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Full Length Research Paper

Use of the response surface methodology for optimizing the action of mashing enzymes on wort reducing sugars of the *Madjeru* sorghum cultivar

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A three factor Doehlert design was used to develop a statistical model to optimize the action of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers protease) on reducing sugars content of the worts of unmalted and malted *Madjeru* sorghum. The response surface methodology revealed that increasing amounts of Hitempase considerably increased reducing sugars content during mashing of unmalted and malted *Madjeru* sorghum grist to about 105.39 g/L and 132.25 g/L respectively. The milling process contributed to about 22 g/L and 54 g/L for the unmalted and malted mash types respectively. Increasing amounts of Bioglucanase was virtually insignificant, while for Brewers protease, reducing sugar yields rather decreased to nil for both the unmalted and malted mash types. Optimization of the concerted actions of the three enzymes for reducing sugars content of unmalted *Madjeru* sorghum mash gave a combination of 1995 U, 89.31 BGU and 28.86 mg for Hitempase, Bioglucanase and Brewers Protease respectively. This gave a maximal reducing sugars content of 108.78 g/L. This combination was 3000 U, 0 BGU and 49.69 mg for malted *Madjeru* sorghum mash, giving a maximal reducing sugars yield of 153.15 g/L.

Key words: Response surface methodology, optimization, mashing enzymes, *Madjeru*, reducing sugars.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is an important tropical cereal-bearing monocotyledonous plant found in the semi-arid areas of the world. It is a vital calorie-based food component in humane nutrition in some parts of Africa (Taylor, 2004).

Besides this fundamental function, sorghum is used in the production of traditional opaque beer and non-alcoholic drinks in developing countries and, until recently, in the production of industrial beer (Palmer, 1989; Taylor and Dewar, 2001). The low synthesis and development of principal hydrolytic enzymes during malting of this cereal is however a limiting factor for easy mashing of its malts as compared to barley malt (EtokAkpan and Palmer, 1990; EtokAkpan, 1992). This was ascribed to the malting procedures and varietal types of sorghum used. Work on some popular sorghum cultivars of Northern Cameroon used in brewing the traditional beer *Bili-Bili* confirmed that the profile of hydrolytic enzymes and the levels of fermentable sugars during mashing were indeed cultivar-dependent. The *Madjeru* sorghum cultivar was shown to be richer in starch than the *Safrari* and *S.35* cultivars, but had poorer hydrolytic enzymes and fermentable sugar potentials (Nso et al., 2003; 2006).

The use of commercial enzymes when mashing with sorghum in order to obtain better wort specifications for

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Table 1. Characteristics of commercial enzyme preparations used in mashing.

	Organism of origin	Activity	Description	Optimum temperature	Optimum pH	Recommended application level in adjuncts	Form
Hitempase 2XL	*Bacillus licheniformis*	4416.29 ± 19.34 U/ml	α-amylase	60 – 95°C	4 – 8	60 U/g	Solution
BioglucanaseTX	*Trichodermareesei*	750 BG U/ml	β-glucanase	60°C	4.5 – 6.5	0.01 et 0.025 % (v/w)	Solution
Brewers Protease	*Bacillus amyloliquefaciens*	1842.2 ± 1.8 mg FAN/min/mL	Protease	45 – 50°C (denatured at 85°C)	6.5 – 7.5	0.4 – 2 g/Kg	Solution

Hitempase 2XL and Bioglucanase TX were obtained from Kerry Bioscience; Kilnagleary, Carrigaline, Co. Cork, Ireland. Brewers Protease was obtained from DSM Food Specialities, Cedex France.

beer brewing has however become common practice (Bajomo and Young, 1992, Agu and Palmer, 1998; Goode et al., 2002; 2003; Goode and Arendt, 2003,).

It is not however clear how far mashing enzyme supplements in *Madjeru* sorghum cultivar mashes could help alleviate the levels of fermentable sugars in its worts. In this work, the action of three principal commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers Protease) on the reducing sugars content of worts from the *Madjeru* sorghum cultivar (unmalted and malted) was modeled and optimized using the response surface methodology (RSM).

MATERIALS AND METHODS

Enzymes

The characteristics of the commercial enzymes used (Hitempase 2XL, a thermo stable α-amylase from *Baccillus licheniformis,* Brewers Protease from *Bacillus amyloliquefaciens* and Bioglucanase TX, from an enzymatic composition of β-glucanase and hemicellulases from *Trichoderma reesei*) and sources are presented in Table 1.

Sorghum cultivar

The *Madjeru* sorghum cultivar was obtained from the Institute of Research and Agronomic Development (IRAD) Maroua, Cameroon.

Modeling

Modeling was carried out as previously described (Desobgo et al., 2010). A Doehlert matrix design of 3 factors representing Hitempase 2XL (X_1), Bioglucanase TX (X_2) and Brewers Protease (X_3) at ranges of [0-3000 U], [0-937.5 BGU] and [0-100 mg] respectively, was used. The transformed matrix of coded variables to an experimental matrix and the desired response (reducing sugars) are shown in Table 2. Mathematical models describing the relationships among the process dependent variable and the independent variables in a second-order equation were developed (Giovanni, 1983).

Design-based experimental data were matched according to the following second-order polynomial Equation 1.

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum\sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

Where, I and j, are linear and quadratic coefficients respectively, while 'β', the regression coefficient, k the number of factors studied and optimized in the experiment and 'ε' is random error. The coefficients of the models and the models were obtained using the Systat version 12 software (Systat Software, Inc., San Jose, USA). This software also gives a statistical analysis on the model. Lastly, the curves were plotted using Sigmaplot version11 build 11.0.0.77 software (WPCubed, GmbH, Germany).

Validation of models

The quality of fit of the second order equations was expressed by the coefficient of determination R^2. The models were validated using two differents methods. The first method was the absolute average deviation (AAD) method (Bas and Boyac, 2007), while the second method consisted in applying the bias factor and accuracy factor (Ross, 1996; Baranyi et al., 1999).

Malting

One kilogram of *Madjeru* sorghum cultivar grains was washed three times using 3 L of distilled water to remove dirt and foreign bodies. The grains were steeped in 3 L of distilled water for 48 h at room temperature (≈25°C) with 3 changes of water at intervals of 12 h before steep out. Germination was carried out for 4 days in a Heraeus type oven (D-63450 Hanau, Germany) at a temperature of 25°C with water sprinkled on the grains on daily basis. The malt was then air dried at 40°C for 4 days using a CKA 2000

Table 2. Matrices of Doehlert coded and transformed experimental values.

Coded values			Transformed experimental values			Reducing sugars content(g/L)(*Madjeru* wort cultivar)					
Hit	Bio	Brew Prot	Hit (U)	Bio (BGU)	Brew Prot (mg)	Unmalted			Malted		
X_1	X_2	X_3	X_1	X_2	X_3	Exp[a]	Theo[b]	Res[c]	Exp	Theo	Res
0.000	0.000	0.000	1500	468.75	50	100.81	102.57	-1.76	124.00	123.08	0.92
1.000	0.000	0.000	3000	468.75	50	85.55	78.61	6.94	137.59	132.66	4.93
0.500	0.866	0.000	2250	937.5	50	87.56	87.51	0.05	106.21	109.81	-3.60
-0.500	-0.866	0.000	750	0.00	50	80.34	73.74	6.60	76.30	77.63	-1.33
0.500	-0.866	0.000	2250	0.00	50	104.64	105.30	-0.66	140.30	142.90	-2.60
-0.500	0.866	0.000	750	937.5	50	76.98	76.93	0.05	99.27	96.23	3.04
0.500	0.289	0.816	2250	615.18	100	79.65	82.49	-2.84	114.47	118.89	-4.42
-0.500	-0.289	-0.816	750	312.32	0.0	88.61	75.79	12.82	93.99	96.95	-2.96
0.500	-0.289	-0.816	2250	312.32	0.0	94.01	100.67	-6.66	115.48	117.55	-2.07
0.000	0.577	-0.816	1500	781.07	0.0	89.28	90.00	-0.72	88.89	87.90	0.99
-0.500	0.289	0.816	750	615.18	100	70.97	65.23	5.74	63.37	60.65	2.72
0.000	-0.577	0.816	1500	156.43	100	80.02	82.93	-2.91	79.34	77.66	1.68
0.000	0.000	0.000	1500	468.75	50	97.32	102.57	-5.25	126.00	123.08	2.92
-1.000	0.000	0.000	0.000	468.75	50	31.20	36.47	-5.27	47.65	53.82	-6.17
-1.000	-0.866	-0.816	0.000	0.0	0.0	17.40	22.85	-5.45	58.95	54.94	4.01
0.000	0.000	0.000	1500	468.75	50	100.03	102.57	-2.54	125.00	123.08	1.92
0.000	0.000	0.000	1500	468.75	50	104.39	102.57	1.82	123.00	123.08	-0.08

[a]Experimental result values. [b]Theretical values (values coming from mathematical models). [c] Residue. Hit: Hitempase 2XL, Bio: Bioglucanase TX, Brew Pro: Brewers Protease.

AUF-type dryer Ngaoundere, Cameroon. The malt was rubbed-off of its rootlets and stored until further use.

Mashing

Two hundred and fifty (250) ml of distilled water were put into a 600 ml beaker and 50 g of sorghum (malted or unmalted) flour (Ø < 1 mm) added with continuous stirring until a homogenous mixture was obtained. This mixture was incubated at 45°C for 1 h in a water bath with intermittent stirring at intervals of 5 min. The mix was allowed to decant and 50 ml of the supernatant withdrawn and kept aside. The temperature of the mash was then raised to boiling so as to gelatinize sorghum starch during 40 min with intermittent stirring at intervals of 5 min before cooling to 65°C. The 50 ml of supernatant to the which commercial enzyme/s is/are added according to the Doehlert matrix design of 3 factors, were added to the mash and allowed to incubate for 1 h 30 min with intermittent stirring at intervals of 10 min. The mash was filtered at 25°C during 1h 30 min using Whatmann paper N° 42.

Determination of reducing sugars contents

The reducing sugars content was determined using DNS reagent (Miller, 1959).

Optimization of models

Models were optimized as previously described (Desobgo et al., 2010). The intersection of the curves, representing the optimal zone, was highlighted.

RESULTS AND DISCUSSION

Optimization of the action of mashing enzymes on reducing sugars was carried out by modeling the experimental design required for manipulation in laboratory. Table 2 shows the results obtained for reducing sugars after mashing unmalted and malted *Madjeru* using Hitempase 2XL (α-amylase), Bioglucanase TX (β-glucanase) and Brewers Protease (Protease).

Modeling and validation of results

The mathematical models (Equations 2 and 3) obtained for reducing sugars after mashing unmalted and malted *Madjeru* were as follows respectively:

$$Y_{MadSRed}(X_1, X_2, X_3) = 102.573 + 21.070X_1 - 4.217X_2 - 7.312X_3 - 12.118X_1X_2 - 0.378X_1X_3 + 5.688X_2X_3 - 45.031X_1^2 - 7.261X_2^2 - 16.534X_3^2 \quad (2)$$

$$Y_{MadMSRed}(X_1, X_2, X_3) = 123.077 + 39.421X_1 - 4.181X_2 - 9.230X_3 - 29.849X_1X_2 + 33.635X_1X_3 + 28.583X_2X_3 - 29.835X_1^2 - 11.971X_2^2 - 34.318X_3^2 \quad (3)$$

Table 3. Estimation of regression coefficients and enzyme contributions for the reducing sugars content of *Madjeru*.

Effects	Coefficients		Standard error		t		Probability		Enzyme contributions(%)	
	Unmalted	malted	Unmalted	malted	Unmalted	malted	Unmalted	malted	Unmalted	malted
Constant	102.573	123.077	3.79	2.304	27.066	53.413	0.000	0.000		
X_1	21.07	39.421	3.898	2.37	5.405	16.631	0.001	0.000	18	18
X_2	-4.217	-4.181	3.399	2.067	-1.074	-1.752	0.318	0.123	3	2
X_3	-7.312	-9.23	3.212	1.953	-1.858	-3.856	0.106	0.006	6	4
X_1^2	-45.031	-29.835	6.683	4.064	-6.738	-7.342	0.000	0.000	38	13
X_2^2	-7.261	-11.971	4.864	2.958	-1.12	-3.036	0.300	0.019	6	5
X_3^2	-16.534	-34.318	4.196	2.551	-2.624	-8.957	0.034	0.000	14	16
X_1*X_2	-12.118	-29.849	7.63	4.639	-1.375	-5.572	0.211	0.001	10	14
X_2*X_3	5.688	28.583	6.715	4.083	0.599	4.947	0.568	0.002	0	15
X_1*X_3	-0.378	33.635	7.951	4.834	-0.039	5.677	0.970	0.001	5	13

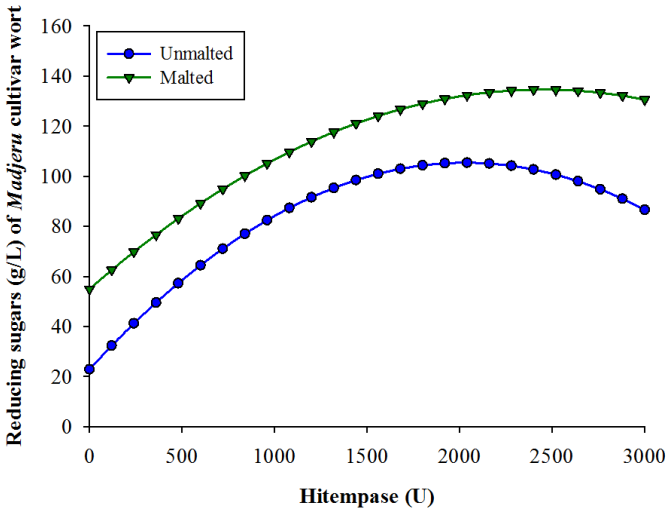

Figure 1A. Effect of concentration of Hitempase (α-amylase) as sole mashing enzyme on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

With :$Y_{MadSRed}(X_1, X_2, X_3)$ representing the mathematical model for unmalted *Madjeru*; $Y_{MadMSRed}(X_1, X_2, X_3)$, the model for malted *Madjeru*: X_1, Hitempase; X_2, Bioglucanase and X_3, Brewers Protease.

These mathematical models are polynomials having several variables with correlation coefficients R^2 = 0.951 for unmalted *Madjeru* and R^2 = 0.987 for malted *Madjeru*. These coefficients, coupled to AAD values of 0.067 and 0.032 obtained by the method (Bas and Boyac, 2007) for unmalted and malted *Madjeru* respectively, allowed for the validation of the models for the wort reducing sugars. In addition, a bias factor (1.01 and 1 for unmalted and malted *Madjeru*), coupled to accuracy factors of 1.07 and 1.03 for both unmalted and malted *Madjeru* respectively, also allowed for validation of the models according to the method described (Ross, 1996; Baranyi et al., 1999). The factors of the models were linear or of first degree (X_1, X_2 and X_3), quadratic or of the second degree (X_1^2, X_2^2 and X_3^2) and of interaction form (X_1X_2, X_1X_3, X_2X_3). They were statistically considered significant or not if the probability (P) of increasing reducing sugars content was ≤ 0.05 or ≥ 0.05 respectively (Table 3).

Effect of Hitempase 2XL on reducing sugars production

The impact of Hitempase 2XL (α-amylase) as sole mashing enzyme on the reducing sugars content of unmalted and malted *Madjeru* is shown in Figure1A. Reducing sugars content increased with increasing concentration of enzyme for unmalted and malted *Madjeru* mash types to attain maxima of 105.39 g/L and 132.25 g/L respectively, at an enzyme concentrations of 2031 U and 2451 U respectively. This was followed by a slight and steady decrease thereafter for the unmalted *Madjeru* mash. The measurable reducing sugar contents could not however be ascribed entirely to the action of Hitempase supplements, as in the absence of the enzyme, Figure 1A clearly showed that the contents for the unmalted and malted *Madjeru* mash types were about 22 g/L and 54 g/L respectively. These amounts of reducing sugars could be attributed to the milling process in the case of the unmalted *Madjeru* mash type, and to milling and malting processes for the malted *Madjeru* mash type. When the mathematical models were applied to predict the impact of a combination of the three enzymes (Bioglucanase and Brewers protease added at fixed amounts of 750 BGU and 60 mg, respectively) on the reducing sugar contents, the same trends as described above in Figure 1A were observed with increasing amounts of Hitempase for both *Madjeru* mash types

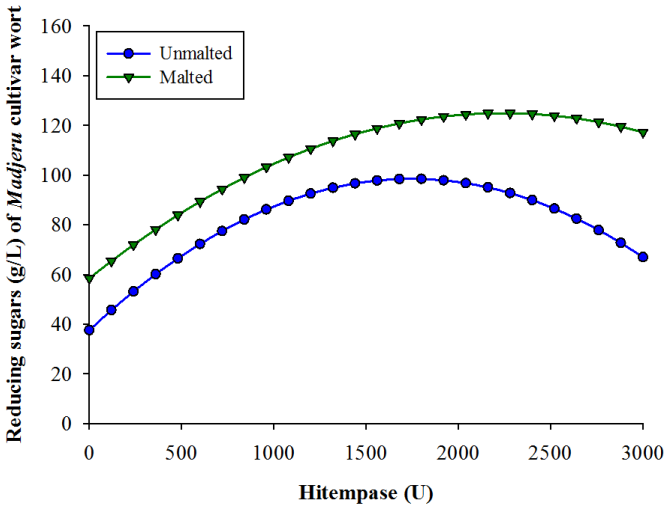

Figure 1B. Effect of concentration of Hitempase (α-amylase) in the presence of fixed concentrations of Bioglucanase (750 BGU) and Brewers protease (60 mg) on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

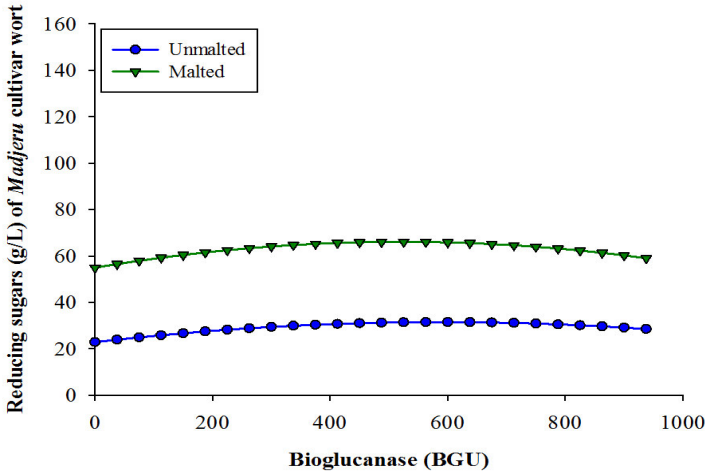

Figure 2A. Effect of concentration of Bioglucanase as sole mashing enzyme on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

(Figure 1B). In fact, starch is the main macromolecule of sorghum and the main substrate of α-amylase. It could therefore be expected that Hitempase contributes for the greatest amount of soluble materials in the form of reducing sugars that could be found in resulting worts due to its action on starch (Goode et al., 2003, Phiarais et al., 2006, Desobgo et al., 2010). The reducing sugars content for the unmalted *Madjeru* mash type slightly reduced beyond the maxima concentrations of the enzyme (Figures 1A and 1B). This could be explained by the fact that traces of amino-acids and other soluble nitrogenous materials present in the wort reacted with some of the reducing sugars (Hough et al., 1982), thus decreasing the measurable content. These results also demonstrated that the presence of the key mashing enzyme (α-amylase) developed during malting boosted the mashing efficiency of this cereal (Figure 1A). From the mathematical model, it was shown that in its linear form (X_1), the action of Hitempase contributed for 18% of reducing sugars for both unmalted *Madjeru* and malted *Madjeru* (Table 3). Statistical analyses also showed that this contribution was significant (P = 0.001 and 0.000 for the two mash types, respectively) (Table 3). In its quadratic form (X_1^2), the action of Hitempase remained statistically significant (P = 0.000) for both unmalted and malted *Madjeru*. This confirmed the above biological observation according to which supplements of this enzyme in both the unmalted and malted mashes of *Madjeru* are important. Its contribution to reducing sugars in its quadratic form (X_1^2) (excess of α-amylase in principle) is indeed 38 and 13% for unmalted and malted *Madjeru* respectively (Table 3).

Effect of Bioglucanase TX on reducing sugars production

Figure 2A shows the effect of mashing unmalted and malted *Madjeru* using Bioglucanase TX as sole mashing enzyme on reducing sugars content. There was no significant effect of Bioglucanase on the reducing sugars content of both unmalted and malted *Madjeru* mashes (Table 3). Reducing sugars were present at 22.85 and 54.94 g/L for the two mash types respectively in the absence of this mashing enzyme. Almost all of the measurable soluble reducing sugars therefore seemed to be due to the milling operation. In the presence of Bioglucanase, reducing sugars contents were maximal at 22.86 and 54.95 g/L at concentrations of 590.24 and 521.74 BGU for unmalted and malted *Madjeru* respectively with increasing enzyme concentration. The small reducing sugars yields due to Bioglucanase's action can be attributed to its hydrolysis of β-glucans into glucose and other soluble carbohydrates. Bioglucanase was therefore not a backbone enzyme for production of reducing sugars during mashing (Phiarais et al., 2006, Desobgo et al., 2010). A similar application of the mathematical models as carried out above for Hitempase's action, using 60 mg of Brewers protease and 1875 U of Hitempase, predicted that supplementing these two key mashing enzymes could provide higher reducing sugars content for both unmalted and malted *Madjeru* mashes (Figure 2B). Hitempase once more demonstrated that it was the backbone enzyme that contributed most in reducing sugars' yields. Figure 2B indeed showed that even in the absence of Bioglucanase, but in the presence of Hitempase, the reducing sugars content increased 5 folds for unmalted

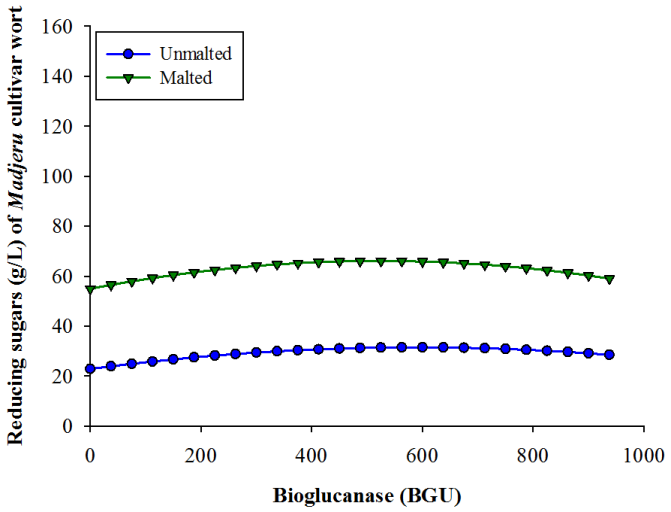

Figure 2A. Effect of concentration of Bioglucanase as sole mashing enzyme on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

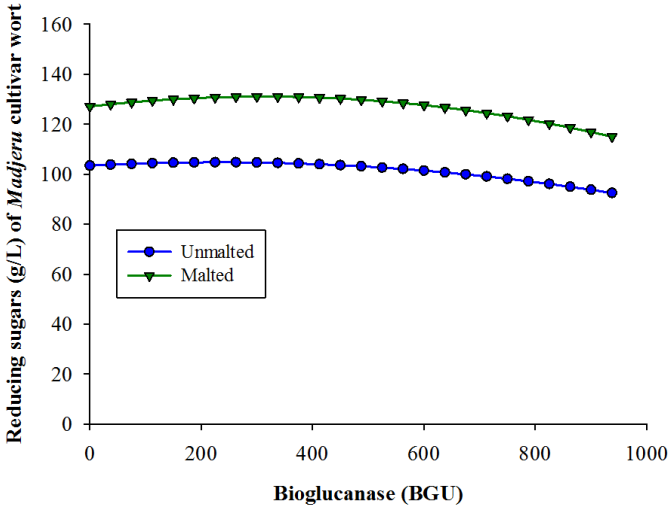

Figure 2B. Effect of concentration of Bioglucanase (β-glucanase) in the presence of fixed concentrations of Hitempase (1875 U) and Brewers protease (60 mg) on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

Madjeru mashes and 3 folds for malted *Madjeru* mashes. These observations were all statistically confirmed. Indeed, in its linear form (X_2), Bioglucanase's action was not significant (P = 0.318 and 0.123 for unmalted and malted *Madjeru* respectively) (Table 3). Table 3 showed that this enzyme contributed to merely 3 and 2% of reducing sugars content for unmalted and malted *Madjeru* mashes respectively. In its quadratic form (X_2^2) (excess of enzyme in principle), Bioglucanase contributed to 6 and 5 % of reducing sugars for unmalted and malted *Madjeru* mashes respectively (Table 3). This contribution was not statistically significant for unmalted *Madjeru* mash, but significant for malted *Madjeru* mash (P = 0.300 and 0.019) (Table 3).

Once more, the highest values of reducing sugars obtained for malted *Madjeru* mash, as compared to unmalted *Madjeru* mash, could be attributed to the impact of hydrolytic enzymes developed during malting.

Effect of Brewers protease on reducing sugars production

The effect of mashing unmalted and malted *Madjeru* on reducing sugars content using as sole mashing enzyme, Brewers protease, is shown in Figure 3A. There was no contribution of reducing sugars for both unmalted and malted *Madjeru* by this enzyme.

All the measurable soluble reducing sugars seemed to be due to the milling operation for the unmalted mash type, and the malting and milling operations, for the malted mash type. The decrease of yields of reducing sugars with increase of Brewers protease for unmalted and malted *Madjeru* mashes could be attributed to reactions between nitrogenous functions and some of the reducing sugars (Hough et al., 1982). The mathematical model was once more used to predict the reducing sugars content in the presence of fixed amounts of the two other key mashing enzymes. Thus, using 1875 U of Hitempase and 750 BGU of Bioglucanase with increasing amounts of Brewers protease, the results showed that higher amounts of reducing sugars could be obtained for both unmalted and malted *Madjeru* mashes (Figure 3B). These observations were statistically confirmed using the mathematical model. In its first degree form (X_3), the impact of Brewers protease was not significant for unmalted *Madjeru* mash but significant for malted *Madjeru* mash (P= 0.106 and 0.006 respectively) (Table 3). Its contribution to reducing sugars content was 6 and 4% respectively for both mashes (Table 3). The impact of the enzyme in its quadratic form (X_3^2), was significant for both mashes (P= 0.034 and 0.000 respectively). Its contribution to reducing sugars content was 14 and 16% respectively (Table 3).

Effect of enzymes interactions on reducing sugars production

The models were further exploited to predict the impacts of the interactions (X_1X_2, X_1X_3 and X_2X_3) of these enzymes on reducing sugars content. The results are shown in Table 3. They were statistically not significant

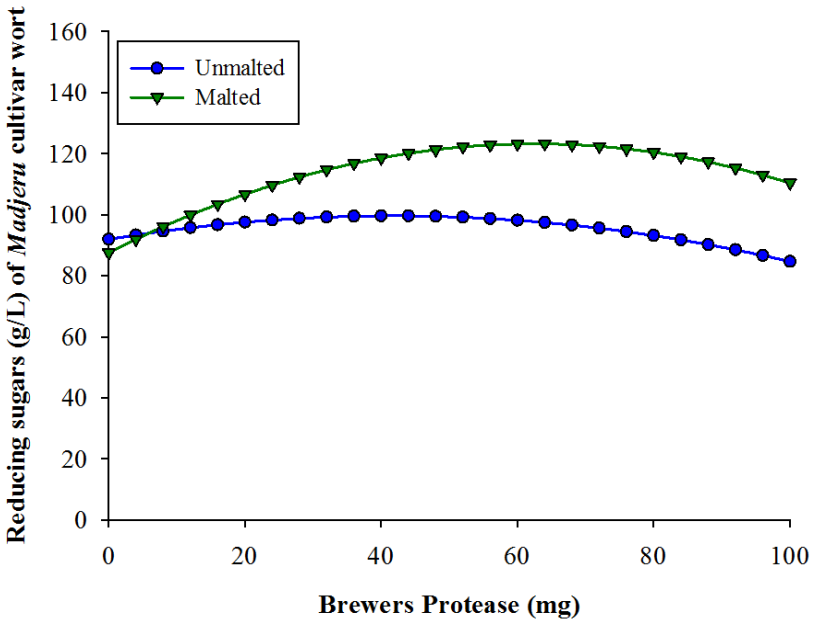

Figure 3B. Effect of concentration of Brewers protease in the presence of fixed concentrations of Hitempase (1875 U) and Bioglucanase (750 BGU) on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

Table 4. ANOVA for the reducing sugars content of *Madjeru*.

Source	DF	Sum square		Mean square		F		P	
		Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Regression	9	8675.21	12655.782	963.912	1406.198	15.034	59.323	0.001	0.000
Linear	3	4174.58	8636.535	1391.53	2878.845	21.704	121.45	0.001	0.000
Quadratic	3	4337.98	2342.886	1446	780.962	22.553	32.946	0.001	0.000
Interactions	3	162.641	1676.361	54.214	558.787	0.846	23.574	0.511	0.000
Residual error	7	448.802	165.928	64.115	23.704				
Total error	16	9124.01	12812.71						

DF: Degree of freedom

for unmalted *Madjeru* mashes (P = 0.511), but were for malted *Madjeru* mashes (P=0.000) (Table 4). The interaction X_1X_2 (Hitempase/Bioglucanase) had no significant impact on unmalted *Madjeru* mash but had for malted *Madjeru* (P = 0.211 and 0.001) respectively (Table 3). It contributed to 10% of reducing sugars content for unmalted *Madjeru* mash and to 14% for malted *Madjeru* mash (Table 3). Though known to be the backbone starch hydrolyzing enzyme, the action of Hitempase is best exploited when the cell walls of cereal grains are broken down by β-glucanases, hemicellulases and cellulases, to liberate starch granules. This sequence of events during malting was confirmed by the mathematical models above.

The interaction X_1X_3, corresponding to the couple Hitempase/Brewers protease, also had no significant impact on the reducing sugars content of unmalted *Madjeru* mash (P = 0.970), but had a significant impact on the reducing sugars content of malted *Madjeru* mash (P = 0.001) (Table 3). Its contribution to reducing sugars content was 0 and 15% respectively (Table 3). This result was once more in conformity with the biological sequence occurring during malting. Efficient starch hydrolysis by α-amylase indeed occurs only after the breakdown of cell walls by β-glucanase, followed by liberation of starch granules due to proteolysis of the protein matrix enrobing them. The interaction Bioglucanase/Brewers protease (X_2X_3) had no significant impact on reducing sugars content for unmalted *Madjeru* mashes, but had for malted *Madjeru* mashes (P = 0.568 and 0.002 respectively)

Table 5. ANOVA for comparing reducing sugars content of unmalted and malted *Madjeru* worts.

Source	DF	Sum square	Mean square	F	P
Inter-groups	1	3224.25	3224.25	4.7	0.037
Intra-groups	32	21945.7	685.804		
Total	33	25170			

DF : Degree of freedom.

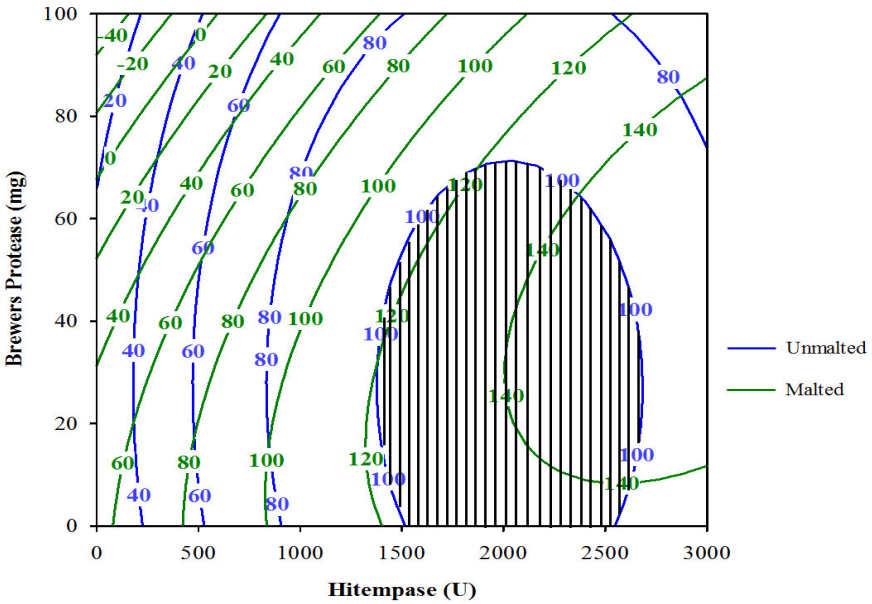

Figure 4. Response surface curves for the enzyme combinations providing for optimal reducing sugars content (g/L) for unmalted and malted sorghum wort cultivar *Madjeru*.

(Table 3). Its contribution to reducing sugars content was 5 and 13% respectively for both mash types (Table 3). These low contributions by the couple (Bioglucanase/ Brewers protease) were expected, as the two enzymes only play a supporting role in starch hydrolysis during mashing (Desobgo et al., 2010).

Optimization of the concerted mashing enzymes action on the production of reducing sugars

The results obtained for the action of the enzymes on reducing sugars yields after mashing on the basis of the models, were optimized to define a satisfactory domain of compromise for the action of the mashing enzymes. This domain was obtained for a reducing sugars content ≥ 100 g/L. The theoretical optimal combination of enzyme action for unmalted *Madjeru* gave the following triplet of coded variables for reducing sugars content: 0.330, – 0.701 and – 0.345 (1995 U, 89.31 BGU and 28.86 mg real variables) for Hitempase, Bioglucanase and Brewers protease respectively. This triplet allowed for a maximal reducing sugars content of 108.78 g/L. The triplet for malted *Madjeru* was 1, – 0.866 and – 0.005 (3000 U, 0 BGU and 49.69 mg real variables). It allowed for a maximal extract of 153.15 g/L. The optimal enzyme combinations were thus different and gave different results. These results once more confirmed the fact that commercial enzymes supplements for mashing of unmalted and malted *Madjeru* were necessary to obtain maximum reducing sugars contents. The significant difference (P = 0.037) between reducing sugars content of unmalted and malted *Madjeru* worts is shown in Table 5. By fixing Bioglucanase concentration at 0 BGU (according to the optimization), the minimal reducing sugars content (100 g/L) could be obtained and the necessary enzyme combination could therefore permit highlight the optimal domain (Figure 4).

Conclusion

Optimizing the amounts of the enzyme needed for alleviating the levels of reducing sugars during mashing

demonstrated that Hitempase 2XL was the most important enzyme component in terms of both amounts and efficiency for the unmalted and malted mash types of the *Madjeru* sorghum cultivar. Optimizing studies also showed that Brewers Protease was the next important enzyme in increasing reducing sugars yields, as its need in both mash types was demonstrated. Bioglucanase TX was not indispensable for the malted mash type as shown by the optimal triplet of mashing enzymes obtained for this sample. The role of the milling process also independently facilitated the dissolution of some of these reducing sugars during mashing of both unmalted and malted *Madjeru* grist. Finally, the response surface methodology used in this work permitted to confirm the specific virtues always allotted to the malting process as an unavoidable route in providing the best malt types that can be used in mashing and beer brewing.

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REFERENCES

Agu RC, Palmer GH (1998). Effect of mashing with commercial enzymes on the properties of sorghum worts. World J. Micro-biol. Biotechnol., 14: 43-48.

Bajomo MF, Young TW (1992). Development of a mashing profile for the use of microbial enzymes in brewing with raw sorghum (80%) and malted barley or sorghum malt (20%). J. Inst. Brew., 98: 515-523.

Baranyi J, Pin C, Ross T (1999). Validating and comparing predictive models. Int. J. Food Microbiol., 48: 159-166.

Bas D, Boyac IH (2007). Modeling and optimization I: Usability of response surface methodology. J. Food Eng., 78: 836-845.

Desobgo ZSC, Nso EJ, Tenin D, Kayem GJ (2010). Modelling and optimizing of mashing enzymes-effect on yield of filtrate of unmalted sorghum by use of response surface methodology. J. Inst. Brew., 116: 62-69.

EtokAkpan OU (1992). Amylolytic potentials and wort fermenting components of nigerian sorghums and barley. World J. Microbiol. Biotechnol., 8: 287-289.

EtokAkpan OU, Palmer GH (1990). Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. World J. Microbiol. Biotechnol., l 6:408-417.

Giovanni M (1983). Response surface methodology and product optimization. Food Technol., 37:41-45.

Goode DL, Arendt EK (2003). Pilot scale production of lager beer from grist containing 50% unmalted sorghum. J. Institute of Brew., 109: 208-217.

Goode DL, Halbert C, Arendt EK (2002). Mashing studies with unmalted sorghum and malted barley. J. Institute of Brewing 108:465-473.

Goode DL, Halbert C, Arendt EK (2003). Optimisation of mashing conditions when mashing with unmalted sorghum and commercial enzymes. J. Am. Soc. Brew. Chem., 61: 69-78.

Hough JS, Briggs DE, Stevens R, Young TW (1982). In: Malting and brewing science. London: Chapman and Hall.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem., 31: 426-428.

Nso EJ, Ajebesone PE, Mbofung CM, Palmer GH (2003). Properties of three sorghum cultivars used for the production of *Bili-Bili* beverage in Northern Cameroon. J. Inst. Brew., 109: 245-250.

Nso EJ, Nanadoum M, Palmer GH (2006). The effect of formaldehyde on enzyme development in sorghum malts. MBAA TQ 43:177-182.

Palmer GH (1989). Cereal in malting and brewing. In: Cereal science and technology(Palmer G ed) Aberdeen, Scotland: Aberdeen University Press.

Phiarais NB, Schehl BD, Oliveira JC, Arendt EK (2006). Use of surface response methodology to investigate the effectiveness of commercial enzymes on buckwheat malt for brewing purpose. J. Institute of Brew., 112: 324-332.

Ross T (1996). Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol., 81: 501-508.

Taylor JRN (2004). Overview: Importance of sorghum in Africa. In: Department of Food Science, Pretoria, South Africa: University of Pretoria. p. 21

Taylor JRN, Dewar J (2001). Developments in sorghum food technologies. In: Advances in Food and Nutrition Research (Taylor, J., ed), San Diego, USA: Academic Press, pp. 217-264.