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Full Length Research Paper

Modeling the action of technical mashing enzymes on extracts and free-amino nitrogen yields of the *Madjeru* sorghum cultivar

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The action of three technical mashing enzymes (hitempase 2XL, bioglucanase-TX and brewers protease) on yields of extract and free amino nitrogen (FAN) of the worts of mashes of unmalted and malted *Madjeru* sorghum was modeled and analyzed using the response surface methodology. The analysis showed that increasing amounts of hitempase 2XL considerably increased yields of extract during mashing of unmalted *Madjeru* sorghum grist. The use of bioglucanase-TX was not indispensable, while Brewers' protease contributed very little. Increasing amounts of hitempase contributed approximately 45% of the free amino nitrogen, while Brewers' protease influence amounted to not more than 15%. Bioglucanase's action was globally nil. Addition of the three enzymes into malted *Madjeru* sorghum mashes had no significant effect on the yields of extracts and FAN, but the milling operation singularly liberated more than 50% of FAN for both mash types. Optimization of the concerted actions of the three enzymes for extract yield for unmalted *Madjeru* sorghum mash gave a combination of (1960.5 U; 132.61 BGU and 28.86 mg) for hitempase, bioglucanase and brewers protease respectively). This gave a maximal extract yield of 16.55 °P. This combination was: 2610 U; 0 BGU and 40.44 mg for malted *Madjeru* sorghum mash, giving a maximal extract yield of 16.35 °P. Optimization for free amino nitrogen for unmalted *Madjeru* sorghum mash gave a combination of: 3000 U; 0 BGU and 100 mg for hitempase, bioglucanase and brewers protease respectively). This gave maximal FAN of 93.55 mg/L. The combination was: 3000 U; 0 BGU and 100 mg for malted *Madjeru* sorghum mash, giving a maximal FAN of 144.48 mg/L.

Key words: Modeling, technical mashing enzymes, yields of extract, free-amino-nitrogen, *Madjeru*, optimization.

INTRODUCTION

Sorghum in its malted form or as adjuncts has become a potential brewing cereal particularly in the tropics where barley is not grown (Taylor, 1983; Aisien and Muts, 1987; Arri, 1989; Palmer, 1989; Adejemilua, 1995). Its low contents of potential mashing enzymes (Aisien, 1982; EtokAkpan and Palmer, 1990; Nso et al., 2003, 2006) due to their poor development during malting has often triggered the use of technical mashing enzymes as supplements (Agu and Palmer, 1998; Goode et al., 2002, 2003; Goode and Arendt, 2003) to achieve higher yields in extracts and other important wort specifications in beer brewing. Modeling and optimization approaches to ameliorate wort properties of sorghum grist and buckwheat malts has recently thrown more light into the precise role played by supplements of technical mashing enzymes (Goode et al., 2003, Phiarais et al., 2006, Desobgo et al., 2010). It is not however clear whether the use of technical mashing enzymes to obtain optimal mashing and brewing specifications for worts when using malted

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sorghum is indispensable. A series of work using two popular sorghum cultivars used in northern Cameroon for the production of the traditional beer Bili-Bili is currently taking place. This will help to have a clear understanding on the necessity of applying or not, technical enzymes when mashing with these cultivars. Some of the findings were recently reported for yields of reducing sugars for these cultivars (Desobgo et al., 2011a, b).

As a follow up, the effect of the same three technical mashing enzymes (Hitempase 2XL, Bioglucanase B-10L and Brewers protease) were modeled and optimized in this work for yields of extracts and free amino nitrogen during mashing of *Madjeru,* one of the popular sorghum cultivars of Northern Cameroon, using the response surface methodology (RSM).

MATERIALS AND METHODS

Enzymes

The characteristics of the technical enzymes used (Hitempase 2XL, a thermo stable α-amylase from *Baccillus licheniformis,* Brewers protease from *Bacillus amyloliquefaciens* and Bioglucanase TX, an enzymatic composition of β-glucanase and hemicellulases from *Trichodermareesei*) 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.

Modelling

Modelling was carried out as previously described (Desobgo et al., 2011a, b).

Validation of models

The models were validated as previously described (Desobgo et al., 2011a, b).

Mashing

Mashing was carried out as described (Desobgo et al., 2011a, b).

Determination of extract

Extract was determined as described in analytica-EBC, 1998.

Determination of free amino nitrogen

Free amino nitrogen (FAN) was determined as described in analytica-EBC, 1998.

Optimization of models

Models were optimized as previously described (Desobgo et al., 2011a, b).

RESULTS AND DISCUSSION

The modeling and optimization of the action of mashing enzymes on the two key wort properties: extract and free amino nitrogen (FAN), was carried out for the experimental design required for manipulation in the laboratory. Table 2 shows the results obtained for extracts and free amino nitrogen (FAN) after mashing unmalted and malted *Madjeru* using the technical enzymes hitempase 2XL (α-amylase), bioglucanase TX (β-glucanase) and brewers protease (protease).

Modeling and validation of results of yields of extract

The mathematical models obtained for extracts after mashing unmalted and malted *Madjeru* were as follows respectively:

$$Y_{MadEX}\ (X_1,\ X_2,\ X_3) = 15.134 + 4.477X_1 - 1.397X_2 - 1.729X_3 + 0.980X_1X_2 - 2.155X_1X_3 + 0.296X_2X_3 - 7.507X_1^2 - 0.964X_2^2 - 3.732X_3^2 \quad (1)$$

$$Y_{MadMEX}\ (X_1,\ X_2,\ X_3) = 15.566 + 1.036X_1 - 0.197X_2 - 0.304X_3 - 0.842X_1X_2 + 0.784X_1X_3 + 0.623X_2X_3 - 1.11X_1^2 - 0.14X_2^2 - 0.843X_3^2 \quad (2)$$

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

The mathematical models were polynomials having several variables with coefficients of determination $R^2 = 0.940$ for unmalted *Madjeru* and $R^2 = 0.980$ for malted *Madjeru*. These coefficients, coupled to AAD values of 0.091 and 0.006 for unmalted and malted *Madjeru* respectively, allowed for the validation of the models for yields of extract of the worts. In addition, a bias factor of 1.05 and 1 for unmalted and malted *Madjeru* respectively, coupled to exactitude factors of 1.19 and 1.01 for both unmalted and malted *Madjeru* respectively, also allowed for validation of the models according to the method described (Ross, 1996). 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 yields of extracts was ≤ 0.05 or ≥ 0.05 respectively (Table 3).

Effect of hitempase 2XL on yields of extract

The impact of hitempase 2XL as sole mashing enzyme on the yield of extract of unmalted and malted *Madjeru* is shown in Figure 1A. Extract yield increased from 2 °P with increasing concentration of enzyme for unmalted *Madjeru* mash to attain a maximum level (15.72°P) at

Table 1. Characteristics of commercial mashing enzyme preparations.

Commercial mashing enzyme	Organism of origin	Activity	Description	Temperature optima	pH optima	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
Bioglucanase TX	*Trichoderma reesei*	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			*Madjeru*											
Hit	Bio	Brew Prot	Hit (U)	Bio (BGU)	Brew Prot (mg)	Unmalted						malted					
						Extract (°P)			FAN (mg/L)			Extract (°P)			FAN (mg/L)		
X_1	X_2	X_3	X_1	X_2	X_3	Exp[a]	Theo[b]	Res[c]	Exp	Theo	Res	Exp	Theo	Res	Exp	Theo	Res
0.000	0.000	0.000	1500	468.75	50	15.07	15.13	-0.06	64.34	63.50	0.84	15.60	15.57	0.03	94.00	94.81	-0.81
1.000	0.000	0.000	3000	468.75	50	13.93	12.10	1.83	71.79	71.17	0.62	15.70	15.49	0.21	107.70	107.32	0.38
0.500	0.866	0.000	2250	937.5	50	13.10	13.99	-0.89	98.00	94.42	3.58	15.08	15.17	-0.09	147.00	141.67	5.33
-0.500	-0.866	0.000	750	0.00	50	13.61	11.93	1.68	65.38	64.31	1.07	14.56	14.47	0.09	98.10	95.82	2.28
0.500	-0.866	0.000	2250	0.00	50	15.09	15.56	-0.47	83.08	83.01	0.07	16.12	16.24	-0.12	126.00	125.54	0.46
-0.500	0.866	0.000	750	937.5	50	9.05	8.66	0.39	81.79	82.30	-0.51	14.97	14.86	0.11	122.70	123.85	-1.15
0.500	0.289	0.816	2250	615.18	100	10.00	10.45	-0.45	84.78	86.08	-1.30	15.14	15.27	-0.13	130.00	130.96	-0.96
-0.500	-0.289	-0.816	750	312.32	0.0	11.25	9.60	1.65	44.00	35.71	8.29	14.97	14.85	0.12	66.00	53.61	12.39
0.500	-0.289	-0.816	2250	312.32	0.0	14.14	15.55	-1.41	48.00	48.97	-0.97	15.39	15.49	-0.10	72.00	73.27	-1.27
0.000	0.577	-0.816	1500	781.07	0.0	13.20	12.79	0.41	49.07	52.58	-3.51	14.76	14.80	-0.04	73.60	78.48	-4.88
-0.500	0.289	0.816	750	615.18	100	8.74	7.45	1.29	68.86	68.53	0.33	13.93	13.84	0.09	103.30	103.07	0.23
0.000	-0.577	0.816	1500	156.43	100	10.73	11.58	-0.85	73.80	72.84	0.96	14.56	14.53	0.03	110.70	109.97	0.73
0.000	0.000	0.000	1500	468.75	50	14.56	15.13	-0.57	62.27	63.50	-1.23	15.60	15.57	0.03	93.40	94.81	-1.41
-1.000	0.000	0.000	0.000	468.75	50	1.52	3.15	-1.63	40.88	40.35	0.53	13.20	13.42	-0.22	61.30	59.78	1.52
-1.000	-0.866	-0.816	0.000	0.0	0.0	1.20	1.86	-0.66	33.31	37.13	-3.82	13.52	13.52	0.00	50.00	56.24	-6.24
0.000	0.000	0.000	1500	468.75	50	14.90	15.13	-0.23	62.00	63.50	-1.50	15.60	15.57	0.03	93.00	94.81	-1.81
0.000	0.000	0.000	1500	468.75	50	15.08	15.13	-0.05	60.00	63.50	-3.50	15.46	15.57	-0.11	90.00	94.81	-4.81

[a]Experimental result values. [b]Theoretical values (values coming from mathematical models). [c] Residue.

about 2038 U, followed by a slight and steady decrease thereafter. It was already high (13.52 °P) in the absence of Hitempase for malted *Madjeru* mash and only increased slightly to attain a maximal level (16.04 °P) at 2268 U of enzyme concentration. When the mathematical models were applied to predict the impact of supplements of bioglucanase and brewers protease at concentrations of 750 BGU and 60 mg respectively and in the presence of Hitempase, it was observed that the profile of extract yields remained similar to that in Figure 1A (compare with

Table 3. Estimation of regression coefficients for the extracts of umalted and malted *Madjeru*.

Effects	Coefficient		Std. deviation		t-statistics		P-value	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Constant	15.134	15.566	0.766	0.08	19.768	195.176	0.000	0.000
X_1	4.477	1.036	0.788	0.082	5.684	12.63	0.001	0.000
X_2	-1.397	-0.197	0.687	0.072	-1.762	-2.387	0.121	0.048
X_3	-1.729	-0.304	0.649	0.068	-2.174	-3.67	0.066	0.008
$X_{1\ 2}$	-7.507	-1.11	1.35	0.141	-5.56	-7.895	0.001	0.000
$X_{2\ 2}$	-0.964	-0.14	0.983	0.102	-0.736	-1.027	0.486	0.339
$X_{3\ 2}$	-3.732	-0.843	0.848	0.088	-2.932	-6.357	0.022	0.000
X_1*X_2	0.98	-0.842	1.541	0.161	0.55	-4.543	0.599	0.003
X_2*X_3	0.296	0.623	1.357	0.141	0.154	3.115	0.882	0.017
X_1*X_3	-2.155	0.784	1.606	0.167	-1.095	3.823	0.310	0.007

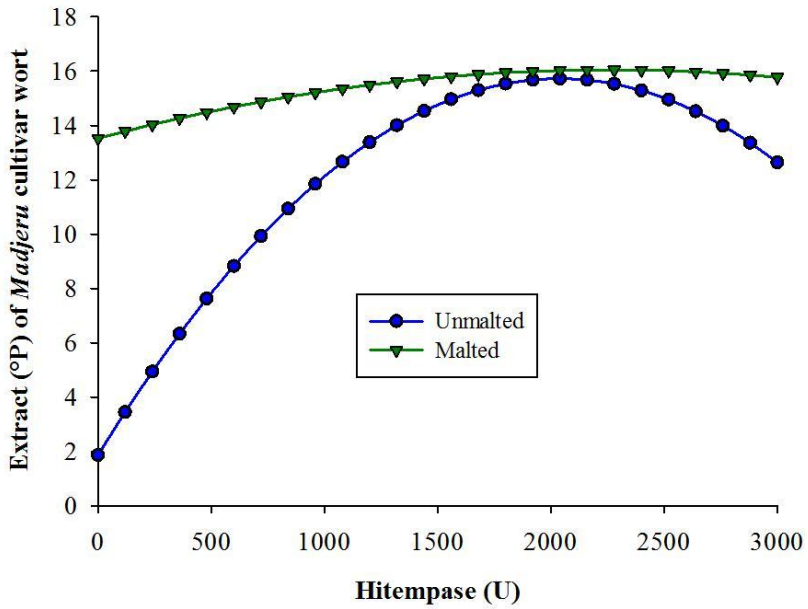

Figure 1A. Effect of concentration of hitempase (α-amylase) as sole mashing enzyme on yield of wort extract of sorghum cultivar *Madjeru*.

Figure 1B). Starch is indeed the main macromolecule of cereals and the main substrate of α-amylase. It is therefore expected that this enzyme contributes to the greatest amount of soluble materials 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). Figures 1 A and B also showed that supplements of hitempase in unmalted *Madjeru* mash was by far more useful than for the malted *Madjeru* mash type. The important role of milling in obtaining instantaneous dissolution of soluble materials at the beginning of mashing was better displayed for the malted *Madjeru* mash type than for the unmalted *Madjeru* mash type (93 and 14% of extract yields respectively). The soluble nitrogenous compounds and reducing sugars (Hough et al., 1982) in the medium (Figures 1A and B).

From the mathematical models, it was shown that in its linear form (X_1), hitempase contributed 19 and 18% of extract yields for unmalted and malted *Madjeru* respectively (Figures 4A and B). This more or less equitable contribu-tion of extracts in the worts of the two mash types, pairs with the observations made earlier whereby extract levels were virtually the same for both. Moreover, statistical analyses also showed that this contribution was significant (P = 0.001 and 0.000 for unmalted and malted *Madjeru* respectively (Table 3). In its quadratic form (X_1^2), hitempase remained statistically significant for mashing both unmalted *Madjeru* and malted *Madjeru* (P = 0.001 and 0.000 respectively). This confirmed the earlier biolo-gical observation according to which supplements of this

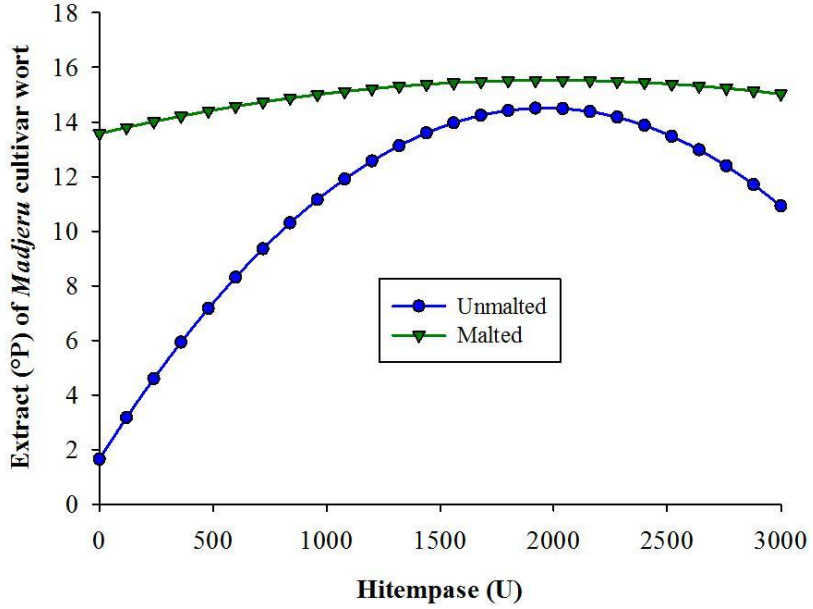

Figure 1B. Effect of concentration of hitempase (α-amylase) in the presence of fixed concentrations of bioglucanase (750 BGU) and brewers protease (60 mg) on yield of wort extract of sorghum cultivar *Madjeru*

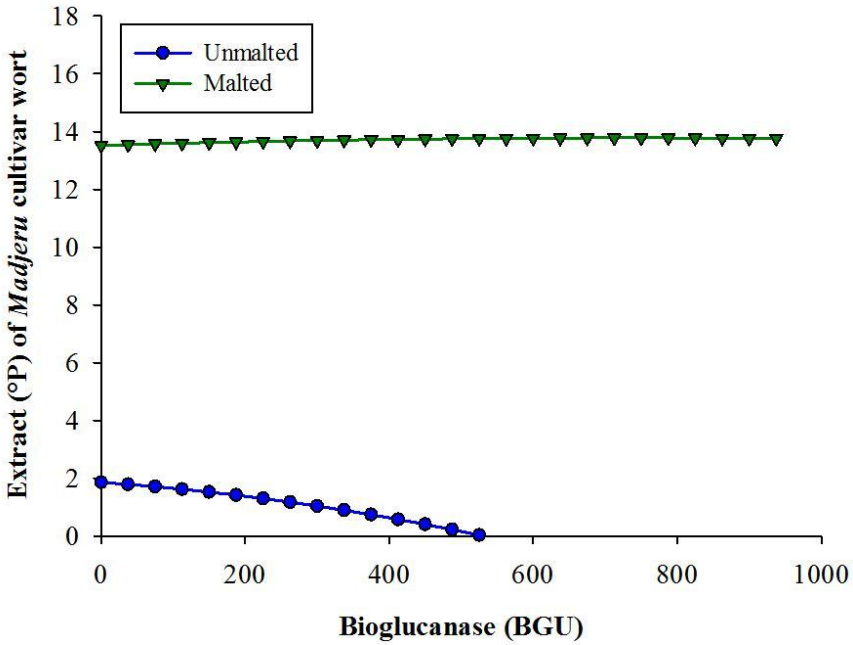

Figure 2A. Effect of concentration of bioglucanase (β-glucanase) as sole mashing enzyme on yield of wort extract of sorghum cultivar *Madjeru*.

enzyme in malted mashes of *Madjeru,* was also necessary. Its contribution in increasing extract yields in its quadratic form (X_1^2) (excess of α-amylase in principle) was indeed 33 and 19% for unmalted and malted *Madjeru* respectively (Figures 4A and B).

Effect of bioglucanase TX on yields of extract

Figure 2A shows the effect of mashing unmalted and malted *Madjeru* using bioglucanase, as sole mashing enzyme, on yields of extract. There was a progressive

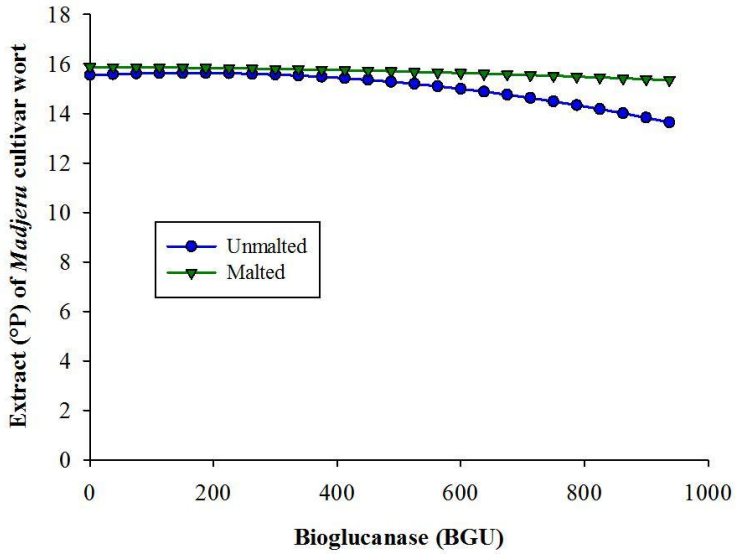

Figure 2B. Effect of concentration of bioglucanase (β-glucanase) in the presence of fixed concentrations of hitempase (1875 U) and brewers protease (60 mg) on yield of wort extract of sorghum cultivar *Madjeru*.

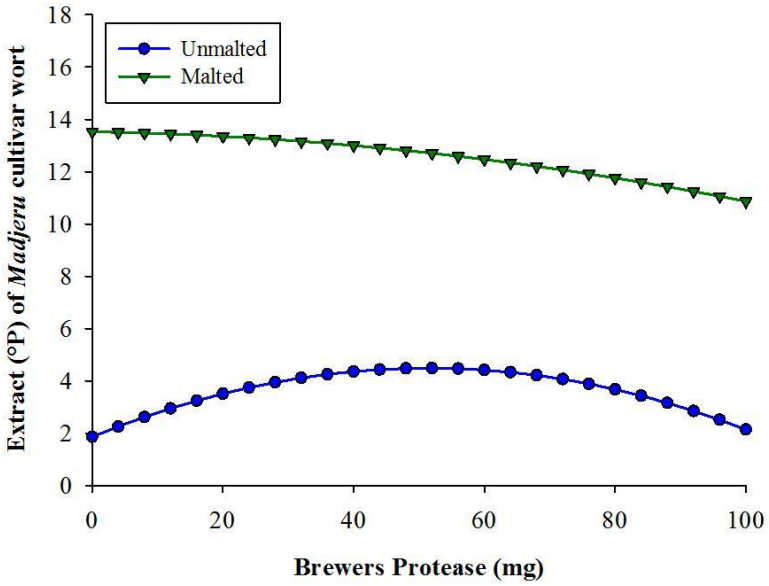

Figure 3A. Effect of concentration of brewers protease as sole mashing enzyme on yield of wort extract of sorghum cultivar *Madjeru*.

decrease of yield of extract from 1.86 °P to nil as enzyme concentration increased for unmalted *Madjeru* wort. This figure also shows that the extract obtained (1.86 °P) was probably completely due to milling, suggesting that the enzyme plays no important role in production of extract from the grist of the unmalted *Madjeru* cultivar during mashing. The yield of extract for malted *Madjeru* mash was virtually at its maximal level (13.78 °P) even in the absence of bioglucanase and remained virtually constant with increasing enzyme concentration. Bioglucanase was therefore not a backbone enzyme for extract production during mashing. A similar application of the mathematical models as carried out earlier for hitempase's action, using 60 mg of brewers protease and 1875 U hitempase, predicted that the supplementation of these two mashing enzymes could provide similar results in extract yields for both unmalted and malted *Madjeru* mashes (Figure 2B). Hitempase once more revealed that it was the real

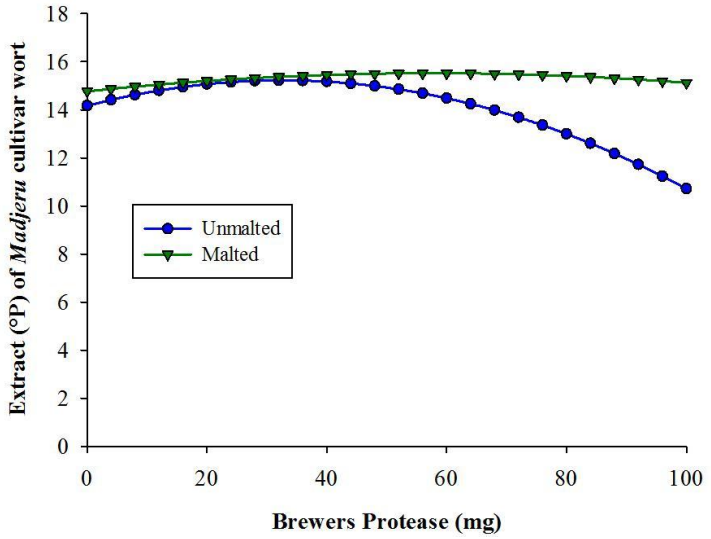

Figure 3B. Effect of concentration of brewers protease in the presence of fixed concentrations of hitempase (1875 U) and bioglucanase (750 BGU) on yield of wort extract of sorghum cultivar *Madjeru*.

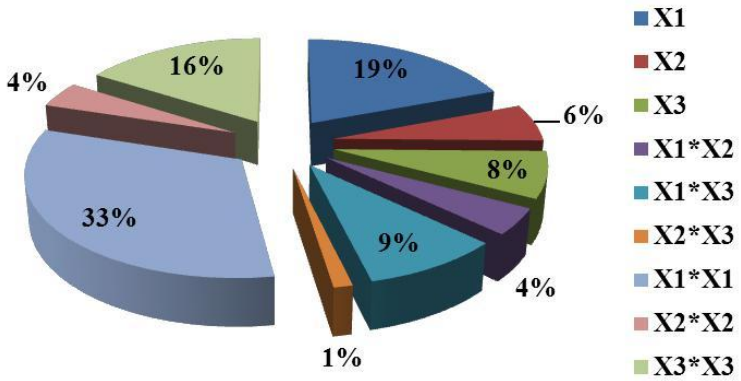

Figure 4A. Contribution to yield of wort extract (°P) of each factor in its linear, quadratic and interaction (combined) forms for unmalted sorghum cultivar *Madjeru*.

backbone enzyme that contributed for most of the yields of extract. The level of extract yields reached almost 15.54 °P for unmalted *Madjeru* and 15.87 °P for malted *Madjeru* mash types. From the two figures, it is also important to underline the natural virtues of the malting procedure in rendering the Madjeru grains potentially mash-able to reasonable extract yields in the absence of all these enzymes. These observations were all statistically confirmed. Indeed, in its linear form (X_2), bioglucanase's action was not significant (P = 0.121) for unmalted but significant for malted *Madjeru* (P = 0.048) (Table 3).

Figures 4A and B showed that this enzyme contributed barely for 6 and 3% of extract yields for unmalted and malted *Madjeru* respectively. In its quadratic form (X_2^2) (excess of enzyme in principle), bioglucanase contributed 4 and 3% of extract yield for unmalted and malted *Madjeru* respectively (Figures 4A and B). Similarly, these contributions were statistically not significant for both unmalted and malted *Madjeru* mash types (p = 0.486 and 0.0.339 respectively) (Table 3).

Effect of brewers protease on yields of extract

The effect of mashing unmalted and malted *Madjeru* on yields of extract using as sole mashing enzyme, "brewers protease", is shown in Figure 3A. Yields of extracts

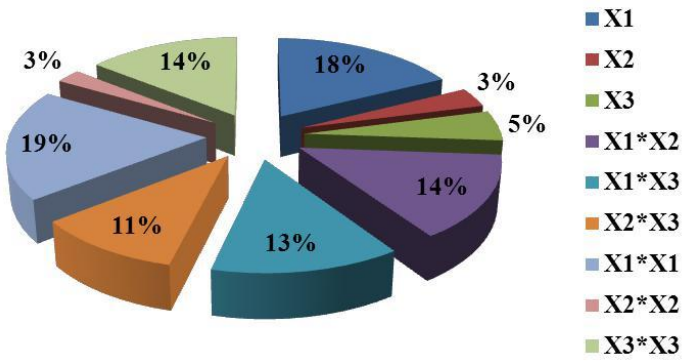

Figure 4B. Contribution to yield of wort extract (°P) of each factor in its linear, quadratic and interaction (combined) forms for malted sorghum cultivar *Madjeru*.

Table 4. ANOVA for the extracts of umalted and malted *Madjeru*.

Source	Ddl	Sum square		Mean square		F-value		P-value	
		Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Regression	9	285.91	9.965	31.768	1.107	12.14	38.993	0.002	0.000
Linear	3	156.256	6.507	52.085	2.169	19.905	76.379	0.001	0.000
Quadratic	3	126.512	2.496	42.171	0.832	16.116	29.299	0.002	0.000
Interactions	3	3.141	0.963	1.047	0.321	0.400	11.302	0.757	0.004
Residual error	7	18.317	0.199	2.617	0.028				
Total error	16	304.227	10.164						

gradually increased from 1.86 °P with increasing amounts of enzyme to reach a maximum of 4.48 °P at enzyme concentration of 51.39 mg. This small yield of extract due to the action of this enzyme could be attributed to its capacity of hydrolyzing proteins into soluble amino acids and peptides (Briggs et al., 2004) during which additional soluble sugars could also be liberated. Figure 3A however shows once more that in the absence of supplements of "brewers protease", maximal extract was obtained when mashing with malted *Madjeru*. This could be once more attributed to the virtues well known to the malting process as explained earlier. These results confirmed earlier observations (Desobgo et al., 2011a, b). The gradual and slight decrease of extract yields with increasing amounts of "brewers protease" for malted *Madjeru* and unmalted *Madjeru* (as from 51.39 mg concentration) mash types could once more be attributed to reactions between soluble nitrogenous functions and some of the soluble sugars. The mathematical models were once more used to predict the yield in extract as carried out earlier for hitempase and bioglucanase actions. Thus, using 1875 U hitempase and 750 BGU of bioglucanase with increasing amounts of "brewers protease", the models once more showed that the adding of these mashing enzymes could provide similar results in extract yields for both unmalted and malted *Madjeru* mashes (Figure 3B). The aforementioned 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 was for malted *Madjeru* mash (P = 0.066 and 0.008 respectively) (Table 3). Its contribution to extract yield was 8 and 5% respectively for both unmalted and malted mashes (Figures 4A and B).

The impact of the enzyme in its quadratic form (X_3^2) was significant for both unmalted and malted mashes (P = 0.022 and 0.000 respectively) (Table 3). Its contribution to extract yield was 16 and 14% respectively (Figures 4A and 4).

Effect of enzymes' interactions on yields of extract

The models were further exploited to predict the impacts of enzyme interactions (X_1X_2, X_1X_3 and X_2X_3) on yields of extract. The results are shown in Figures 4A and B. They were globally not statistically significant for unmalted *Madjeru* mashes (P = 0.757), but were for malted *Madjeru* mashes (P = 0.004) (Table 4). The interaction X_1X_2 (hitempase/bioglucanase) had no significant impact on unmalted *Madjeru* mash, but had for malted *Madjeru* (P = 0.599 and 0.003 respectively (Table 3). It contributed

Table 5. ANOVA for comparing extracts of unmalted and malted *Madjeru* worts.

Source	DF	Sum of squares	Mean of squares	F-value	P-value
Inter-groups	1	102.344	102.344	10.420	0.003
Intra-groups	32	314.391	9.824		
Total	33	416.735			

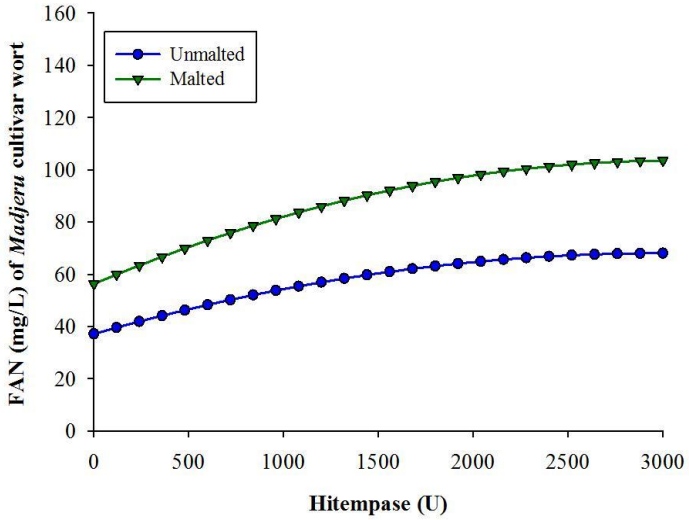

Figure 5A. Effect of concentration of hitempase (α-amylase) as sole mashing enzyme on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

for merely 4% of extract for unmalted *Madjeru* mash, but up to 14% for malted *Madjeru* mash (Figures 4A and B). It is however significant to underline that this important contribution could be attributed to the intrinsic virtues that malting offers when mashing with malted *Madjeru* and not to the hitempase/bioglucanase interaction as such. 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 aforementioned (Desobgo et al., 2010). The interaction X_1X_3 corresponding to the couple hitempase/brewers protease, also had no significant impact on extract yields of unmalted *Madjeru* mash (P = 0.310), but had on malted *Madjeru* mash (P = 0.007) (Table 3). Its contribution to extract yield was 9 and 13% respectively (Figures 4A and B). 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 extract yields for unmalted *Madjeru* mash (P = 0.882), but had for malted *Madjeru* mashes (P = 0.017) (Table 3). Its contribution to extract yields was 1 and 11% respectively for both mash types (Figures 4A and B). 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).

Table 5 statistically confirms the observation that malted *Madjeru* worts associated with the technical enzymes have better yields of extracts than unmalted *Madjeru* worts (P = 0.003).

Modeling and validation of results of free amino nitrogen (FAN)

The mathematical models obtained for free amino nitrogen (FAN) for mashing unmalted and malted *Madjeru* were as follows respectively:

$$Y_{MadAAL}(X_1, X_2, X_3) = 63.504 + 15.411X_1 + 8.487X_2 + 18.418X_3 - 3.798X_1X_2 + 3.976X_1X_3 + 7.807X_2X_3 - 7.747X_1^2 + 25.922X_2^2 - 8.636X_3^2 \quad (3)$$

$$Y_{MadMAAL}(X_1, X_2, X_3) = 94.807 + 23.771X_1 + 12.745X_2$$

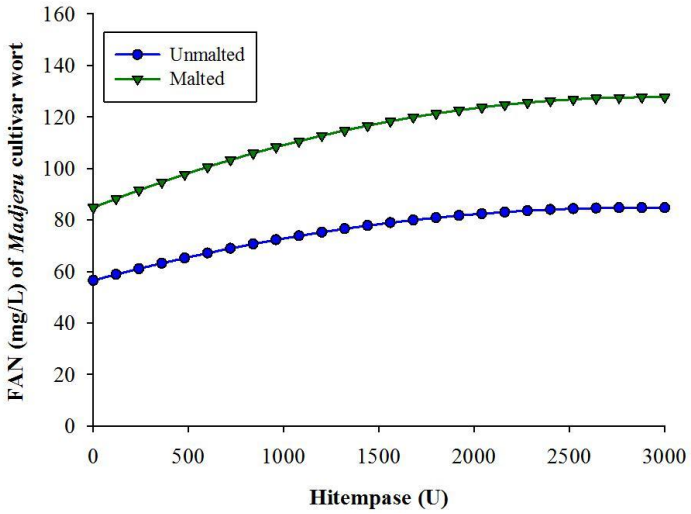

Figure 5B. Effect of concentration of hitempase (α-amylase) in the presence of fixed concentrations of bioglucanase (750 BGU) and brewers protease (60 mg) on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

Table 6. Estimation of regression coefficients for free amino nitrogen of umalted and malted *Madjeru*.

Effects	Coefficient		Std. deviation		t-statistics		P-value	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Constant	63.504	94.807	2.047	3.024	31.026	31.347	0.000	0.000
X_1	15.411	23.771	2.106	3.111	7.319	7.641	0.000	0.000
X_2	8.487	12.745	1.836	2.713	4.003	4.069	0.005	0.005
X_3	18.418	28.311	1.735	2.563	8.663	9.012	0.000	0.000
X_1 $_2$	-7.747	-11.26	3.61	5.334	-2.146	-2.111	0.069	0.073
X_2 $_2$	25.922	36.639	2.627	3.882	7.4	7.658	0.000	0.000
X_3 $_2$	-8.636	-11.98	2.266	3.348	-2.538	-2.383	0.039	0.049
X_1*X_2	-3.798	-6.875	4.121	6.089	-0.798	-0.978	0.451	0.361
X_2*X_3	7.807	12.315	3.627	5.359	1.521	1.624	0.172	0.148
X_1*X_3	3.976	7.478	4.294	6.345	0.756	0.962	0.475	0.368

$$+28.311X_3 - 6.875X_1X_2 + 7.478X_1X_3 + 12.315X_2X_3 - 11.261X_1^2 + 39.639X_2^2 - 11.983X_3^2 \quad (4)$$

With: Y_{MadAAL} (X_1, X_2, X_3) representing the mathematical model for unmalted *Madjeru*; $Y_{MadMAAL}$ (X_1, X_2, X_3) for malted *Madjeru;* X_1, hitempase; X_2, bioglucanase and X_3, brewers protease (protéase). These mathematical models were once more polynomials having several variables with determination coefficients of R^2 = 0.973 for unmalted *Madjeru* and R^2 = 0.974 for malted *Madjeru*. These coefficients, coupled to AAD values of 0.037 and 0.036 for unmalted and malted *Madjeru* respectively, allowed for the validation of the models for assessment of the wort free amino nitrogen content. In addition, a bias factor of 1 for both unmalted and malted *Madjeru* mash types, coupled to exactitude factors of 1.00 for both mash types, also allowed for validation of the models according to the method described (Ross, 1996). The factors of the models were once more 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) or of interaction form (X_1X_2, X_1X_3, and X_2X_3). They were statistically considered significant or not if the probability (P) in increasing yields of FAN was ≤ 0.05 or ≥ 0.05 respectively (Table 6).

Effect of hitempase 2XL on yields of free amino nitrogen (FAN)

The impact of hitempase as sole mashing enzyme onwort FAN for unmalted and malted *Madjeru* is shown in Figure 5A. Free amino nitrogen content of wort gradually increased from 37.12 mg/L with increasing enzyme

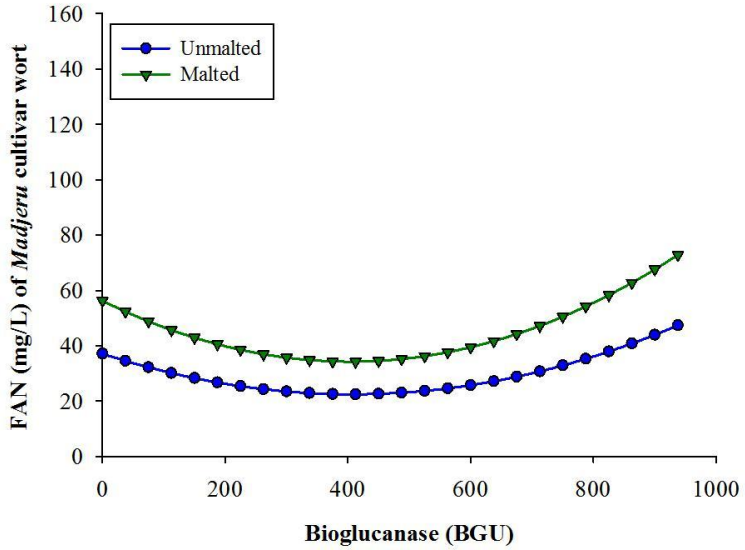

Figure 6A. Effect of concentration of bioglucanase (β-glucanase) as sole mashing enzyme on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

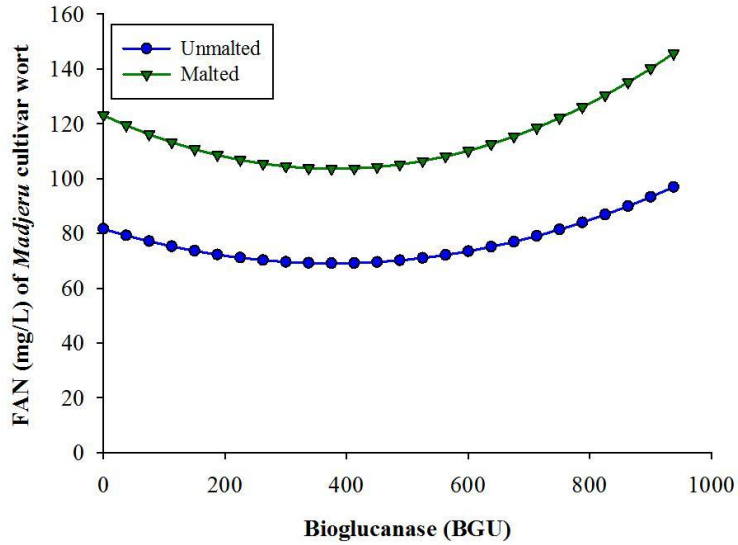

Figure 6B. Effect of concentration of bioglucanase (β-glucanase) in the presence of fixed concentrations of hitempase (1875 U) and brewers protease (60 mg) on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

concentration to reach a maximum of 68.04 mg/L for both unmalted mashes and from 56.26 mg/L to reach a maximum of 103.51 mg/L for malted *Madjeru* mashes. The Figure also showed that for both mash types, the yields in FAN in the absence of the enzyme represented more than 50% of the final yields. This suggests that the milling operation was at the basis of the instantaneous dissolution of these considerable amounts of free amino nitrogen at the start of mashing. Although hitempase is not a protein hydrolyzing enzyme, it exposes more free amino nitrogen functions upon acting on globular proteins and starch granules. This could explain the increase in FAN observed with increase in enzyme concentration. The higher FAN content for malted *Madjeru* mash compared to unmalted *Madjeru* mash is once more to be attributed to the natural virtues that the grains incur during

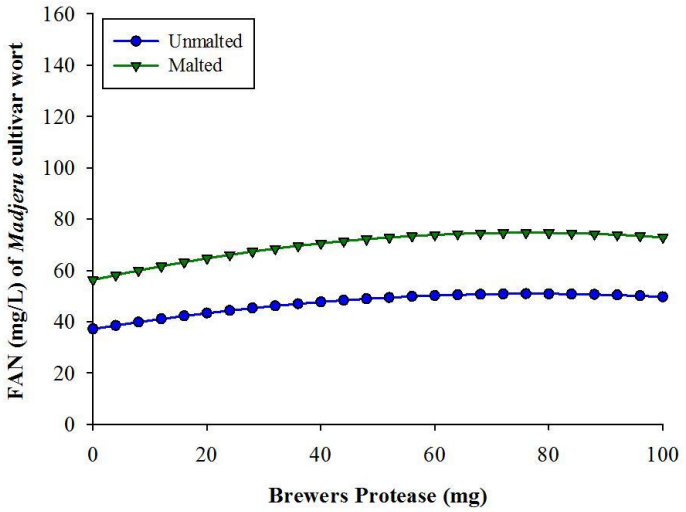

Figure 7A. Effect of concentration of brewers protease as sole mashing enzyme on yield of wort free amino nitrogen of sorghum cultivar *Madjeru.*

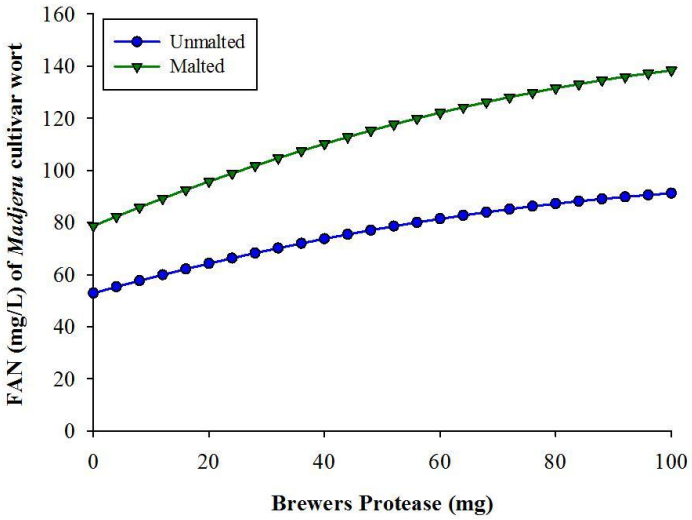

Figure 7B. Effect of concentration of brewers protease in the presence of fixed concentrations of hitempase (1875 U) and bioglucanase (750 BGU) on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

during the malting process. Use of the models to predict the profile of FAN content of worts if the mashing enzymes bioglucanase (at 750 BGU) and brewers protease (at 60 mg) were coupled to hitempase's action, showed a profile of increases in yields of FAN similar to that observed in Figure 5B with increments contributing to amounts equivalent to roughly 15%. The models also showed that hitempase (X_1), in its first degree form, contributed for 15% of the FAN content of both the unmalted and malted *Madjeru* mashes (Figures 8A and B). This contribution was statistically significant for the two mash types (P = 0.000) (Table 6).

Similarly, in its quadratic form (X_1^2), hitempase's effect was not significant for the two mash types (P = 0.069 and 0.073 respectively) (Table 6). Its contribution in this form was 8 and 7% respectively (Figures 8A and B).

Effect of bioglucanase TX on yields of free amino nitrogen (FAN)

Figure 6A shows the effect of bioglucanase on the FAN content as sole mashing enzyme in unmalted and malted *Madjeru*. This content initially decreased with increasing enzyme concentration in both mash types, dropping from

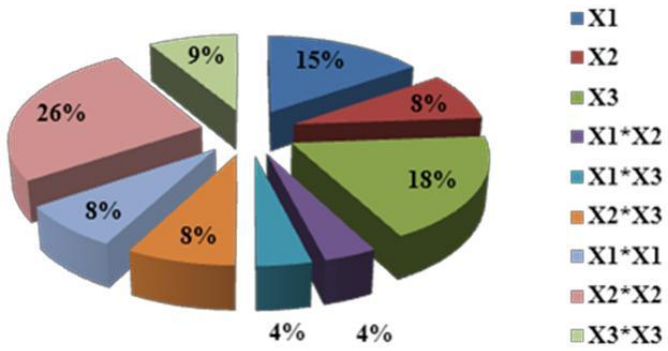

Figure 8A. Contribution to yield of wort free amino nitrogen (FAN) of each factor in its linear, quadratic and interaction (combined) forms for unmalted sorghum cultivar *Madjeru*.

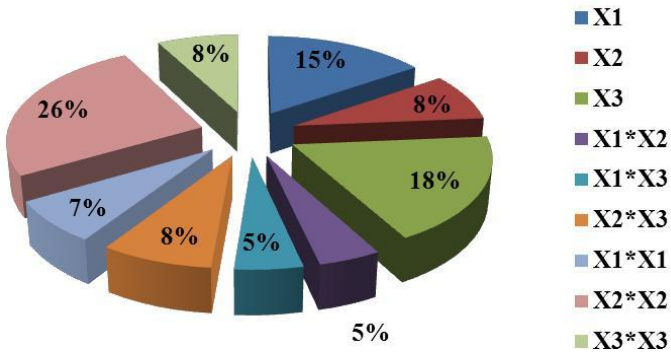

Figure 8B. Contribution to yield of wort free amino nitrogen (FAN) of each factor in its linear, quadratic and interaction (combined) forms for malted sorghum cultivar *Madjeru*.

37.12 to a minimal of 22.47 mg/L for unmalted *Madjeru*, and from 56.26 to 34.25 mg/L for malted *Madjeru,* and henceforth gradually increased with increases in enzyme concentration as from 407 BGU. This observation indicated that bioglucanase is not a protein hydrolyzing enzyme. The initial decrease in FAN content, followed by a subsequent increase, could be explained by the fact that, the free amino nitrogen instantaneously dissolved in the mash after the milling process, reacted with soluble sugars, after which the hydrolyzing action of cell wall components by bioglucanase, kinetically become perceptible to permit observing the liberation of extra FAN molecules. Upon using the model to predict the amounts of FAN if mashed in the presence of hitempase (at 1875 U) and brewers protease (at 60 mg), the same profile as in Figure 6A were observed, but with the levels of free amino nitrogen contents for both mash types increasing 2 times as compared to the original contents (Figure 6B). This confirmed the need to have all mashing enzymes present in appropriate proportions during mashing to permit obtaining substantial amounts of FAN in worts. These observations once more displayed the role of the malting process in guaranteeing worts of higher brewing properties.

According to the models, bioglucanase (X_2), in its linear form, contributed to 8% of the FAN content for both the unmalted and malted *Madjeru* mash types (Figures 8A and B). This contribution was statistically significant for the two mash types (P = 0.005) (Table 6). Similarly, in its quadratic form (X_2^2), the effect of the enzyme remained significant for both mash types (P = 0.000) (Table 6). Its contribution in this form was 26% for both mash types (Figures 8A and B).

Effect of brewers protease on yields of free amino nitrogen (FAN)

The effect of brewers protease as sole mashing enzyme on FAN content for unmalted and malted *Madjeru* is presented in Figure 7A. Free amino nitrogen content increased very slightly from 37.12 to 49.66 mg/L for unmalted *Madjeru* worts and from 56.26 to 72.86 mg/L for malted *Madjeru* worts with increasing enzyme concen-trations. These levels were maintained virtually constant as from about 60 mg of enzyme input thereof. The models predicted that coupling hitempase (at 1875 U)

Table 7. ANOVA for free amino nitrogen of umalted and malted *Madjeru*.

Source	DF	Sum of squares		Mean of squares		F-value		P-value	
		Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Regression	9	4697.665	10845.612	521.963	1025.068	27.908	29.51	0.000	0.000
Linear	3	3323.582	7688.072	1107.861	2562.691	59.235	62.756	0.000	0.000
Quadratic	3	1311.862	2987.332	437.287	995.777	23.381	24.385	0.001	0.000
Interactions	3	62.221	170.208	20.74	56.736	1.109	1.389	0.407	0.323
Residual error	7	130.919	285.848	18.703	40.835				
Total error	16	4828.585	11131.46						

Table 8. ANOVA for comparing free amino nitrogen of unmalted and malted *Madjeru* worts.

Source	DF	Sum of squares	Mean of squares	F-value	P-value
Inter-groups	1	8813.2	8813.2	17.67	0.000
Intra-groups	32	15960	498.751		
Total	33	24773.2			

and bioglucanase (at 750 BGU) to the action of brewers protease would induce a steady increase in FAN content (Figure 7B). This once more indicates the need of having all mashing enzymes present in order to obtain higher FAN yields. The additional FAN content observed for malted *Madjeru* mash as compared to unmalted *Madjeru* mash could once more be attributed to the natural virtues that the malting process contributed to the grains used. The mathematical model statistically showed that the action of brewers protease was in its linear form significant for both mash types (P = 0.000 for both (Table 6). Its contribution to FAN content in this form (X_3) was 18% for both the unmalted and malted *Madjeru* worts (Figures 8A and B).

The effect of the enzyme in its quadratic form (X_3^2) however remained significant for both mash types (P = 0.039 and 0.049 respectively) (Table 6). Its contribution was 9% for unmalted *Madjeru* worts and 8% for malted *Madjeru* worts (Figures 8A and B).

Effect of enzymes interactions on yields of free amino nitrogen (FAN)

The global action of the enzymes' interaction or as coupled forms (X_1X_2, X_1X_3 and X_2X_3) on the FAN content was statistically not significant (P = 0.407 for unmalted *Madjeru* and P = 0.323 for malted *Madjeru*) (Table 7). Their contributions of FAN content are shown in Figures 8A and B. The effect of the X_1X_2 (hitempase 2XL/bioglucanase TX) interaction was not significant for both wort types (P = 0.451 for unmalted *Madjeru* and P = 0.361 for malted *Madjeru*) (Table 6). Its contribution of FAN content in both mash types was 4 and 5% respectively) (Figures 8A and B). Similarly, the action of the couple X_1X_3 (hitempase 2XL/brewers protease) was also not significant for both mash types (P = 0.475 for unmalted *Madjeru* and P = 0.368 for malted *Madjeru*) (Table 6). Its contribution of FAN content in both mash types was 5 and 4% respectively (Figures 8A and B). Finally, for the couple X_2X_3 (BIOGLUCANASETX/brewers protease), its action was also not significant for both wort types (P = 0.172 for unmalted *Madjeru* and P = 0.148 for malted *Madjeru*) (Table 6). The contribution of FAN content in both wort types was 8% for each (Figures 8A and B).

Table 8 statistically confirmed the biological assertion that malted type samples were more potential raw materials for mashing in terms of FAN contents than the combination of unmalted grains and commercial enzymes (P = 0.000).

Optimization of the concerted mashing enzymes' action on yields of extracts and free amino nitrogen

The results obtained for the action of the enzymes on extract and free amino nitrogen yields after mashing on the basis of the models were optimized to define satisfactory domains of compromise for the mashing enzymes. These domains were obtained for the two key brewing parameters by fixing the wort conditions at: extract ≥ 12 °P and free amino nitrogen ≥ 80 mg/L. The theoretical optimal combination of enzyme action for unmalted *Madjeru* gave the following triplet of real variables for extract: 1960.5 U; 132.61 BGU and 28.86 mg for hitempase 2XL, Bioglucanase TX and brewers protease respectively. This triplet allowed for a maximal extract of 16.55 °P. The triplet for malted *Madjeru* was: 2610 U; 0 BGU and 40.44 mg. It allowed for a maximal extract of 16.35 °P. The optimal enzyme combinations were thus different particularly with regards to

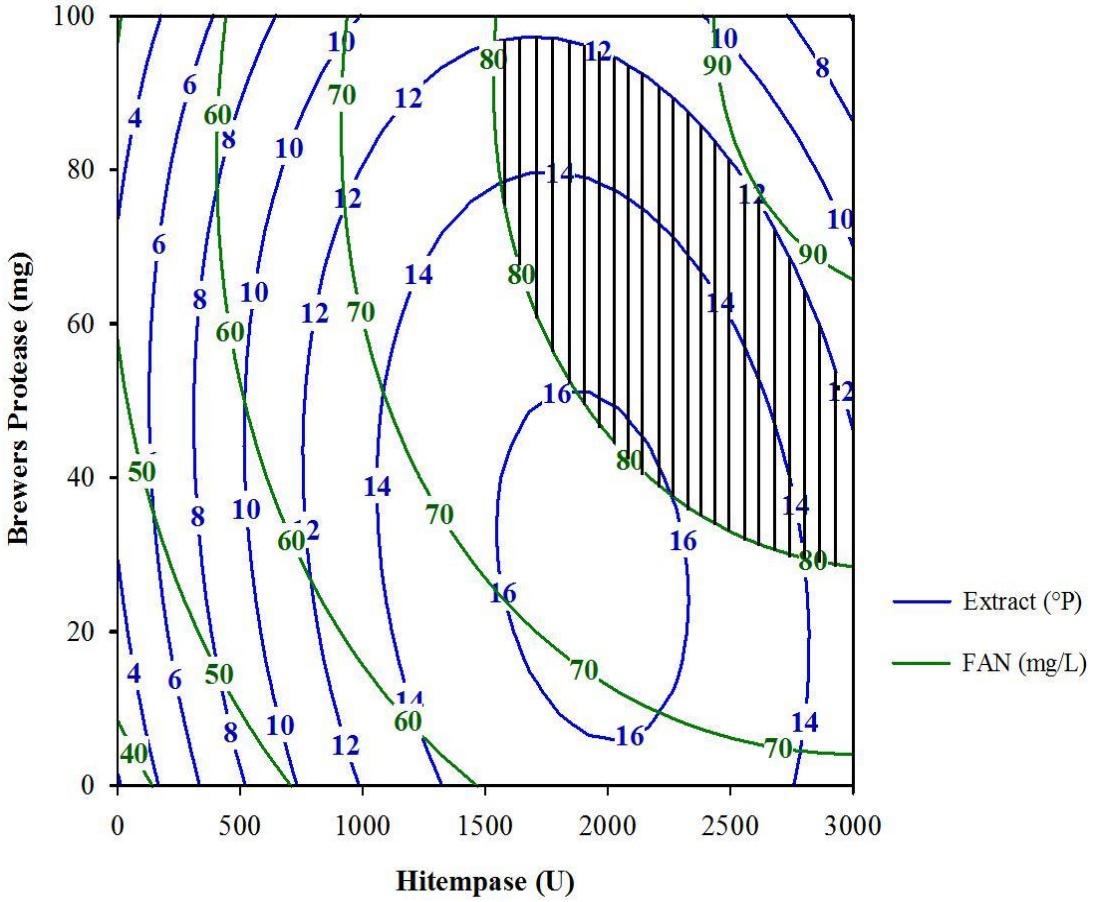

Figure 9A. Response surface curves for the enzyme combinations providing for optimal yields in extract and free amino nitrogen for unmalted sorghum cultivar *Madjeru.*

bioglucanase TX but both gave comparable yields of extracts. These results show that bioglucanase TX supplements for mashing malted *Madjeru* is not indispensable for obtaining required yields in extract, whereas the three enzymes are needed as supplements when mashing unmalted *Madjeru*. Figure 9A shows the response surface areas exploitable for efficient mashing capable of giving optimal results of yields of extract. For free amino nitrogen, the theoretical optimal combination of enzymes' action for unmalted *Madjeru* gave as triplet of real variables: 3000 U; 0 BGU and 100 mg for hitempase 2XL, bioglucanase TX and brewers protease respectively. This triplet allowed for maximal free amino nitrogen of 93.55 mg/L. The triplet for malted *Madjeru* was: 3000 U; 0 BGU and 100 mg. It allowed for maximal free amino nitrogen content of 144.48 mg/L. These results show that bioglucanase TX is not an indispensable mashing enzyme when seeking for appreciable amounts of free amino nitrogen in the worts of *Madjeru*, be it malted or not.

Figure 9B shows the response surface areas exploitable for efficient mashing giving optimal results of free amino nitrogen content.

Conclusions

The effects of three technical mashing enzymes (hitempase 2XL, bioglucanase TX and brewers protease) on yields of extract and free amino nitrogen were studied during the mashing of unmalted and malted *Madjeru* grist. Hitempase 2XL was principally responsible for extract yields in unmalted *Madjeru* mash but its impact on malted *Madjeru* mash type was mild. Bioglucanase TX played no role, while brewers protease showed limited contributions to yields of extract. Hitempase 2XL and brewers protease individually contributed to yields in free amino nitrogen in both unmalted and malted *Madjeru* mashes, though the milling operation contributed to FAN yields for more than 50% in both mashes. This study shows that proper malting and mashing of this sorghum cultivar could lead to satisfactory worts properties in terms of extract and free amino nitrogen for brewing purposes. Supplements of technical mashing enzymes to boost their yields of extract in particular, are thus not indispensable when mashing with malted *Madjeru.* Optimization of mashing properties through models clearly describing the actions of individual technical mashing

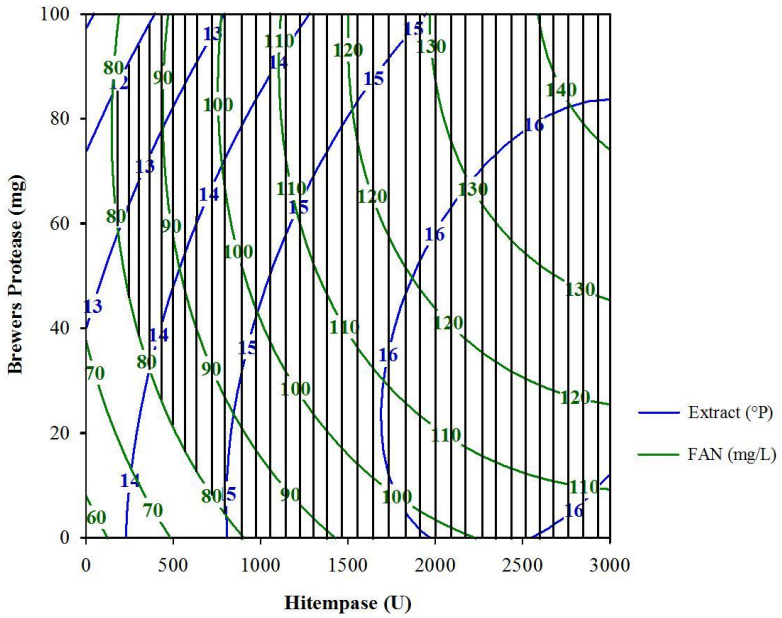

Figure 9B. Response surface curves for the enzyme combinations providing for optimal yields in extract and free amino nitrogen for malted sorghum cultivar *Madjeru.*

enzymes, as displayed in this study using the response surface methodology is however of interest particularly when mashing with high amounts of sorghum adjuncts. Further studies on the fermentability of worts obtained after such studies would be of importance in order to assess the exploitability of the results for improved brewing practices with this sorghum cultivar.

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