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Full Length Research Paper

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

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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 *Safrari* sorghum. The response surface methodology revealed that increasing amounts of Hitempase considerably increased reducing sugars content during mashing of unmalted *Safrari* sorghum grist to about 90 g/L. Limited amounts of reducing sugars were obtained with increasing concentrations of both Bioglucanase (≈ 40 g/L) and Brewers Protease (≈ 30 g/L). The milling process facilitated the dissolution of about 10 g/L in yields of reducing sugars for the unmalted *Safrari* sorghum mash type without the help of enzyme. None of the three enzymes as sole mashing enzyme appeared to be of use in mashing malted *Safrari*, as reducing sugars yields were at maximum (168 g/L) after dissolving the grist in water and rather decreased with increasing amounts of enzyme supplements. Optimizing the concerted actions of the three enzymes for reducing sugars content of unmalted *Safrari* sorghum mash gave a combination of 2163 U, 937.5 BGU and 0 mg for Hitempase, Bioglucanase and Brewers Protease respectively. This gave a maximal reducing sugars content of 126.57 g/L. This combination was 0 U, 137.48 BGU and 0 mg for malted *Safrari* sorghum mash, giving a maximal reducing sugars yield of 168.56 g/L.

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

INTRODUCTION

Sorghum (*Sorghum bicolor (L.) Moench)* is a vital calorie-based food component in human nutrition in some parts of Africa (Taylor, 2004). Besides this fundamental function, sorghum is used in the production of beer, traditional opaque beer and non-alcoholic drinks in developing countries, and also industrial beer (Palmer, 1989; Taylor and Dewar, 2001). The poor developmental profile of the principal hydrolytic enzymes during malting of this cereal is however a limiting factor to 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 *Safrari* and *S.35* sorghum cultivars were shown to be poorer in starch than the *Madjeru* cultivar, but had higher hydrolytic enzymes profiles and fermentable sugars potentials (Nso et al., 2003; Nso et al., 2006). The use of commercial enzymes when mashing with sorghum in order to obtain better wort specifications for beer brewing has however become a common practice (MacFadden and Clayton, 1989;Dale et al., 1990; Bajomo and Young, 1992; Agu and Palmer, 1998; Goode et al., 2002; Goode and Arendt, 2003; Goode et al., 2003). However, it is not clear whether the use of mashing enzyme supplements in *Safrari* sorghum

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cultivar mashes are indispensable in alleviating 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 *Safrari* sorghum cultivar (unmalted and malted) was modeled and optimized using the response surface methodology (RSM) to verify the necessity of mashing enzymes in mashing with this cultivar.

MATERIALS AND METHODS

Enzymes

The characteristics of the commercial mashing 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 their sources are presented in Table 1.

Sorghum cultivar

The *Safrari* 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 to 3000 U], [0 to 937.5 BGU] and [0 to 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 Safrari 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 incubator (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 AUF-type dryer Ngaoundere; Cameroon. The malt was rubbed-off of its rootlets and stored in plastics sachets at -18°C until further use.

Mashing

Two hundred and fifty millilitres 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 was withdrawn and kept aside. The temperature of the mash was then raised to boiling so as to gelatinize sorghum starch for 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 (at 65°C) for 1 h 30 min with intermittent stirring at intervals of 10 min. The mash was filtered at 25°C for one 1 h 30 min using a Whatmann paper NO 42.

Determination of the content of Reducing Sugars

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

Modeling and validation of results

Optimisation of the action of mashing enzymes on the reducing sugars yields was carried out by modeling the experimental design required for laboratory purposes. Table 2 shows the results obtained after mashing unmalted and malted *Safrari* using the commercial mashing enzymes Hitempase (α-amylase), Bioglucanase (β-glucanase) and Brewers Protease (Protease). The mathematical models obtained for reducing sugars after mashing unmalted and malted *Safrari* were as follows respectively:

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 BGU/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.

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

Coded values			Transformed experimental values			Reducing sugars content (*Safrari* 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	99.00	99.80	-0.80	130.50	131.98	-1.48
1.000	0.000	0.000	3000	468.75	50	94.00	84.36	9.64	110.50	109.91	0.59
0.500	0.866	0.000	2250	937.5	50	110.00	110.47	-0.47	108.73	104.90	3.83
-0.500	-0.866	0.000	750	0.00	50	71.00	63.88	7.12	108.91	113.31	-4.40
0.500	-0.866	0.000	2250	0.00	50	89.54	89.97	-0.43	107.93	105.45	2.48
-0.500	0.866	0.000	750	937.5	50	80.00	78.54	1.46	75.90	78.35	-2.45
0.500	0.289	0.816	2250	615.18	100	95.00	96.45	-1.45	141.50	146.27	-4.77
-0.500	-0.289	-0.816	750	312.32	0.0	79.62	68.95	10.67	161.67	157.74	3.93
0.500	-0.289	-0.816	2250	312.32	0.0	96.71	101.72	-5.01	92.50	95.54	-3.04
0.000	0.577	-0.816	1500	781.07	0.0	114.00	113.95	0.05	109.13	110.48	-1.35
-0.500	0.289	0.816	750	615.18	100	77.00	71.20	5.80	68.50	65.38	3.12
0.000	-0.577	0.816	1500	156.43	100	92.00	94.86	-2.86	109.07	107.42	1.65
0.000	0.000	0.000	1500	468.75	50	96.20	99.80	-3.60	131.00	131.98	-0.98
-1.000	0.000	0.000	0.000	468.75	50	24.00	26.35	-2.35	90.50	91.23	-0.73
-1.000	-0.866	-0.816	0.000	0.0	0.0	05.00	06.21	-1.21	166.98	166.53	0.45
0.000	0.000	0.000	1500	468.75	50	98.70	99.80	-1.10	133.05	131.98	1.07
0.000	0.000	0.000	1500	468.75	50	99.35	99.80	-0.45	134.00	131.98	2.02

$$Y_{SafSRed}(X_1, X_2, X_3) = 99.804 + 29.007X_1 + 10.153X_2 - 4.522X_3 + 3.376X_1X_2 - 5.800X_1X_3 - 13.731X_2X_3 - 41.448X_1^2 - 3.968X_2^2 - 0.818X_3^2 \quad [2]$$

$$Y_{SafMSRed}(X_1, X_2, X_3) = 131.976 + 9.344X_1 - 10.252X_2 - 9.124X_3 + 19.873X_1X_2 + 80.639X_1X_3 + 10.301X_2X_3 - 31.406X_1^2 - 31.500X_2^2 - 11.548X_3^2 \quad [3]$$

with: $Y_{SafSRed}(X_1, X_2, X_3)$, representing the mathematical model for unmalted *Safrari*; $Y_{SafMSRed}(X_1, X_2, X_3)$, the model for malted *Safrari*; X_1, Hitempase; X_2, Bioglucanase and X3

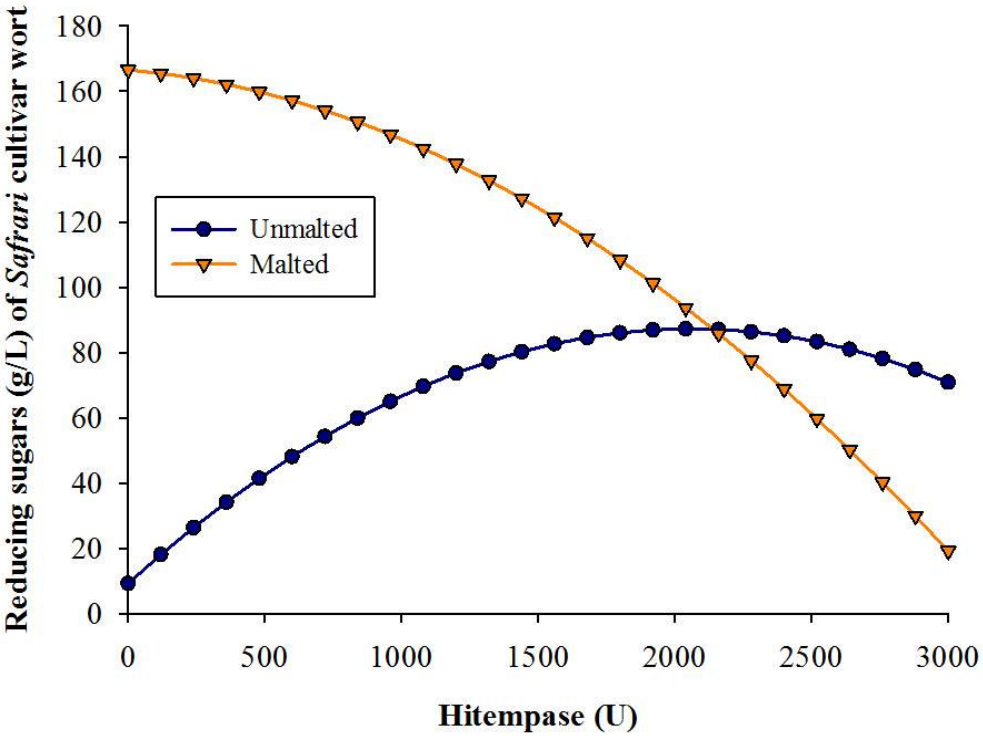

Figure 1A. Effect of concentration of Hitempase (α-amylase) as sole mashing enzyme on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

Brewers Protease.

These mathematical models are polynomials having several variables with correlation coefficients R2 = 0.975 for unmalted Safrari and R2 = 0.990 for malted Safrari. These coefficients, coupled to AAD values of 0.101 and 0.021 (Bas and Boyac, 2007) for unmalted and malted Safrari respectively, allowed for the validation of the models for the wort reducing sugars yields. In addition, bias factors of 1.04 and 1.00 for unmalted and malted Safrari respectively, coupled to accuracy factors of 1.08 and 1.02 for unmalted and malted Safrari 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 (X1, X2 and X3), quadratic or of the second degree (X12, X22 and X32) and of interacting form (X1X2, X1X3, X2X3). They were statistically considered significant or not if the probability (P) of increasing reducing sugars yields was ≤ 0.05 or ≥ 0.05 respectively (Table 3).

Effect of Hitempase 2XL on reducing sugars production

The impact of Hitempase as the sole mashing enzyme on the yields of reducing sugars of unmalted and malted Safrari is shown in Figure 1A. Reducing sugars yields increased with increasing amounts of enzyme for unmalted Safrari mash to attain a maximum level (≈ 90 g/L) at about 2000 U, followed by a slight and steady decrease thereafter. The reducing sugars yield was maximal (≈ 168 g/L) even in absence of Hitempase for malted Safrari mash. Indeed, increasing concentrations of enzyme instead resulted into a steady decrease of reducing sugars content for malted Safrari mash. The maximal reducing sugars content observed for malted Safrari mash in absence of Hitempase was however attributed to the natural virtues that the malting process imparts to mashing cereals in terms of synthesis and development of hydrolytic enzymes. This explains why though known to be the principal mashing enzyme, supplements of Hitempase (α-amylase) seemed to be of no use in producing reducing sugars as the natural enzyme synthesized during malting seemed to be in sufficient amounts to ensure starch hydrolysis. When the mathematical models were applied to predict the impact of supplements of Bioglucanase and Brewers Protease at concentrations of 400 BGU and 60 mg respectively as accompanying mashing enzymes to Hitempase, it was observed that the profile of reducing sugars increased with increasing amounts of Hitempase for malted *Safrari* mash though the amounts in the absence of enzyme dropped by roughly two folds (Figure 1B). In both mash types, the milling operation facilitated the dissolution of reducing sugars as could be seen in Figure 1A. This

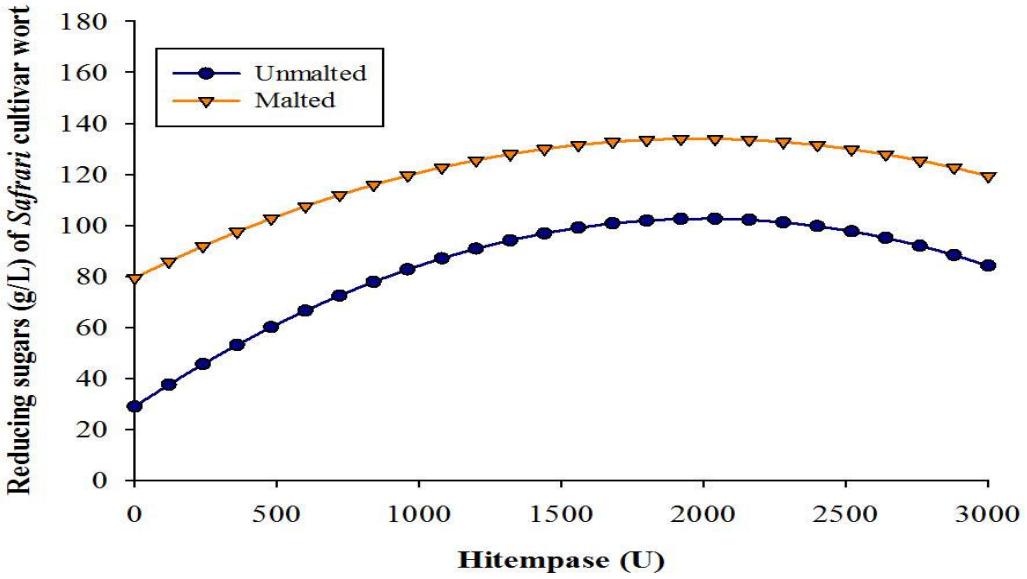

Figure 1B. Effect of concentration of Hitempase (α-amylase) in the presence of fixed concentrations of Bioglucanase (400 BGU) and Brewers protease (60 mg) on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

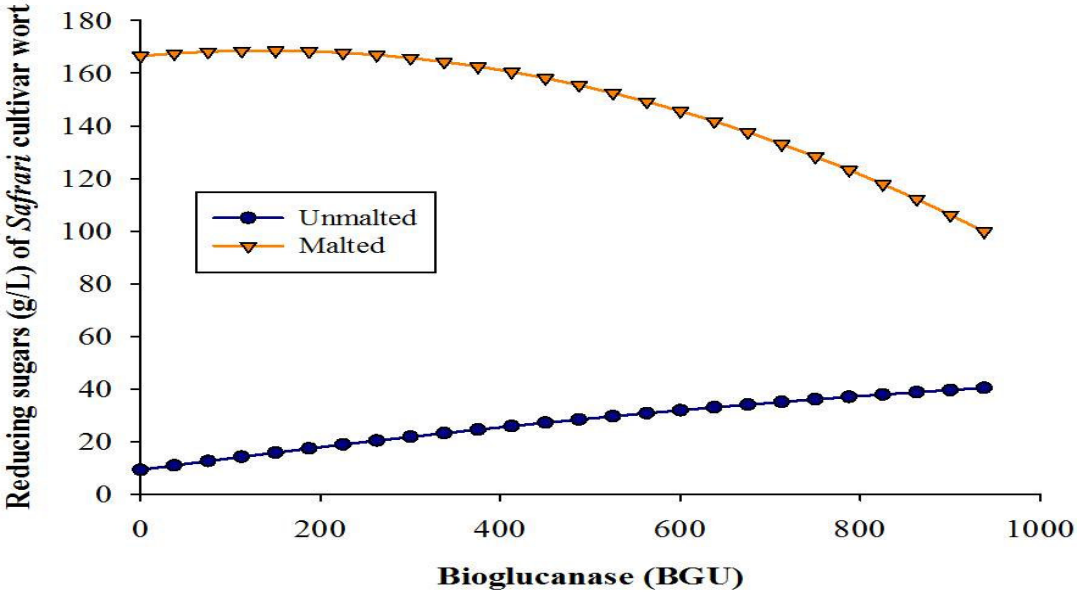

Figure 2A. Effect of concentration of Bioglucanase as sole mashing enzyme on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

dissolution accounted for total reducing sugars contents (168 g/L) for malted *Safrari* mash and for 10 g/L for unmalted Safrari mash. Starch is indeed the main macromolecule of cereals and the main substrate of α-amylase. It is therefore expected that Hitempase contributes to the greatest amounts of reducing sugars in resulting worts due to its action on starch (Goode et al., 2003; Phiarais et al., 2006; Desobgo et al., 2010). The steady decrease in yields of reducing sugars of malted mash could be attributed to Milliard reactions between soluble nitrogenous compounds and reducing sugars obtained earlier due to the action of hydrolytic enzymes synthesized during malting (Figure 1A) (Hough et al., 1982). From the mathematical models, it was shown that

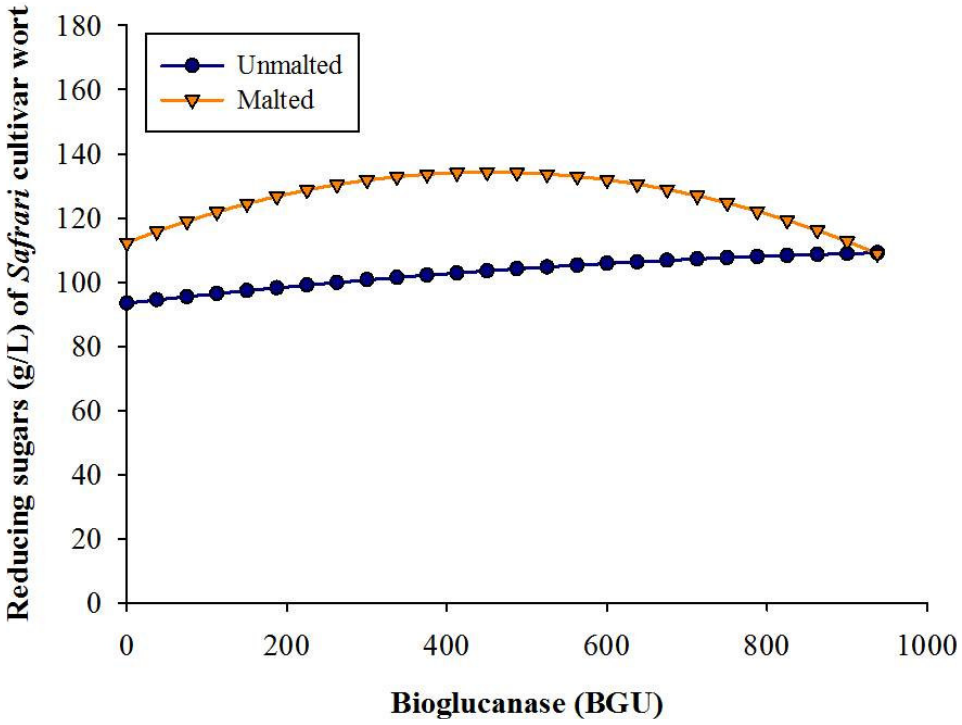

Figure 2B. Effect of concentration of Bioglucanase (β-glucanase) in the presence of fixed concentrations of Hitempase (2000 U) and Brewers protease (60 mg) on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

in its linear form (X_1), Hitempase's impact on reducing sugars yields was significant (P = 0.000 and 0.002) (Table 3) for both unmalted and malted *Safrari* mash types. This action contributed to 26and 4% for unmalted and malted *Safrari* respectively (Table 3). In its quadratic form (X_1^2), Hitempase's action remained statistically significant for both mash types (P = 0.000) (Table 3). Its contribution to increasing reducing sugar yields in this quadratic form (X_1^2) (excess of α-amylase in principle) is indeed 37 and 15% for unmalted and malted *Safrari* respectively (Table 3).

Effect of Bioglucanase TX on reducing sugars production

Figure 2A shows the effect of mashing unmalted and malted *Safrari* using Bioglucanase as only mashing enzyme on reducing sugars yields. There was a slight but constant increase in reducing sugars yield (≈ 40 g/L) as enzyme concentration increased for unmalted *Safrari* mash. This small yield of reducing sugars due to Bioglucanase's action on unmalted *Safrari* mash can be attributed to its ability to hydrolyse β-glucans into glucose and other soluble carbohydrates. Contrary to Hitempase, Bioglucanase was therefore not a backbone enzyme for reducing sugars production during mashing. The reducing sugars yield for malted *Safrari* mash was maximal even in the absence of Bioglucanase, and steadily decreased with increase in enzyme concentration. A similar application of the mathematical models as carried out above for Hitempase's action, using 60 mg of Brewers Protease and 2000 U Hitempase as accompanying enzymes, predicted that supplements of these two key mashing enzymes could provide similar results in reducing sugar yields for both unmalted and malted *Safrari* mashes (Figure 2B). Hitempase once more demonstrated that it was the backbone enzyme contributing to most of the reducing sugars. Figure 2B indeed showed that in the absence of Bioglucanase, but in the presence of Hitempase, yields of reducing sugars became comparable for both unmalted and malted *Safrari* mashes. In addition, the facilitator role of dissolving reducing sugars by the milling process was considerably accentuated. These observations were all statistically confirmed. Indeed, in its linear form (X_2), Bioglucanase's action was significant (P = 0.018 and 0.001 for unmalted and malted *Safrari* respectively (Table 3)). Table 3 showed that this enzyme contributed for 9 and 5% of reducing sugars for unmalted and malted *Safrari* respectively. In its quadratic form (X_2^2) (excess of enzyme in principle), Bioglucanase contributed for 3and 15% of reducing sugars for unmalted and malted *Safrari* respectively (Table 3). These contributions were not statistically significant for unmalted *Safrari* mash, but were for malted *safrari* mash (P = 0.492 and 0.000)

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

Effects	Coefficients		Standard Error		t		Probability		Enzyme Contributions (%)	
	Unmalted	malted	Unmalted	malted	Unmalted	malted	Unmalted	malted	Unmalted	malted
Constant	99.804	131.976	3.197	1.938	31.217	68.108	0.000	0.000		
X_1	29.007	9.344	3.289	1.993	8.82	4.688	0.000	0.002	26	4
X_2	10.153	-10.252	2.868	1.738	3.066	-5.108	0.018	0.001	9	5
X_3	-4.522	-9.124	2.71	1.642	-1.362	-4.533	0.216	0.003	4	4
X_1^2	-41.448	-31.406	5.638	3.417	-7.351	-9.19	0.000	0.000	37	15
X_2^2	-3.968	-31.5	4.103	2.487	-0.725	-9.499	0.492	0.000	3	15
X_3^2	-0.818	-11.548	3.54	2.145	-0.154	-3.584	0.882	0.009	1	5
X_1*X_2	3.376	19.873	6.437	3.901	0.454	4.411	0.663	0.003	3	9
X_2*X_3	-13.731	10.301	5.665	3.434	-1.713	2.12	0.130	0.072	12	5
X_1*X_3	-5.8	80.639	6.707	4.065	-0.706	16.186	0.503	0.000	5	38

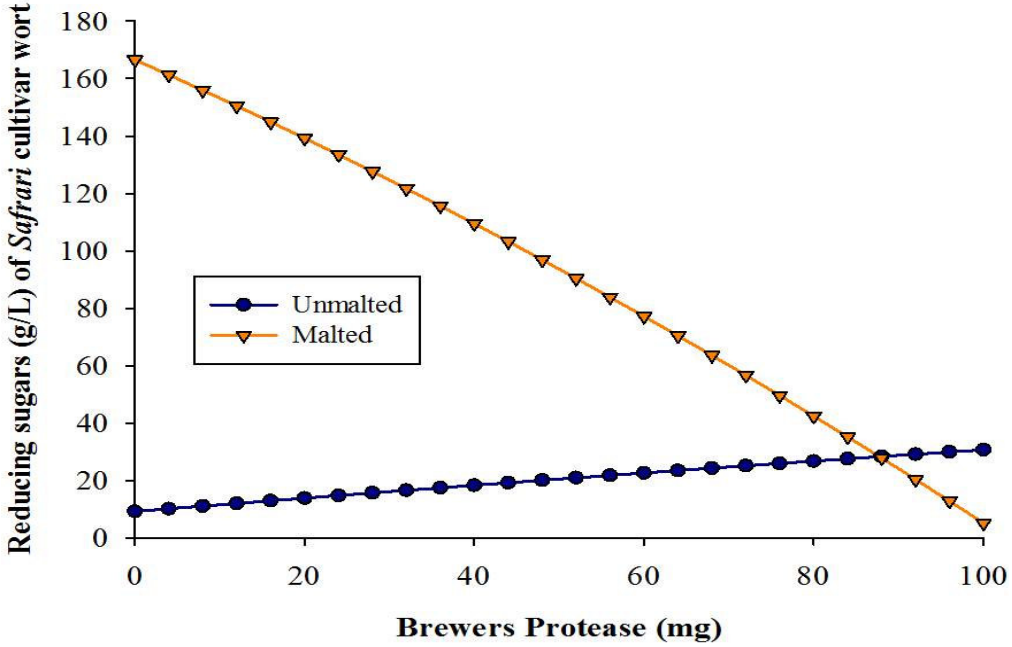

Figure 3A. Effect of concentration of Brewers Protease as sole mashing enzyme on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

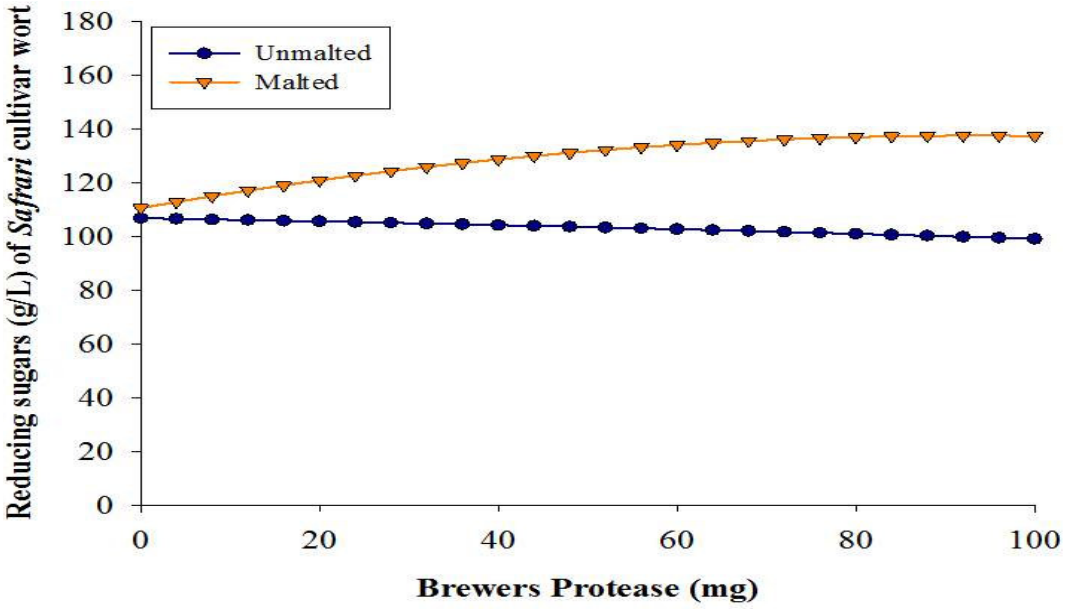

Figure 3B. Effect of concentration of Brewers protease in the presence of fixed concentrations of Hitempase (1875 U) and Bioglucanase (750 BGU) on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

(Table 3). This 1 to 5 ratio difference in reducing sugars content between the unmalted and malted *Safrari* mash types could once more be attributed to the natural virtues of the malting process.

Effect of Brewers Protease on reducing sugars production

The effect of mashing unmalted and malted *Safrari* on yields of reducing sugars using sole mashing enzyme, Brewers Protease, is shown in Figure 3A. A small but constant increase in reducing sugars content with increasing enzyme concentrations was observed (≈ 30 g/L) for unmalted *Safrari* mash. The milling process once more contributed to about 10 g/L of these reducing sugars amounts. As observed for Bioglucanase, the maximal reducing sugars amounts were obtained when mashing with malted *Safrari* in the absence of supplements of Brewers Protease. This could once more be attributed to the virtues known to the malting process as earlier explained above. A high rate of decrease of reducing sugars content with increasing concentrations of Brewers Protease for the malted *Safrari* mash was observed. This could be explained by the fact that this enzyme released into the medium soluble nitrogenous materials (amino acids and others) which reacted with the reducing sugars (produced during the malting process), displaying as such the rapid decrease in the amounts of the sugars as observed in Figure 3A (Hough et al., 1982). The mathematical models were once more used to predict the yield in reducing sugars as carried out above for Hitempase and Bioglucanase actions. Thus, using 2000 U of Hitempase and 400 BGU of Bioglucanase as accompanying mashing enzymes with increasing amounts of Brewers Protease, similar results in reducing sugars yields for both unmalted and malted *Safrari* mashes were once more observed (Figure 3B). These observations were statistically confirmed. In its first degree form (X_3), the impact of Brewers Protease was not significant for unmalted *Safrari* mash but significant for malted *Safrari* mash (P = 0.216 and 0.003 respectively) (Table 3). Its contribution to reducing sugars was barely 4% for both mash types (Table 3). The impact of the enzyme in its quadratic form (X_3^2), was not significant for unmalted *Safrari* mash but significant for malted *Safrari* mash (P = 0.882 and 0.009 respectively) (Table 3). Its contribution to reducing sugars was 1 and 5% respectively (Table 3).

Effect of enzyme interactions on the production of reducing sugars

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 yields of reducing sugars. The results are shown in Table 3. Globally, they were statistically not significant for unmalted *Safrari* mashes (P = 0.339), but were for malted *Safrari* mashes (P = 0.000) (Table 4). The interaction X_1X_2 (Hitempase/Bioglucanase) had no significant impact on unmalted *Safrari* mash, but had for

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

Source	dl	Sum square		Mean square		F		Probability	
		Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Regression	9	12650.765	11623.844	1405.641	1291.538	30.804	77.047	0.000	0.000
Linear	3	8527.165	2482.605	2842.388	827.535	62.29	49.367	0.000	0.000
Quadratic	3	3941.669	583.306	1313.89	194.435	28.793	11.599	0.000	0.004
Interactions	3	181.932	8557.933	60.644	2852.644	1.329	170.175	0.339	0.000
Residual error	7	319.423	117.341	45.632	16.763				
Total error	16	12970.188	11741.185						

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

Source	dl	Sum square	Mean square	F	P
Inter-groups	1	9200.22	9200.22	11.91	0.0016
Intra-groups	32	24711.4	772.23		
Total	33	33911.6			

malted *Safrari* (P = 0.663 and 0.003 respectively (Table 3). It contributed for merely 3% of reducing sugars for unmalted *Safrari* and 9% for malted *Safrari* mash (Table 3). The interaction X_1X_3, corresponding to the couple Hitempase/Brewers Protease, also had no significant impact on yields of reducing sugars of unmalted *Safrari,* but had a significant impact on those of malted *Safrari* mashes (P = 0.503 and 0.000 respectively) (Table 3). Its contribution to reducing sugar yields was merely 5% for unmalted *Safrari*, but as much as 38% for malted *Safrari* (Table 3). This remarkable difference was once more to be attributed to the virtues of the malting process and not simply to supplements of the Hitempase/Brewers Protease couple as such. Efficient starch hydrolysis by α-amylase indeed occurs only after the breakdown of cereal grain cell walls by β-glucanase, followed by liberation of starch granules due to proteolysis of the protein matrix enrobing them. This should be the natural sequence of events which accounted for the results obtained for the malted sample type as compared to the unmalted *Safrari* sample type. The interaction Bioglucanase/Brewers Protease (X_2X_3) had no significant impact on reducing sugars yields for both the unmalted *Safrari* and malted *Safrari* mashes (P = 0.130 and 0.072 respectively) (Table 3). Its contribution to reducing sugars yields was 12 and 5% respectively for both mash types (Table 3). The Bioglucanase/Brewers Protease combination plays a supporting role in starch hydrolysis during mashing (Desobgo et al., 2010). Table 4 statistically confirmed the observation that *Safrari* sorghum malted type samples were more potential mashing materials than unmalted *Safrari* sorghum adjuncts to which commercial enzymes are supplemented for the production of worts of higher reducing sugars yields (P = 0.001).

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 ≥ 90 g/L. The theoretical optimal combination of enzyme action for unmalted *Safrari* gave the following triplet of coded variables for reducing sugars content: 0.442, 0.866 and – 0.816 (2163 U, 937.5 BGU and 0 mg real variables) for Hitempase, Bioglucanase and Brewers Protease respectively.

This triplet allowed for a maximal reducing sugars content of 126.57 g/L. The triplet for malted *Safrari* was – 1, – 0.612 and – 0,816 (0 U, 137.48 BGU and 0 mg real variables). It allowed for a maximal reducing sugars content of 168.56 g/L. The optimal enzyme combinations were thus different and gave different results for the two mash types. These results showed that maximal reducing sugars contents could be obtained using Hitempase and Bioglucanase but not Brewers Protease supplements when mashing unmalted *Safrari*, whereas only Bioglucanase, and in limited amounts, could be used for mashing malted *Safrari*. The significant difference (P = 0.001) between reducing sugars content of unmalted and malted *Safrari* worts is shown in Table 5. Minimal reducing sugars content (90 g/L) could be obtained under these conditions permitting the highlight of the optimal domain (Figure 4).

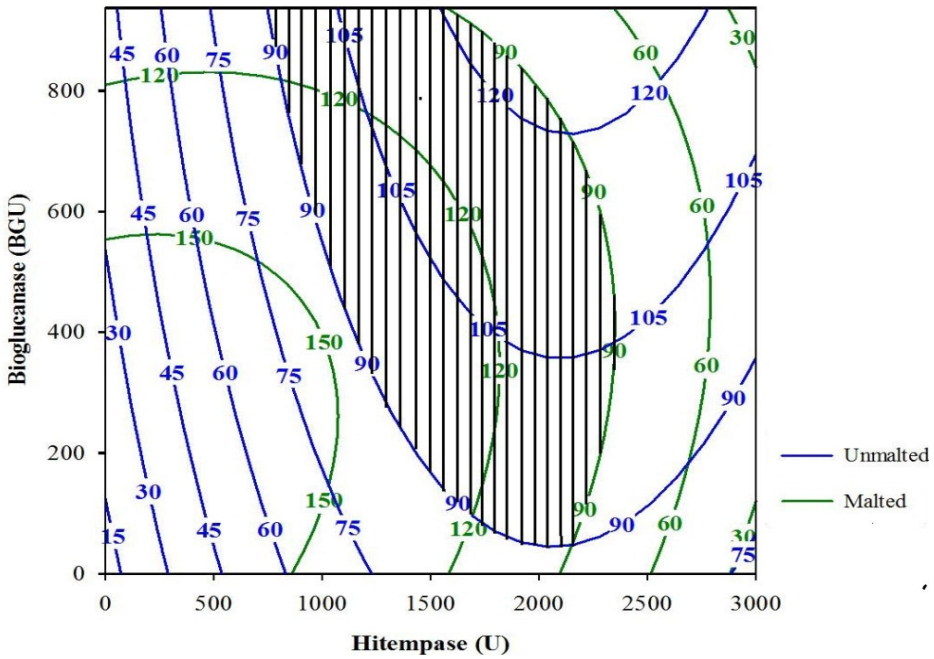

Figure 4. Response surface curves for the enzyme combinations providing for optimal reducing sugars content (g/L) for unmalted and malted sorghum worts of the cultivar *Safrari*.

Conclusion

This work clearly showed that though Hitempase 2XL was the most important enzyme component for producing significant amounts of reducing sugars during mashing of unmalted *Safrari* grist, it appeared to be indispensable for mashing malted *Safrari* grist. Bioglucanase TX and Brewers Protease were merely supporting enzymes to Hitampase 2XL only for mashing unmalted *Safrari* grist, as they too showed little or no participatory role in increasing reducing sugars amounts when used as supplements when mashing with malted *Safrari* grist - in general, the malting process seems to be auto-sufficient in accounting for the required natural mashing enzymes in terms of both amounts and quality, for producing the amounts of reducing sugars obtainable when mashing malted *Safrari* grist.

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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%). Journal of the Institute of Brewing, 98: 515-523.

Baranyi J, Pin C, Ross T (1999). Validating and comparing predictive models. International Journal of Food Microbiology 48:159-166.

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

Dale CJ, Young TW, Omole, T (1990). Small scale mashing experiments with grist containing high proportions of raw sorghum. J. Inst. Brew., 96: 403–409.

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. Micro-biol. Biotechnol., 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. Inst. Brew, 109: 208-217.

Goode DL, Halbert C, Arendt EK (2002). Mashing studies with unmalted sorghum and malted barley. J. of the Institut of Brew 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.MacFadden DP, Clayton M (1989). Brewing with sorghum – Use of exogenous enzymes. Brewing and Beverage Industry International 1:71–81.

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. Brewing, 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. Inst. 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: In importance of sorghum in Africa. Department of Food Science, Pretoria, South Africa: University of Pretoria.
Taylor JRN, Dewar J (2001). Developments in sorghum food technologies. In: Advances in Food and Nutrition Research(Taylor, J., ed), pp. 217-264 San Diego, USA: Academic Press.