Modelling and Optimizing of Mashing Enzymes – Effect on Yield of Filtrate of Unmalted Sorghum by Use of Response Surface Methodology

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ABSTRACT

J. Inst. Brew. 116(1), 62–69, 2010

The effect of commercial enzymes on liquefaction of starch from unmalted sorghum was studied. The effects which these enzymes had on rates of filtration were evaluated. Models were developed, validated and optimized to establish the actions of enzymes, either alone or in combination. Preliminary studies on the sorghum cultivars *Safrari, Madjeru* and *S.35* showed that α-amylase was the backbone enzyme for starch liquefaction among the enzymes used (α-amylase, Filtrase, protease and β-amylase). Models confirmed this observation as α-amylase individually in its first order (X_1) contributed 25, 11 and 17%, and in its sum of first and second orders ($X_1+X_1^2$) contributed a 29, 31 and 36% yield of filtrate for *Safrari, Madjeru* and *S.35* respectively. The ease of starch liquefaction, assessed by summing the first and second orders of individual intervention of all enzymes, was found to be in the order of *Madjeru, S.35* and *Safrari* (79, 70 and 56% of yield of filtrate respectively). The importance of the enzyme combination in starch liquefaction in *Safrari, S.35* and *Madjeru* was shown to be 44, 30 and 21% respectively. Enzyme combinations giving maximal starch liquefaction, as identified from a Doehlert experimental matrix, displayed a similar yield of filtrate (*Safrari:* 85 mL, *Madjeru:* 84 mL and *S.35:* 81 mL) after filtration of a 130 mL mash during 1 h. Validation of the models revealed the model developed for *Madjeru* was the most reliable ($R^2 = 0.994$), while those developed for *Safrari* ($R^2 = 0.987$) and *S.35* ($R^2 = 0.976$) were slightly less reliable. Model optimization gave theoretical enzyme (Brewers Amyliq TS, Filtrase NLC, Brewers Protease and β-amylase) combinations of 25 mg, 5.68 mg, 100 mg and 67.4 U for *Safrari,* 15.06 mg, 0.51 mg, 24.32 mg and 53.8U for *Madjeru* and 19.01 mg, 6.36 mg, 58.76 mg and 43.48 U for *S.35,* with a resulting yield of filtrate of 94, 87.7 and 83.8 mL respectively.

Key words: Mashing enzymes, model validation, modeling, optimization, unmalted sorghum, yields of filtrates.

INTRODUCTION

The addition of enzymes to increase fermentable sugars and free amino acids, and to facilitate filtration when mashing with poorly malted or unmalted cereals, is an established practice in beer brewing[1,2,6,7,10,12,15]. One of the most important technological parameters to which brewers pay attention during mashing is the ease with which the mash filters[8,10,17,19,20]. Studies carried out on malts of three sorghum cultivars of North Cameroon showed that, whether malted traditionally or under controlled laboratory conditions to brew the traditional beer *Bili-Bili*, the mashes of *Madjeru* cultivar filtered slower than the cultivars *Safrari* and *S.35*[17]. Although the proportions of fermentable sugars of worts of the three cultivars were comparable, that of maltose was less than 50% for *Madjeru,* when compared with *Safrari* and *S.35*[17]. These observations were partly attributed to limited amounts of starch hydrolyzing enzymes such as α-amylase and β-amylase present in the *Madjeru* malts[17]. This is indeed one of the major problems encountered in mashing with some malted sorghum cultivars[3-5,8,9,13,14,17,18,21]. However, it is known that acceptable worts for beer brewing can be obtained from 100% unmalted sorghum by supplementing the mashes with optimal amounts of thermostable α-amylase, fungal α-amylase and bacterial proteases[11]. In contrast, what is not known is the effect of singular or combined contributions of these enzymes on starch liquefaction of unmalted sorghum grains. In this paper, we report validated and optimised mathematical models for mashing unmalted grains of the *Safrari, S.35* and *Madjeru* sorghum cultivars using as mashing enzymes the following: Brewer Amyliq (α-amylase), Filtrase NLC (β-glucanase and hemicellulase), Brewers protease and β-amylase in order to assess the singular or combined contributions of these enzymes in mash liquefaction.

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Publication no. G-2010-0316-1048

MATERIALS AND METHODS

Chemicals

The characteristics of the commercial enzymes used (Brewers Amyliq TS from *Baccillus licheniformis,* Filtrase NLC, Brewers protease from *Bacillus amyloliquefaciens* and β-amylase type II-B from crude barley) are presented in Table I. The amounts used for the first three ranged from 0–25 mg, 0–10 mg and 0–100 mg respectively, while for β-amylase the range used was 0–80 U.

Table I. Characteristics of commercial enzyme preparations used in mashing[a].

	Organism of origin	Activity	Description	Temperature optima	pH optimum	Recommended application level in adjuncts	Form
Brewers Amyliq TS	*Bacillus licheniformis*	27.7 ± 6.5 U/mg of solid	α-amylase	93–95°C	5.5–6.5	0.3 g/Kg	Powder
β-amylase (E 3.2.1.2)	Type raw II-B of barley	23–80 U/mg of protein	β-amylase	*NI[b]	*NI	*NI	Powder
Filtrase NLC	*NI	*NI	β-glucanase and hemicellulase	*NI	*NI	0.15–0.2 g/Kg	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

[a] All the commercial enzymes used in this study were obtained from DSM Food Specialities, Cedex France, apart from β-amylase which was sourced from SIGMA CHIMIE, Cedex, France.
[b] Not indicated by DSM Food Specialities France/SIGMA.

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

Coded values				Transformed experimental values				*Madjeru*			*Safrari*			*S.35*		
α-amylase	Filtrase	Proteases	β-amylase	α-amylase (mg)	Filtrase (mg)	Proteases (mg)	β-amylase (U)									
X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	Exp[a]	Theo[b]	Res[c]	Exp	Theo	Res	Exp	Theo	Res
1.000	0.000	0.000	0.000	25	5	50	40	68	65	3	85	83	2	76	76.1	–0.1
–1.000	0.000	0.000	0.000	0	5	50	40	24	27	–3	36	38	–2	35	34.9	0.1
0.500	0.866	0.000	0.000	18.75	10	50	40	61	60.405	0.595	67	70.001	–3	77	78.404	–1.4
–0.500	–0.866	0.000	0.000	6.25	0	50	40	64	64.596	–0.59	60	56.999	3.001	60	58.602	1.398
0.500	–0.866	0.000	0.000	18.75	0	50	40	84	83.097	0.903	71	71.5	–0.5	69	67.702	1.298
–0.500	0.866	0.000	0.000	6.25	10	50	40	40	40.905	–0.9	40	39.502	0.498	45	46.304	–1.3
0.500	0.289	0.816	0.000	18.75	6.66	100	40	55	57.088	–2.08	75	75.698	–0.69	80	79.993	0.007
–0.500	–0.289	–0.816	0.000	6.25	3.33	0	40	63	60.885	2.115	59	58.297	0.703	61	60.991	0.009
0.500	–0.289	–0.816	0.000	18.75	3.33	0	40	74	76.885	–2.88	71	69.789	1.211	77	72.579	4.421
0.000	0.577	–0.816	0.000	12.5	8.33	0	40	61	60.29	0.71	64	62.805	1.195	70	69.897	0.103
–0.500	0.289	0.816	0.000	6.25	6.66	100	40	38	35.088	2.912	41	42.19	–1.19	46	50.381	–4.38
0.000	–0.577	0.816	0.000	12.5	1.66	100	40	60	60.685	–0.68	66	67.205	–1.2	69	69.097	–0.09
0.500	0.289	0.204	0.791	18.75	6.66	62.5	80	55	56.459	–1.45	75	73.301	1.699	81	79.483	1.517
–0.500	–0.289	–0.204	–0.791	6.25	3.33	37.5	0	35	33.458	1.542	47	48.7	–1.7	45	46.483	–1.48
0.500	–0.289	–0.204	–0.791	18.75	3.33	37.5	0	63	63.97	–0.97	65	67.693	–2.69	67	72.583	–5.58
0.000	0.577	–0.204	–0.791	12.5	8.33	37.5	0	42	42.372	–0.37	53	51.701	1.299	65	62.394	2.606
0.000	0.000	0.612	–0.791	12.5	5	87.5	0	49	49.078	–0.07	54	50.894	3.106	67	62.491	4.509
–0.500	0.289	0.204	0.791	6.25	6.66	62.5	80	50	48.971	1.029	50	47.293	2.707	70	64.383	5.617
0.000	–0.577	0.204	0.791	12.5	1.66	62.5	80	70	69.565	0.435	62	63.299	–1.29	73	75.592	–2.59
0.000	0.000	–0.612	0.791	12.5	5	12.5	80	76	75.876	0.124	55	58.093	–3.09	72	76.489	–4.48
0.000	0.000	0.000	0.000	12.5	5	50	40	78	77.25	0.75	63	64.25	–1.25	79	78.25	0.75
0.000	0.000	0.000	0.000	12.5	5	50	40	79	77.25	1.75	65	64.25	0.75	77	78.25	–1.25
0.000	0.000	0.000	0.000	12.5	5	50	40	75	77.25	–2.25	63	64.25	–1.25	77	78.25	–1.25
0.000	0.000	0.000	0.000	12.5	5	50	40	77	77.25	–0.25	66	64.25	1.75	80	78.25	1.75

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

Sorghum cultivars

The *Safrari, Madjeru* and *S.35* sorghum cultivars were obtained from the Institute of Research and agronomic development (IRAD) Maroua, Cameroon.

Mashing

Sorghum cultivar grains were milled to particle sizes of 0.7 mm or less using a hammer mill Polymix PX-MFC 90D apparatus type (VWR International S.A.S. Le Périgas 201, rue Carnot, 94126 Fontenay-sous-Bois Cedex, France). Twenty five grams of unmalted sorghum was weighed, placed in a 600 mL beaker and 150 mL of distilled water added. The suspension was homogenised at 24°C by stirring with a glass rod. It was then heated to boiling temperature, at which starch gelatinization was allowed to take place for 20 min, with intermittent stirring at intervals of 5 min, before cooling to 60°C. Mashing was carried out at 60°C for 1 h taking into consideration the conditions indicated in Table I. The mash was cooled to 25°C and filtered for 1 h using Whatman grade 1 filter paper.

Mathematical modelling

The establishment of an experimental matrix was necessary to develop the mathematical model. The Doelhert (number of factors, k = 4)[16] matrix was adopted for this work. Transformation of the matrix of coded variables to the experimental matrix was automatic (Table II), while the quantities of mashing enzymes used were maintained at fixed levels. The amounts of selected enzymes ranged

as follows: Brewers Amyliq TS (0–25 mg); Filtrase NLC (0–10 mg); Protease (0–100 mg); and β-amylase (0–80 U) (β-amylase activity was 31 U/mg of solid).

Doelhert's experiment design (having a homogenous distribution in space) was used to establish the experiment matrix (Table II). This matrix has coded variables and must be converted into an experimental matrix[16] having real variables directly usable in the laboratory. The results of the phenomenon to study are then established and the data considered as "y_{exp}". With the help of the matrix of coded variables and "y_{exp}", the coefficients of the model are obtained. The model is obtainable only from the coded variable matrix because, in this case, there is no need for a counterbalancing effect of the factors in study[16]. The coefficients of the model obtained will thus be effectively linked to the impact of each factor. These coefficients and the model were obtained with the help of the Systat version 12 software (Systat Software, Inc., San Jose, USA). This software also gives a statistical analysis on the model. Lastly, the curves are plotted using Sigmaplot version 11 build 11.0.0.77 software (WPCubed, GmbH, Germany).

Validation of the model was conducted after assays using several enzyme combinations found in the experimental domains not explored by the experimental matrix. Another method consisted of tracing the theoretical results against the experimental results, as the coefficient of correlation R^2 gives an appreciation of the reliability of the model.

Model optimization used Mathcad version 14 software (Parametric Technology Corporation, Massachusetts, USA). Optimal combination was obtained by initially entering the model and then specifying the starting point of each factor. The sweeping interval by the software was given for each factor. Once the data was entered, the software gave a response for a maximum or minimum combination as requested for. This theoretical optimum was then explored for the confirmation of the optimal point.

RESULTS AND DISCUSSION

Before establishing the mathematical model for mashing, trials were carried out to investigate the action of each of the enzyme preparations in starch liquefaction

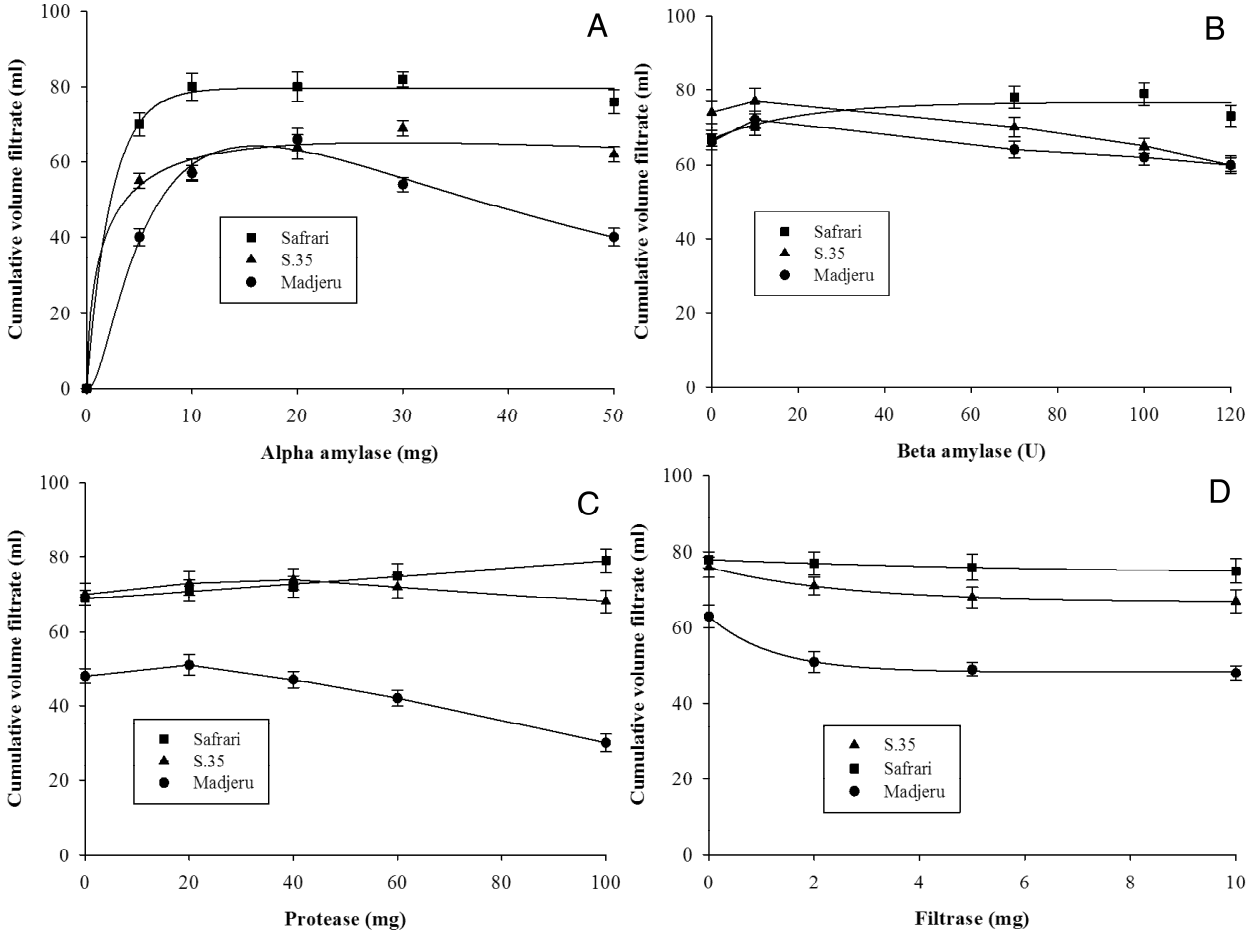

Fig. 1. (A) Effect of α-amylase concentration on filtration of unmalted sorghum mash. The β-amylase, Protease, and Filtrase being constant at 80 U, 60 mg and 10 mg respectively. (B) Effect of β-amylase concentration on filtration of unmalted sorghum mash. The α-amylase, Protease and Filtrase being constant at 20 mg, 60 mg and 10 mg respectively. (C) Effect of Protease concentration on filtration of unmalted sorghum mash. The α-amylase, Filtrase and β-amylase were constant at 20 mg, 10 mg and 80 U respectively. (D) Effect of Filtrase concentration on filtration of unmalted sorghum mash. The α-amylase, Protease and β-amylase were constant at 20 mg, 60 mg and 80 U respectively.

during mashing. Among the enzyme preparations used in this study, it was observed (Fig. 1a) that α-amylase was the principal enzyme of starch liquefaction during mashing[1,7,12]. Figures 1b, 1c and 1d show that the actions of β-amylase, filtrase and protease on starch liquefaction were only noticeable in the presence of α-amylase. This is justified by the fact that filtration was not possible after mashing in the presence of these enzymes, but in the absence of α-amylase (Fig. 1a). These results indicated that mathematical modeling would provide more precise information on the contribution of each enzyme component and combination of enzyme components required to liquefy the mash optimally.

Previous studies on malting and mashing of sorghum cultivars used in this study showed that *Madjeru* mashes filtered slower than those of *Safrari* and *S.35*[17,18]. This slow filtration rate was suggested to be due to the low amounts of α-amylase and β-amylase in *Madjeru* malts compared to *Safrari* and *S.35*. Experimental conditions for the three cultivars in this study were standardized by using unmalted grains and several combinations of the mashing enzymes as shown in Table II. The best enzyme combinations to mash with were identified and the rates of filtration compared under those conditions (Fig. 2). The results showed that the filtration rates, and thus the yields of filtrates for the three varieties were comparable as 85 mL, 84 mL and 81 mL of filtrate was obtained after filtration of 130 mL for 1 h of the *Safrari, Madjeru* and *S.35* mash, respectively. These results confirmed the hypothesis that the slow filtration of mashes of *Madjeru* malts during mashing could be partly attributed to insufficient quantities of α-amylase and β-amylase as suggested previously[17]. A mathematical model was established for each cultivar using a precise matrix. Models obtained by the response surface method established the following equations for mashing the three cultivars.

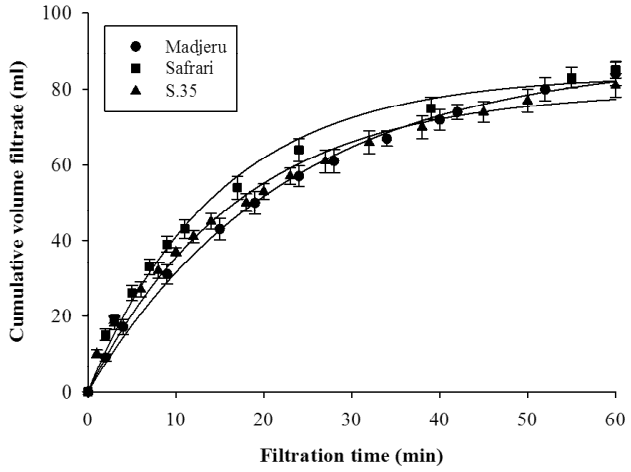

Fig. 2. The effect of best combinations of enzymes on mash filtration: *Madjeru* [α-Amylase (18.75 mg), Filtrase (0 mg), Protease (50 mg), β-amylase (40 U)]; *Safrari* [α-amylase (25 mg), Filtrase (5 mg), Protease (50 mg), β-amylase (40 U)]; *S.35* [α-amylase (18.75 mg), Filtrase (6.66 mg), Protease (62.5 mg), β-amylase (80 U)].

Safrari

$$Y = 64.25 + 22.5X_1 - 5.484X_2 - 1.182X_3 + 3.636X_4 + 9.237X_1X_2 + 10.219X_1X_3 - 1.576X_1X_4 - 5.437X_2X_3 + 3.470X_2X_4 + 8.655X_3X_4 - 3.75X_1^2 - 5.083X_2^2 - 0.169X_3^2 - 8.796X_4^2$$

Madjeru

$$Y = 77.25 + 19X_1 - 13.39X_2 - 9.226X_3 + 9.801X_4 + 0.577X_1X_2 + 3.472X_1X_3 - 15.66X_1X_4 + 3.427X_2X_3 - 0.638X_2X_4 - 14.23X_3X_4 - 31.25X_1^2 - 9.583X_2^2 - 17.96X_3^2 - 23.87X_4^2$$

S.35

$$Y = 78.25 + 20.6X_1 - 0.461X_2 - 0.816X_3 + 8.217X_4 + 13.279X_1X_2 + 6.342X_1X_3 - 13.44X_1X_4 - 1.536X_2X_3 - 0.822X_2X_4 + 1.147X_3X_4 - 22.75X_1^2 - 13.08X_2^2 - 7.692X_3^2 - 8.508X_4^2$$

The three equations were structurally identical and all of second order with interactions. In the models, the factors X_1 and X_4, corresponding to α-amylase and β-amylase respectively had positive coefficients. These indicated that the two enzymes were directly involved in the liquefaction of starch and were thus instrumental contributors to yields of filtrates. Figures 3a, 3b and 3c detail the individual and combined contributions of the various mashing enzyme components on the total yield of filtrates of the liquefied starch. Thus, α-amylase, in its singular expression as first order in the model, contributed 25%, 11% and 17% of the yield of filtrate for the mashes of *Safrari, Madjeru* and *S.35* respectively. Similarly, β-amylase accounted for 4%, 6% and 7% respectively. The factors X_2 and X_3, corresponding to filtrase and protease respectively, had negative coefficients. This suggested that these enzymes were not directly responsible for starch liquefaction, and cannot, a priori, be considered as directly contributing to yield of filtrate during mashing. Filtrase, in its singular expression as first degree in the model, indirectly contributed to 6%, 8% and 1% in yields of filtrates, while protease contributed to 1%, 5% and 1% in *Safrari, Madjeru* and *S.35* mashes respectively. The factors X_1^2, X_2^2, X_3^2 and X_4^2 corresponding to the second order of the components of α-amylase, filtrase, protease and β-amylase respectively, showed that the singular action of all these enzymes expressed to their second order in the model, had a negative coefficient. Their absolute contribution to the yield of filtrate was about 4%, 6%, 0% and 10% for *Safrari,* 20%, 5%, 10% and 14% for *Madjeru* and 19%, 11%, 7% and 7% for S.35 mashes respectively. Table III summarises these results, with the sum totals in percent, of the contribution in yield of filtrate by these enzymes in their first and second orders as expressed by the model. The results corroborated those shown in Fig. 1, wherein α-amylase is evidently the sole enzyme of starch liquefaction during mashing. Indeed, this enzyme in its first and second orders in the model, singularly contributed 29%, 31% and 36% to filtrate yield of the cultivars *Safrari, Madjeru* and *S.35* respectively.

Table III also summarises the results for the contributions of the interactions between various enzyme components used. Thus, the interaction X_1X_2, corresponding to the combined action of α-amylase and filtrase, had a pos-

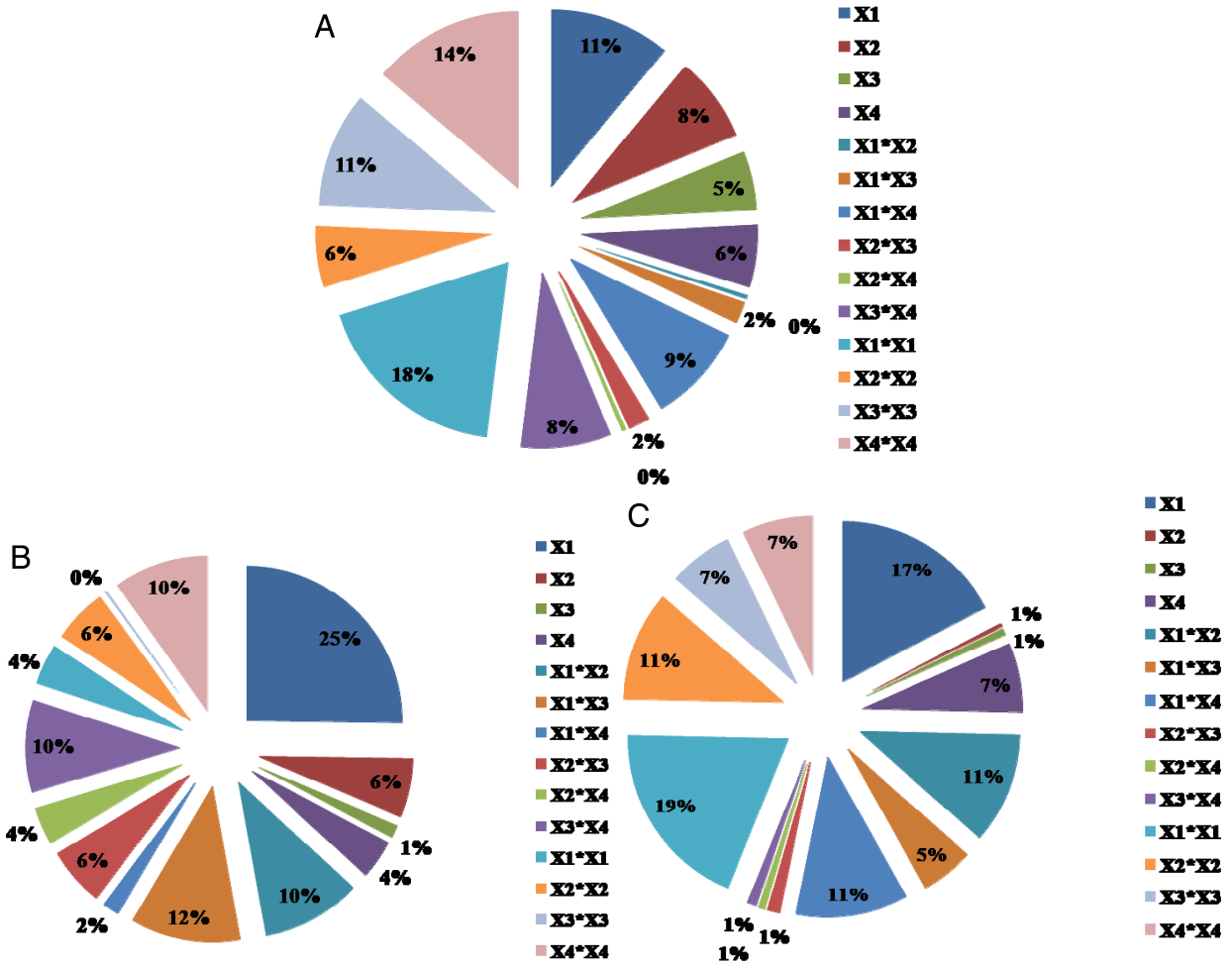

Fig. 3. (A) Contribution of the model factors on the total effect for *Safrari*. (B) Contribution of the model factors on the total effect for *Madjeru.* (C). Contribution of the model factors on the total effect for *S.35*.

itive coefficient, suggesting that the second enzyme contributed to reducing the viscosity of the mash and thus facilitated its filtration. This positive interaction between α-amylase and filtrase roughly contributed to 10%, 0% and 11% in yield of filtrate for *Safrari, Madjeru* and *S.35* mashes respectively. The interaction X_1X_3, corresponding to the combined action of α-amylase and protease, also had a positive coefficient. Similarly to the α-amylase/filtrase interaction, protease facilitates mash hydrolysis by α-amylase. This positive interaction between the two enzymes contributed to about 12%, 2% and 5% in yield of filtrate for *Safrari, Madjeru* and *S.35* mashes respectively. The interaction X_1X_4, corresponding to the combined action of α-amylase and β-amylase had a negative coefficient. Since these two enzymes are starch hydrolysing enzymes, they independently and directly attack free convertible starch granules in suspension without any prerequisites. Whereas starch granules embedded in the protein matrix and surrounded by β-glucano-hemicellulosic cell walls must absolutely be liberated by the action of proteases and β-glucanases in order to allow α-amylase and β-amylase to act on starch[21]. The contribution by this combined effect in yield of filtrate was 2%, 9% and 11% for *Safrari, Madjeru* and *S.35* mashes respectively. Upon adding the contributions of α-amylase's action in its singular first and second orders, to those of its interactions with the other enzymes (α-amylase/filtrase, α-amylase/protease and α-amylase/β-amylase) in the model, we obtained the direct and indirect contribution to starch liquefaction. These contributions amounted to 53%, 42% and 63% for *Safrari, Madjeru* and *S.35* respectively. The interaction X_2X_3, corresponding to the combined action of filtrase and protease, had a negative coefficient for the mashes of the cultivars *Safrari* and *S.35,* but was positive for *Madjeru*. The contribution by this combined effect in yield of filtrate was roughly 6%, 2% and 1% for *Safrari, Madjeru* and *S.35* mashes respectively. These two enzymes are not starch hydrolysing enzymes, and only help to liberate starch granules embedded in the protein matrix and surrounded by β-glucano-hemicellulosic cell walls. The low contribution of this interaction is therefore predictable. Interaction X_2X_4, corresponding to the combined action of filtrase and β-amylase, had a positive coefficient for the cultivar *Safrari,* but was negative for *Madjeru* and *S.35*. The contribution by this combined effect in yield of filtrate was about 4%, 0% and 1% for *Safrari, Madjeru* and *S.35* mashes respectively. Once more, the low contribution of this interaction for the three cultivars suggested that the filtrase/β-amylase combination was not important for filterability. This can be explained by the fact that the

Table III. Contributions of single (1st and 2nd orders), their sums and combined enzyme components to filtrate yields.

Enzyme	Single component as 1st, 2nd degree and sum	Cultivar type *Safrari*	*Madjeru*	*S.35*
α-amylase	X_1	25	11	17
	X_1^2	4	20	19
	$X_1 + X_1^2$	29	31	36
Filtrase	X_2	6	8	1
	X_2^2	6	5	11
	$X_2 + X_2^2$	12	13	12
Protease	X_3	1	5	1
	X_3^2	0	10	7
	$X_3 + X_3^2$	1	15	8
β-amylase	X_4	4	6	7
	X_4^2	10	14	7
	$X_4 + X_4^2$	14	20	14
Total		**56**	**79**	**70**
Enzymes interacting	Combined component	*Safrari*	*Madjeru*	*S.35*
α-amylase / Filtrase	$X_1 X_2$	10	0	11
α-amylase / Protease	$X_1 X_3$	12	2	5
α-amylase / β-amylase	$X_1 X_4$	2	9	11
Filtrase / Protease	$X_2 X_3$	6	2	1
Filtrase / β-amylase	$X_2 X_4$	4	0	1
Protease / β-amylase	$X_3 X_4$	10	8	1
Total		**44**	**21**	**30**

role of filtrase was limited to unmasking starch granules embedded in cells by hydrolysing the β-glucans and hemicelluloses of the walls of these cells, while β-amylase, a saccharifying enzyme, had only limited action on the molecular size of starch material. Finally, the interaction X_3X_4 corresponding to the combined action of protease/β-amylase had a positive coefficient for the cultivars *Safrari* and *S.35,* but negative for the cultivar *Madjeru*. The contribution of this combined effect on yield of filtrate was about 10%, 8% and 1% for *Safrari, Madjeru* and *S.35* respectively. The impact of this enzyme combination on the filtration of mash will depend not only on the extent of hydrolysis of the protein matrix surrounding starch granules, but also on that of the β-amylase component, which will be assessed by the extent of hydrolysis of convertible starch granules. Thus, if proteolysis is effective, but the starch type is more of amylopectin than amylose, the rate of mash filtration would be slower, as the saccharifying action of β-amylase would be reduced. This could explain the disparity in the yield of filtrate observed for these three cultivars of sorghum, with respect to the enzyme combinations.

With respect to the observed effects of the first and second orders as a sum for each of the enzyme components in the model, 56%, 79% and 70% for *Safrari, Madjeru* and *S.35* respectively, it is clear that the ease of mash liquefaction was in the order of *Madjeru, S.35* and *Safrari* (Table III). Also, the model showed that the ease of mash liquefaction on the basis of enzyme combined components was in the order of *Safrari, S.35* and *Madjeru* (44%, 30% and 21% respectively) (Table III). These results suggest that the action of filtrase and protease in liberating protein embedded starch granules surrounded by β-glucano-hemicellulosic cell walls, was of great importance in liquefying and saccharifying starch by α-amylase and β-amylase respectively for *Safrari,* followed by *S.35* and then by *Madjeru*. This also suggests that *Safrari* was richer in proteins and/or β-glucans than *S.35*. This has indeed been shown with respect to the total protein contents of these three cultivars[17].

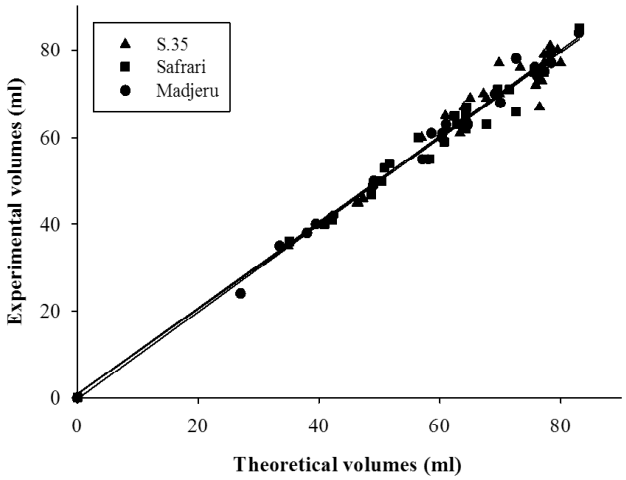

Fig. 4. Validation of mathematical models for *Madjeru, Safrari* and *S.35* with R^2 = 0.994, 0.987 and 0.976 respectively.

The mathematical models were validated using two methods. Firstly, tests were carried out on several combinations of the experimental space which were not explored within the framework of the experimental matrix. The results were then compared with the theoretical results and the errors statistically evaluated. These errors were between 2% and 3.7%, 1.7% and 3%, and 0.1% and 3.4% for *Safrari, Madjeru* and *S.35* respectively. The global error thus was 0.1% to 3.7%. As the highest error limit is approximately 3.7%, it could be concluded that the mathematical models established, satisfactorily describe the observed phenomena. It is however necessary to determine this error and reliability. This can be done by a second method consisting of plotting the theoretical results against the experimental results and determining the

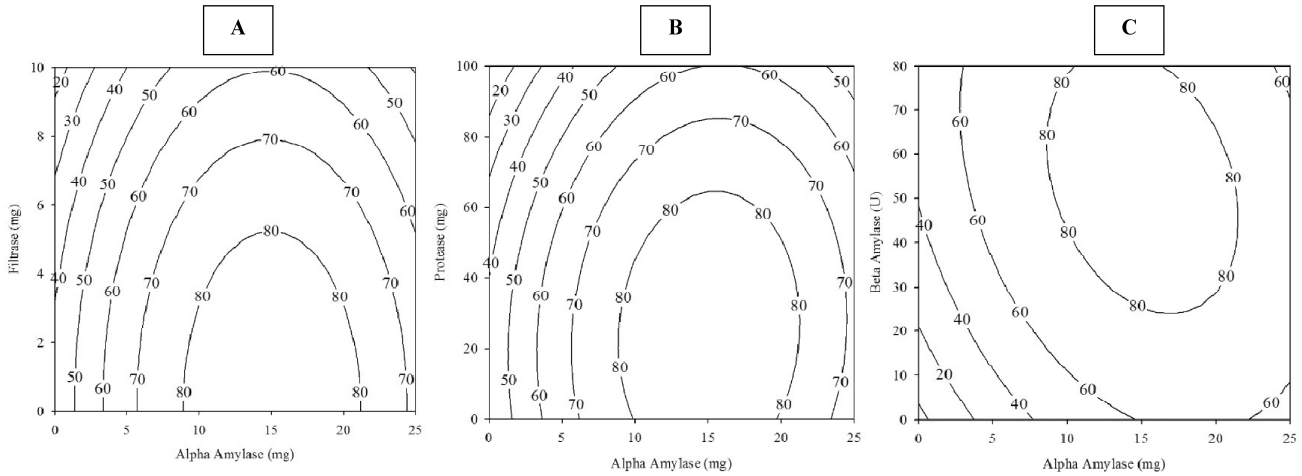

Fig. 5. Response surface curves for *Madjeru* yield in filtrates [all other factors were fixed at optimal quantity: (A) Protease: 24.32 mg; β-amylase: 53.8 U; (B) Filtrase: 0.51 mg; β-amylase: 53.80U; (C) Filtrase: 0.51 mg; Protease: 24.32 mg].

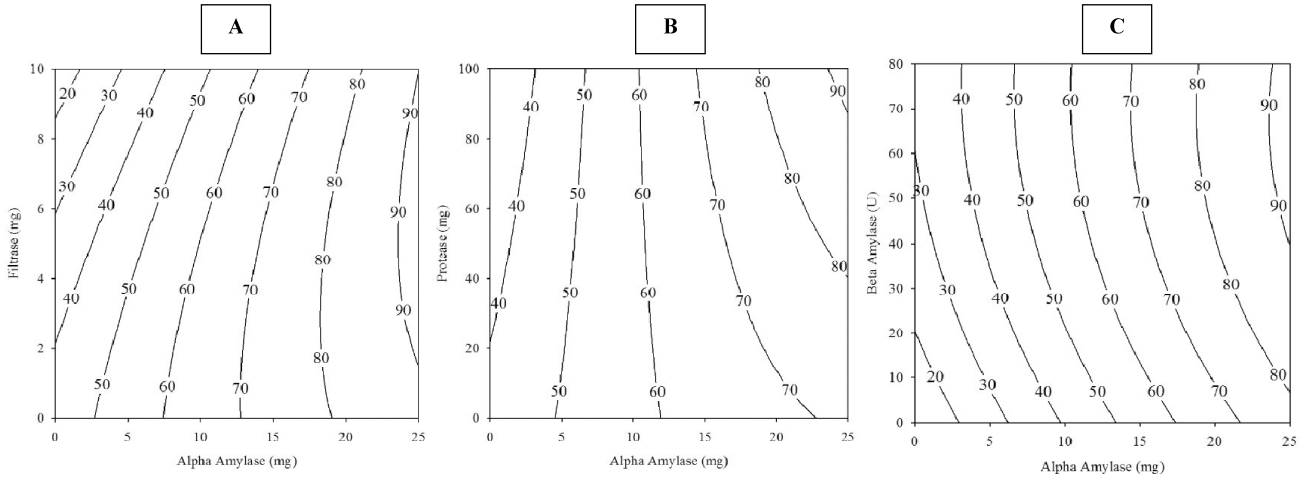

Fig. 6. Response surface curves for *Safrari* yield in filtrates [all other factors were fixed at optimal quantity: (A) Protease: 100 mg; β-amylase: 67.40 U; (B) Filtrase: 5.68 mg; β-amylase: 67.40 U; (C) Filtrase: 5.68 mg; Protease: 100 mg].

coefficient of correlation R^2, in order to appreciate the reliability of the models. The second method of validating the models allowed for the classification of reliability in the following order: first *Madjeru* (R^2 = 0.994), followed by *Safrari* (R^2 = 0.987) and then *S.35* (R^2 = 0.976) (Fig. 4).

Optimization of the outputs in filtrates was logically the final step in this work after validating the models. This was conducted using Mathcad version 14 software (Parametric Technology Corporation, Massachusetts, USA). The theoretical maxima for the combinations in amounts of dispensable mashing enzyme preparations, in order to obtain optimal filtrates in our working conditions, were for α-amylase, filtrase, protease and β-amylase, as follows: 25 mg, 5.68 mg, 100 mg and 67.4 U (or in coded values: 1, 0.118, 0.816, 0.542) respectively for *Safrari,* 15.06 mg, 0.51 mg; 24.32 mg and 53.80 U (or in coded values: 0.205, –0.777, –0.419, 0.273) respectively for *Madjeru* and: 19.01 mg, 6.36 mg, 58.76 mg and 43.48 U (or in coded values: 0.521, 0.236, 0.143, 0.069) respectively for *S.35*. Figs. 5, 6 and 7 show these results in their response surface representations. Thus, the theoretical optimal volumes calculated for the three models were 94, 87.7 and 83.8 mL for the three cultivars respectively. It is however important to note that the optima obtained were with respect to cooking, cooling and then mashing at 60°C as described in the Materials and Methods. The response surface methodology will obviously give different results if other temperature regimes taking into consideration the high thermostability of the α-amylase (temperature optimum at 93–95°C) are applied.

CONCLUSIONS

The α-amylase was the main enzyme component responsible for starch liquefaction during the mashing of unmalted sorghum. The β-amylase, Filtrase and protease served basically as supporting enzymes. The release and hydrolysis (liquefaction) of starch were facilitated by the sequential actions of filtrase, which hydrolysed cell wall materials, and protease, which facilitated the breakdown of released protein materials and thus enhanced starch release. The β-amylase complements the dominant action of α-amylase in starch liquefaction and extract develop-

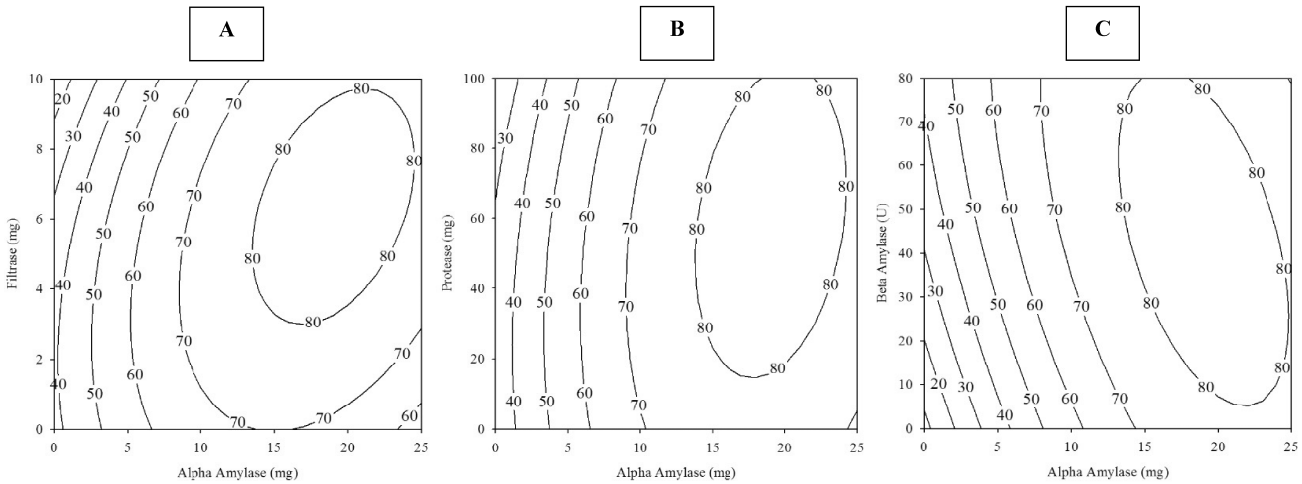

Fig. 7. Response surface curves for *S.35* yield in filtrates [all other factors were fixed at optimal quantity: (A) Protease: 58.76 mg; β-amylase: 43.48 U. (B) Filtrase: 6.36 mg; β-amylase: 43.48 U. (C) Filtrase: 6.36 mg; Protease: 58.76 mg].

ment. In general, the response surface methodology appears to be a very reliable tool in assessing the scope and the actions of mashing enzymes, as single or combined components, in starch liquefaction during mashing. This methodology should also be helpful in predicting the development of important wort parameters such as soluble nitrogen, free α-amino nitrogen, extract content, fermentability and viscosity.

ACKNOWLEDGEMENTS

The authors are grateful to the University of Ngaoundere Research Funding Program (GPRU) for funding this research. They also thank DSM France for supplying the enzyme kits used in this work.

REFERENCES

1. Agu, R. C. and Palmer, G. H., Effect of mashing with commercial enzymes on the properties of sorghum worts. *World J. Microbiol. Biotechnol.,* 1998, **14,** 43-48.
2. Agu, R. C., Ugwu, A. H., Okenchi, M. U., Aneke, I. B. and Anyanwu, T. U., Effect of low kilning temperatures on diastase and cellulase development of Nigerian malted sorghum (Sorghum bicolor). *Process Biochem.,* 1995, **31,** 63-68.
3. Aisien, A. O., Enzymic modification of sorghum endosperm during seedling growth and malting. *J. Sci. Food Agric.,* 1982, **33,** 754-759
4. Aniche, G. N. and Palmer, G. H., Development of amylolytic activities in sorghum and barley malt. *J. Inst. Brew.,* 1990, **96,** 377-379.
5. Arri, B. K., Problems associated with the use of sorghum for lager beer production. Industrial utilization of sorghum: Symposium on the Current Status and Potential of Industrial Uses of Sorghum. ICRISAT: Kano, Nigeria, 1989.
6. Bajomo, M. F. and Young, T. W., Development of profile for the use of microbial enzymes in brewing with raw sorghum (80%) and malted barley of sorghum malt (20%). *J. Inst. Brew.,* 1992, **98,** 515-523.
7. Dale, C. J., Young, T. W. and Omole, T., Small scale mashing experiments with grist containing high proportions of raw sorghum. *J. Inst. Brew.,* 1990, **96,** 403-409.
8. Dufour, J. P. and Melotte, L., Sorghum malts for the production of a lager beer. *J. Am. Soc. Brew. Chem.,* 1992, **50,** 110-119.
9. EtokApan, O. U. and Palmer, G. H., Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. *World J. Microbiol. Biotechnol.,* 1990, **6,** 408-417.
10. Goode, L. D. and Arendt, E. K., Pilot scale production of lager beer from grist containing 50% unmalted sorghum. *J. Inst. Brew.,* 2003, **109,** 208-217.
11. Goode, L. D., Halbert, C. and Arendt, E. K., Mashing studies with unmalted sorghum and malted barley. *J. Inst. Brew.,* 2002, **108,** 465-473.
12. Goode, L. D., Halbert, C. and Arendt, E. K., Optimisation of mashing conditions when mashing with unmalted sorghum and commercial enzymes. *J. Am. Soc. Brew. Chem.,* 2003, **61,** 69-78.
13. Hallgren, L., Lager beers from sorghum. In: Sorghum and millets. Chemistry and Technology DAV Dendy, American Association of Cereal Chemists: Minneapolis, 1995, pp. 283-297.
14. Little, B. T., Alternative cereals for beer production. *Ferment,* 1994, **7,** 163-168.
15. MacFadden, D. P. and Clayton, M., Brewing with sorghum-use of exogenous enzymes. *Brew. Bev. Ind. Int.,* 1989, **1,** 71-81.
16. Mathieu, D., Feneuille, D. and Phan-Tan-Luu, R., Methodologie de la recherche expérimentale: Etude des surfaces de réponse, Laboratoire de Prospective Reactionnelle et d'Analyse de l'Information, IUT de l'Université d'Aix-Marseille, 1977.
17. Nso, E. J., Ajebesone, P. E., Mbofung, C. M. and Palmer, G. H., Properties of three sorghum cultivars used in the production of Bili-Bili beverage in Northern Cameroon. *J. Inst. Brew.,* 2003, **109,** 245-450.
18. Nso, E. J., Nanadum, M. and Palmer, G. H., The effect of formaldehyde on enzyme development in sorghum malts. *Tech. Q. Master Brew. Assoc. Am.,* 2006, **43,** 177-182.
19. Obliana, A. T., Sorghum seed production and grain utilisation in the brewing industries: the Nigerian experience, Proceedings of 2nd Workshop on Seed Production and Utilisation, Lusaka, Zambia, 1990.
20. Okafor, N., Prospects and challenges of the use of local cereals and starchy substrates in brewing. National symposium on the Brewing Industry, Now and the 21st Century. Owerri. Nigeria, 1985, pp. 22-25.
21. Palmer, G. H., Cereals in malting and brewing. In: Cereal Science and Technology. G. H. Palmer Ed., Aberdeen University Press: Aberdeen, Scotland, 1989, pp. 61-462.

(Manuscript accepted for publication February 2010)