribution. It also had no significant impact on the limit dextrinase activity of S.35, with a probability of 0.081, corresponding to 5.17% contribution. This impact on the reduction of limit dextrinase activity was explained by the fact that enzyme proteins are precisely folded polypeptide chains, held together by relatively weak molecular forces. The folded structure determined the integrity of the catalytic site within the enzyme, and this was easily disrupted (denatured) by energy changes in the enzyme's environment by higher temperature (23).

The factor x_2 (pH), as the sole factor, had no significant impact on the limit dextrinase activity of Madjeru cultivar, with a probability of 0.076 and 3.52% contribution. But it had significant impact on the reduction of limit dextrinase activity of Safrari and S.35 with a probability of 0.000 for both and with a contribution

TABLE II
Characterization of Cultivars Safrari, Madjeru, and S.35[a]

Characteristics	Safrari	Madjeru	S.35	Norms (3)
Germination energy, 4 mL (%)	99	98	94	60–99
Germination energy, 8 mL (%)	97	97	90	40–99
Water sensitivity (%)	2	1	4	ND
Germinative capacity (%)	98	99.5	98	92–100
Moisture (%)	10.8 ± 0.5	10.4 ± 0.3	9.96 ± 0.7	≤13

[a] ND = not determined.

of 19.21 and 13.97% (Table III), respectively, for Safrari and S.35. That impact on the reduction of the limit dextrinase activity was observed for *Pokko* and barley malt extract after reaching a pH of 4.5 (28,35). It could be explained by the fact that pH affects the solubility of limit dextrinase and the detailed structure of its active site, and by then impacted on the activity of the enzyme (25,42). If the shape or polarity of the active site changes, that will alter its effectiveness as a catalyst. The pH factor in the second degree (x_2^2) had significant impact on the reduction of limit dextrinase activity of Safrari and Madjeru, with both probabilities of 0.000. It also had no significant impact on the limit dextrinase activity of S.35, with a probability of 0.0764 corresponding to 5.17% contribution.

The factor x_3 (buffer concentration) had a significant impact on the limit dextrinase activity of Safrari, Madjeru, and S.35 cultivars, with a probability of 0.006, 0.039, and 0.011, respectively. The contributions were 6.94, 4.22, and 4.44%, respectively, for Safrari, Madjeru, and S.35 (Table III). This could be explained by the fact that, the larger the difference between the pI and the pH of interest, the greater the net charge on the protein. This implies that the ability of ionic compounds to cause either stabilization (the case of S.35) or destabilization (the case of Safrari and Madjeru) of the protein by binding to specific residues should increase as the difference between pI and pH becomes greater (4). Literature has suggested that any proton transfer event associated with allosteric or catalytic enzyme sites was associated with a partial denaturation that was cooperative within considerable but localized regions of the protein domain (8,39). The buffer concentration factor in the second degree (x_3^2) had no significant impact on the limit dextrinase activity of Safrari and S.35, with respective probabilities of 0.243 corresponding to 4.41% contribution and 0.628 corresponding to 1.28% contribution. It also had significant impact on the increasing limit dextrinase activity of Madjeru, with a probability of 0.013 corresponding to 9.41% contribution. In this system, the higher buffer concentration stabilized the enzyme and contributed to increase the limit dextrinase activity.

The factor x_4 (incubation time) had no significant impact on the limit dextrinase activity of Safrari, Madjeru, and S.35 cultivars, with probabilities of 0.313, 0.093, and 0.732, respectively. The contributions were 2.15, 3.30, and 0.51%, respectively, for Safrari, Madjeru, and S.35 (Table III). Most enzyme activities were relatively constant with varying incubation times (37). The incubation time factor in the second degree (x_4^2) had no significant impact on the limit dextrinase activity of Madjeru and S.35, with respective probabilities of 0.116 corresponding to 5.07% contribution and 0.311 corresponding to 2.53% contribution. It had significant impact on the increasing limit dextrinase activity of Safrari, with a probability of 0.000 corresponding to 18.12% contribution. In this case, the better expression of Safrari limit dextrinase was observed for a longer incubation time.

The interaction x_1x_2 (temperature/pH) had a significant impact on the reduction of the limit dextrinase activity of S.35, as shown in Figure 1, with a probability of 0.005 and a contribution of 13.34%. That impact was not significant for Safrari and Madjeru, because the respective probabilities were 0.957 and 0.081 with respective contributions of 0.29% and 8.91% (Table III). For S.35, at lower pH (4–4.5), the decrease of limit dextrinase activity was significant but lower whatever the temperature, and that reduction became higher at pH higher than 4.5. Increasing the temperature increases the energy of the bonds and atoms in the protein, to the

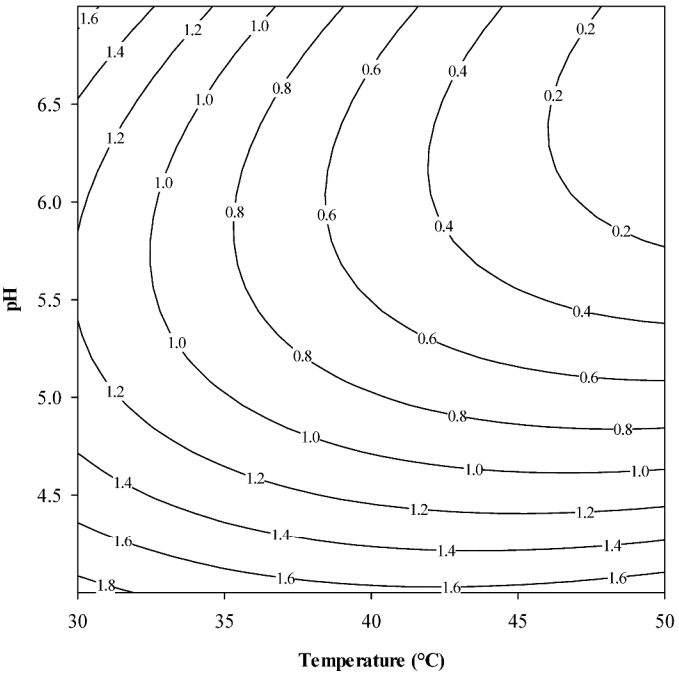

Fig. 1. Impact of temperature/pH interaction on the activity of limit dextrinase (incubation time, 60 min; buffer concentration, 50 mM) for the cultivar S.35.

TABLE III
Estimated Coefficients Impact and Contributions to the Limit Dextrinase Activity[a]

	Safrari			Madjeru			S.35		
Source	**Coefficients**	***P***	**Contribution (%)**	**Coefficients**	***P***	**Contribution (%)**	**Coefficients**	***P***	**Contribution (%)**
A: x_1	0.0615	0.414	1.71	–0.0485	0.283	2.00	0.1713	**0.003**	5.45
B: x_2	–0.0594	**0.000**	19.21	0.0732	0.076	3.52	–0.3802	**0.000**	13.97
C: x_3	–0.2018	**0.006**	6.94	–0.0828	**0.039**	4.22	0.1137	**0.011**	4.44
D: x_4	0.0605	0.313	2.15	–0.0629	0.092	3.30	0.0125	0.732	0.51
AA	–0.402	**0.014**	11.25	–0.1838	**0.044**	7.62	0.1626	0.081	5.17
AB	0.010	0.952	0.29	–0.1855	0.081	8.91	–0.364	**0.005**	13.34
AC	–0.011	0.950	0.37	0.045	0.662	2.37	0.602	**0.000**	23.47
AD	0.069	0.694	2.45	0.101	0.343	5.29	–0.000	0.998	0.03
BB	–0.031	0.764	1.16	–0.4091	**0.000**	22.63	0.5842	**0.000**	24.79
BC	0.095	0.532	3.77	–0.0299	0.739	1.76	0.0601	0.525	2.71
BD	–0.583	**0.003**	23.85	0.0716	0.434	4.34	0.0176	0.852	0.82
CC	–0.1048	0.243	4.41	0.1509	**0.013**	9.41	–0.0262	0.628	1.28
CD	0.099	0.494	4.31	0.0716	**0.004**	19.56	0.0308	0.730	1.50
DD	0.4046	**0.000**	18.12	0.0762	0.116	5.07	–0.0496	0.310	2.53
			100.00			100.00			100.00

[a] Bolded *P* values are significant.

point at which there was enough energy to overcome the force of the intermolecular reactions, resulting in them breaking. Disruption of the interactions in any case led to some of the protein losing its ability to be held in a certain shape, which then reduced its catalytic activity (because catalytic activity relied on the shape). The loss of activity was proportional to the extent of the disruptions, which were in turn proportional to the extent of the change in pH or temperature (9).

The interaction x_1x_3 (temperature/buffer concentration) had a significant impact on the increasing of the limit dextrinase activity of S.35, as shown in Figure 2, with a probability of 0.000 and a contribution of 23.47%. It was not significant for Safrari and Madjeru because their respective probabilities were 0.951 and 0.654 with contributions of 0.37 and 2.37%. This augmentation of limit dextrinase activity was explained by the positive influence of increases in ionic strength (which was attributable to the increase of buffer concentration), which stabilized the enzyme by increasing its thermostability so that whatever temperature was used, activity increased. The same observation was made in the literature on alkaline phosphatase activity in *Escherichia coli* (40).

The interaction x_2x_4 (pH/incubation time) had no significant impact on the limit dextrinase activity of Madjeru and S.35 cultivars (Table III) with respective probabilities of 0.434 and 0.852. However, its impact is significant with Safrari (Fig. 3), with a probability of 0.003, and it tends to slow down the enzyme activity here, given the negative coefficient. This can be explained by the fact that if the pH of the buffer is not at the optimum pH range of the enzyme for a relatively long time, the enzyme activity could decrease due to instability and even loss of activity by the enzyme out of its optimum pH range (38).

The interactions x_1x_4 and x_2x_3 (temperature/incubation time and pH/buffer concentration, respectively) had no significant impact on the limit dextrinase activity of Safrari, Madjeru, and S.35 cultivars, with respective probabilities of 0.694, 0.343, 0.998 and 0.532, 0.739, 0.525. The contributions were 2.45, 5.29, and 0.03% and 3.77, 1.76, and 2.71%, respectively, for Safrari, Madjeru, and S.35 (Table III).

The interaction x_3x_4 (buffer concentration/incubation time) had a significant impact on increasing the limit dextrinase activity of Madjeru as shown in Figure 4, with a probability of 0.004 and a contribution of 19.56%. It was not significant for Safrari and S.35, and the respective probabilities were 0.494 and 0.730 with contributions of 4.31 and 1.50%. Because the buffer concentration contributed to stabilize the limit dextrinase, one observed that as incubation time increased, enzyme activity increased too.

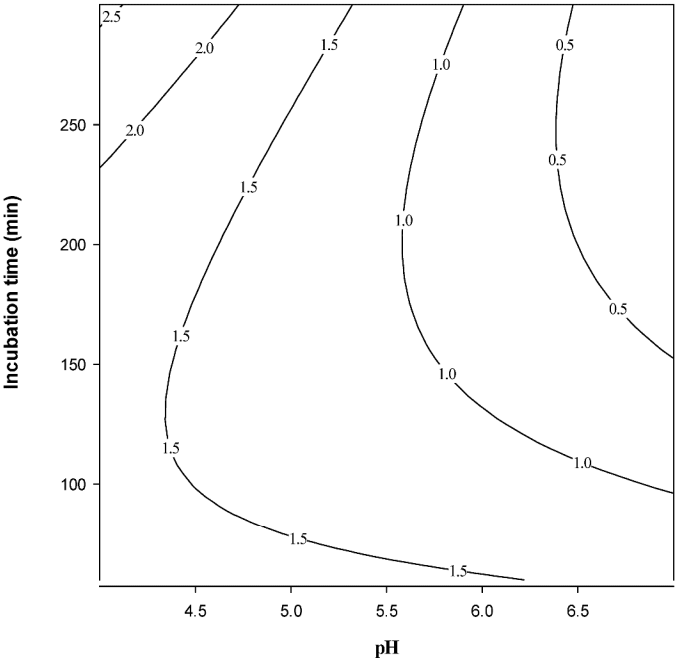

Fig. 3. Impact of incubation time/pH interaction on the activity of limit dextrinase (temperature, 30°C; buffer concentration, 50 mM) for the cultivar Safrari.

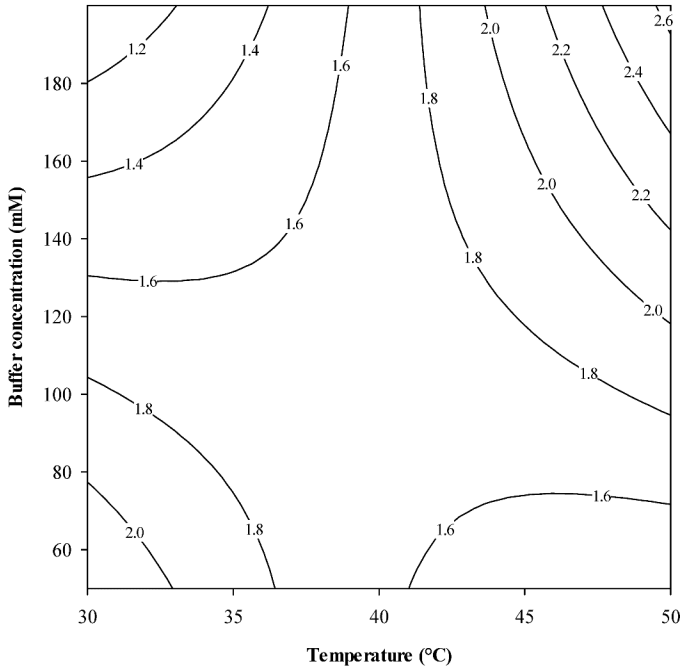

Fig. 2. Impact of temperature/buffer concentration interaction on the activity of limit dextrinase (incubation time, 60 min; pH 4) for the cultivar S.35.

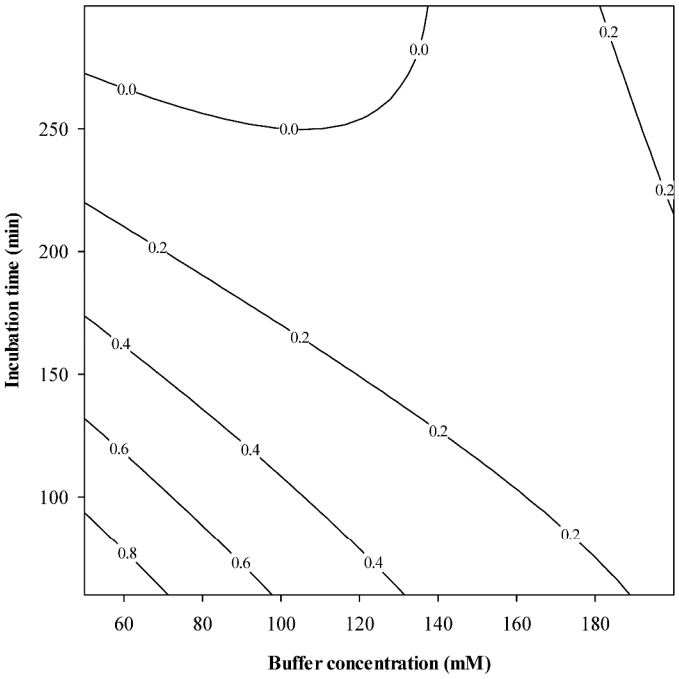

Fig. 4. Impact of buffer concentration/incubation time interaction on the activity of limit dextrinase (temperature, 30°C; pH 4) for the cultivar Madjeru.

TABLE IV
Optimal Extraction Conditions for Limit Dextrinase Activity of Safrari, Madjeru, and S.35 Cultivars

	Safrari		Madjeru		S.35	
Parameter	**Coded**	**Real**	**Coded**	**Real**	**Coded**	**Real**
Temperature (°C)	0.307	43	–0.471	35	0.992	50
pH	–0.865	4.00	0.158	5.77	–0.866	4.00
Buffer concentration (mM)	0.108	135	–0.816	50	0.816	200
Incubation time (min)	0.791	300	–0.791	60	0.199	210
Specific activity (U/μg)	3.10		1.51		3.05	

This observation was also noticed for α-amylase in the literature (13).

Optimization

After modeling and understanding the impact of the factors on limit dextrinase activity, optimization was done to obtain the optimal conditions for limit dextrinase extraction of each sorghum cultivar. The summary appears in Table IV as follows.

According to Table IV, the cultivar Safrari had the stronger limit dextrinase activity, followed by S.35 and Madjeru. Also, the conclusion was that the characteristics of an enzyme depend on the cultivar (29). The optimal temperature conditions obtained were similar to the one for cereal flour (43°C) in general (29) and rice (40°C) in particular (15). The optimal pHs of 4 and 5.7 obtained for Safrari and S.35, respectively, were in agreement with the results obtained from malted barley (19,26). Incubation times of 300 and 210 min for Safrari and S.35, respectively, were obtained.

CONCLUSIONS

This work shows that the factors of temperature, pH, buffer concentration, and incubation time are of different importance on limit dextrinase extraction and activation, and depend on the cultivar used. The modeling permitted understanding the action of each factor and the interactions in the extraction process. By that way, factors implemented for limit dextrinase extraction and optimization are each statistically significant in at least one model or another, apart from the case of the factor incubation time, which was statistically insignificant in all three models. After optimizing the extraction conditions, higher temperature, lower pH, higher buffer concentration, and longer incubation time were found to be best for most cases (Safrari and S.35). That optimization also shows that Safrari and S.35 have closer optima of limit dextrinase activity, with Safrari the highest and Madjeru the lowest.

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LITERATURE CITED

1. Agu, R. C., and Palmer, G. H. The effect of temperature on the modification of sorghum and barley during malting. *Process Biochem.* 32:501-507, 1997.
2. Agu, R. C., and Palmer, G. H. A reassessment of sorghum for lager-beer brewing. *Bioresource Technol.* 66:253-261, 1998.
3. Analytica-EBC. *European Brewery Convention*. Fachverlag Hans Carl, Nürnberg, Germany, 1998.
4. Antosiewiez, J., McCammonb, J. A., and Gilson, M. K. Prediction of pH dependent properties of proteins. *J. Mol. Biol.* 238:415-436, 1994.
5. AOAC. *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington, DC, 1980.
6. Baranyi, J., Pin, C., and Ross, T. Validating and comparing predictive models. *Int. J. Food Microbiol.* 48:159-166, 1999.
7. Beatty, M. K., Rahman, A., Cao, H., Woodman, W., Lee, M., Myers, A. M., and James, M. G. Purification and molecular genetic characterization of ZPU1, a pullulanase-type starch-debranching enzyme from maize. *Plant Physiol.* 119:255-266, 1999.
8. Belrhali, H., Nollert, P., Royant, A., Menzel, C., Rosenbush, J. P., Landau, E. M., and Pebay-Peyroula, E. Protein, lipid and water organization in bacteriorhodopsin crystals: A molecular view of the purple membrane at 1.9 Å resolution. *Structure* 7:909-917, 1999.
9. Bennett, T. P., and Frieden, E. *Modern Topics in Biochemistry*. Macmillan, London, 1969.
10. Desobgo, Z. S. C., Nso, E. J., and Tenin, D. Optimisation of the action of commercial mashing enzymes on wort extracts and free amino nitrogen of the Safrari sorghum cultivar. *Tech. Q. Master Brewers Assoc. Am.* 48:77-86, 2011.
11. Desobgo, Z. S. C., Nso, E. J., and Tenin, D. The response surface methodology as a reliable tool for evaluating the need of commercial mashing enzymes for alleviating the levels of reducing sugars of worts of malted sorghum: Case of the Safrari cultivar. *J. Brew. Distill.* 2:5-15, 2011.
12. Devani, M. B., Shishoo, C. J., Shah, S. A., and Suhagia, B. N. Microchemical methods: Spectrophotometric methods for microdetermination of nitrogen in Kjeldahl digest. *J. Assoc. Anal. Chem.* 72:953-956, 1989.
13. El-Safey, E. M., and Ammar, M. S. Purification and characterization of alpha-amylase isolated from *Aspergillus flavus* var. *columnaris*. *Ass. Univ. Bull. Environ. Res.* 7:94-100, 2004.
14. Ezeogu, L. I., and Okolo, B. N. Effects of air rest period on the mobilization of sorghum reserve proteins. *J. Inst. Brew.* 101:463-468, 1995.
15. Furegon, L., Peruffo, A. D. B., and Curion, A. Immobilization of rice limit dextrinase on γ-alumina beads and its possible use in starch processing. *Process Biochem.* 32:113-120, 1997.
16. Fuwa, H. A new method for microdetermination of amylase activity by the use of amylose as the substrate. *J. Biochem.* 41:583-603, 1954.
17. Giovanni, M. Response surface methodology and product optimization. *J. Food Technol.* 37:41-45, 1983.
18. Hardie, D. G., Manners, D. J., and Yellowlees, D. The limit dextrinase from malted sorghum (*Sorghum vulgare*). *Carbohydr. Res.* 50:75-85, 1976.
19. Heisner, C. B., and Bamforth, C. W. Thioredoxin in barley: Could it have a role in releasing limit dextrinase in brewery mashes? *J. Inst. Brew.* 114:122-126, 2008.
20. Igyor, M. A., Ogbonna, A. C., and Palmer, G. H. Effect of malting temperature and mashing methods on sorghum wort composition and beer flavor. *Process Biochem.* 36:1039-1044, 2001.
21. Igyor, M. A., Ogbonna, A. C., and Palmer, G. H. Effect of malting temperature and time on enzyme development and sorghum wort properties. *J. Inst. Brew.* 104:101-104, 1998.
22. Kristensen, M., Planchot, V., Abe, J., and Svenson, B. Large-scale purification and characterization of barley limit dextrinase, a member of the α-amylase structural family. *Cereal Chem.* 75:473-479, 1998.
23. Law, B. A. The nature of enzymes and their action in foods. In: *Enzymes in Food Technology*. R. J. Whitehurst and B. A. Law, eds. Sheffield Academic Press, Sheffield, U.K., pp. 1-18, 2002.
24. Lee, W. J., and Pyler, R. E. Barley malt limit dextrinase: Varietal, environmental, and malting effects. *J. Am. Soc. Brew. Chem.* 42:11-17, 1984.

25. Longstaff, M. A., and Bryce, J. H. Development of limit dextrinase in germinated barley (*Hordeum vulgare* L.) (Evidence of proteolytic activation). *Plant Physiol.* 101:881-889, 1993.
26. MacGregor, A. W., Macri, L. J., Schoeder, S. W., and Bazin, S. L. Limit dextrinase from malted barley: Extraction, purification, and characterization. *Cereal Chem.* 71:610-617, 1994.
27. Manners, D. J., and Rowe, K. L. Studies of carbohydrate-metabolizing enzymes Part XXV. The debranching enzyme system in germinated barley. *J. Inst. Brew.* 77:358-365, 1971.
28. McCafferty, C. A., Jenkinson, H. R., Brosnan, J. M., and Bryce, J. H. Limit dextrinase—Does its malt activity relate to its activity during brewing? *J. Inst. Brew.* 110:284-296, 2004.
29. McCleary, B. V. Measurement of the content of limit dextrinase in cereal flours. *Carbohydr. Res.* 227:257-268, 1992.
30. Nso, E. J., Ajebesone, P. E., Mbofung, C. M., and Palmer, G. H. Properties of three sorghum cultivars used for the production of *bilibili* beverage in northern Cameroon. *J. Inst. Brew.* 109:245-250, 2003.
31. Odibo, F. J. C., Nwankwo, L. N., and Agu, R. C. Production of malt extract and beer from Nigerian sorghum varieties. *Process Biochem.* 37:851-855, 2002.
32. Okolo, B. N., and Ezeogu, L. I. Enhancement of amylolytic potential of sorghum malts by alkaline steep treatment. *J. Inst. Brew.* 102:79-85, 1996.
33. Owuama, C. I. Brewing beer with sorghum. *J. Inst. Brew.* 105:23-34, 1999.
34. Ross, T. Indices for performance evaluation of predictive models in food microbiology. *J. Appl. Bacteriol.* 81:501-508, 1996.
35. Stenholm, K., and Home, S. A new approach to limit dextrinase and its role in mashing. *J. Inst. Brew.* 105:205-210, 1999.
36. Taylor, J. R. N., Schober, J. T., and Bean, S. R. Novel food and non-food uses for sorghum and millets. *J. Cereal Sci.* 44:252-271, 2006.
37. Verchot, V. L., and Borelli, T. Application of para-nitrophenol (pNP) enzyme assays in degraded tropical soils. *Soil Biol. Biochem.* 37:625-633, 2005.
38. Vidyalakshmi, R., Paranthaman, R., and Indhumathi, J. Amylase production on submerged fermentation by *Bacillus* spp. *World J. Chem.* 4:89-91, 2009.
39. Williams, R. J. P. The problem of proton transfer in membranes. *Theor. Biol.* 219:389-396, 2002.
40. Wilson, I. B., Dayan, J., and Cyr, K. Some properties of alkaline phosphatase from *Escherichia coli*. *J. Biol. Chem.* 239:4182-4185, 1964.
41. Yamasaki, Y., Nakashima, S., and Konno, H. Pullulanase from rice endosperm. *Acta Biochim. Pol.* 55:507-510, 2008.
42. Zhang, N., and Jones, B. L. Characterization of germinated barley endoproteolytic enzymes by two-dimensional gel electrophoresis. *J. Cereal Sci.* 21:145-153, 1995.