Research Article

Optimization of the Impact of Hitempase 2XL, Bioglucanase TX and Brewers Protease on the Turbidity of *Madjeru* sorghum cultivar wort.

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Abstract:— The action of three technical mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers Protease) on the turbidity of the worts of unmalted and malted *Madjeru* sorghum mashes was modeled and analyzed using response surface methodology. The analysis showed that both Hitempase 2XL and Bioglucanase TX had significant impact on unmalted *Madjeru* worts turbidity during mashing of unmalted *Madjeru* sorghum grist, with a contribution of 15% and 11% respectively. Bioglucanase TX and Brewers Protease had significant impact on malted *Madjeru* worts turbidity, they contributed respectively for 7% and 13%. The interaction X_1X_2 also had a significant impact on wort turbidity of unmalted *Madjeru* for which it contributed 23%. The interaction X_1X_3 had a significant impact on wort turbidity of malted *Madjeru* for which it contributed 24%. Optimization of the concerted actions of the three enzymes for turbidity of unmalted *Madjeru* gave a combination of (0 U; 298.25 BGU and 0 mg) for Hitempase 2XL, Bioglucanase TX and Brewers Protease respectively. This gave a minimal turbidity of 0.00 NTU. This combination was: 1387.50 U; 0 BGU and 32.23 mg for malted *Madjeru*, giving a minimal turbidity of 5.27 NTU.

Keywords: enzymes, turbidity, response surface methodology, optimization, Madjeru

I.   INTRODUCTION

Sorghum malt has a lower hydrolytic enzyme and principal extracts potential than the conventional barley malt [1]. However the availability of sorghum in Africa is vital economic development, it is essential that the applied research focuses on the capacity in making this cereal the tool of food safety in Africa. Its small quantity in enzymes due to their insufficient development during malting often imposes the use of the commercial brewery enzymes as supplements to obtain the best wort characteristics for brewing [2-16]. Indeed, differences were reported on the composition and the brewery quality of the cultivars of malted sorghums [17-20]. This however could be allotted to the variety of sorghum used for malting. This report is also observed after mash filtration related to sorghum malt quality [15, 21]. The role of the industrial enzymes in facilitating the brewery processes is based on the fact that they have a great catalytic capacity, even when used in very small quantity (traces). Moreover, they catalyze and reduce the use of chemicals and energy during mashing. These observations, although they show differences in the behavior between the cultivars of sorghum during malting, one could foresee the possibilities of modeling and optimizing the mashing of sorghum. The process of mashing was the main research theme in the field of brewery. An improvement and a control of the manufacturing process of beer led first of all to modeling at the level of the basic stage which is malting. A particular interest was related to the β-glucanes which created enormous problems on the level of mash filtration. The modeling of the action of the β-glucanase on the degradation of the β-glucanes during germination [22] is an illustration. Thereafter, there was a simulation of the modeling of the activity of the enzymes resulting from germination during kilning [23]. Works concerned with the follow-up of the quantity of fermentable sugars by using a complex modeling have been done [24]. Modeling was equally the subject of the follow-up of the activity of the β-glucanase during mashing [25]. One can moreover note the use of modeling in the kinetics of the enzymatic hydrolysis of

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the starch during mashing [26]. Another aspect of research was related to the simulation of the propagation of brewery yeasts [27] and the nonlinear modeling of industrial brewery fermentations [28]. The response surface methodology (RSM) was on the other hand used to study the effectivity of the impact of the industrial enzymes on buckwheat malt for a brewery goal [29]. The models observed in the literature are complex and difficult to implement, and they almost exclusively relate to barley and not to sorghum. The beer of sorghum being known to be opaque makes it necessary to investigate on the turbidity of wort using response surface methodology.

II. MATERIAL AND METHODS

A. Enzymes

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

TABLE I. CHARACTERISTICS OF COMMERCIAL BREWERY ENZYMES USED.

Designation	Origin	Activity	Description	Optimal temperature	Optimal pH	Recommended Quantity	Form
Hitempase 2XL[a]	*Bacillus licheniformis*	4416.29 ± 19.34 U/ml	α-amylase	60-95 ℃	4-8	60 U/g	Solution
Bioglucanase TX[a]	*Trichoderma reesei*	750 BGU/ml	β-glucanase	60 ℃	4.5-6.5	0.01 et 0.025 % (v/m)	Solution
Brewers Protease[b]	*Bacillus amyloliquefaciens*	1842.20 ± 1.8 mg AAL/min/ml	Protease	40-50℃ (denatured at 85℃)	6.5-7.5	0.4-2 g/Kg	Solution

a Hitempase 2XL and Bioglucanase TX were obtained from Kerry Bioscience; Kilnagleary, Carrigaline, Co. Cork, Ireland.

b Brewers protease was obtained from DSM Food Specialities, Cedex France.

B. Sorghum cultivar

The biological material obtained at IRAD (Agricultural Research Institute for Development) center of Maroua (Cameroon) was *Madjeru* cultivar grains.

C. Modeling

Modeling was carried out as previously described [30]. A Doehlert matrix design with three factors (X_1, X_2, X_3) representing Hitempase 2XL (X_1), Bioglucanase TX (X_2) and Brewers Protease (X_3) at ranges of 0–3,000 U, 0–937.5 BGU and 0–100 mg, respectively, was used. The transformed matrix of coded variables to an experimental matrix and desired responses (extract and free amino nitrogen) are shown in Table 2. The coefficients of 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 version 11 build 11.0.0.77 software (WPCubed, GmbH, Germany).

D. Validation of Models

The models were validated using two procedures. The first consisted of coupling the method earlier described [30] to the absolute average deviation (AAD) method [31]. The second procedure consisted of applying the method described [32-34].

E. Malting

Malting was done using the method previously described [35]. About 1 kg of *Madjeru* sorghum cultivar grains were washed three times using 3 L of distilled water to remove dirt and other foreign bodies. The grains were steeped in 3 L of distilled water for 48 h at room temperature ($\approx 25°C$) with three changes of water at intervals of 12 h before steep out. Germination was carried out for 4 days in a Heraeus type oven (D-63450 Hanau, Germany) at a temperature of 25°C with water sprinkled on the grains on daily basis. The malt was then air dried at 40°C for 4 days using a CKA 2000 AUF-type dryer (Ngaoundere, Cameroon). The malt was rubbed-off of its rootlets and stored until further use.

F. Mashing

Mashing was done using the method previously described [35]. Two hundred and fifty ml of distilled water were put into a 600 ml beaker and 50 g of sorghum (malted or unmalted) flour (Ø < 1 mm) added with continuous stirring until a homogenous mixture was obtained. This mixture was incubated at 45°C for 1 h in a water bath with intermittent stirring at intervals of 5 min. The mix was allowed to decant and 50 ml of the supernatant withdrawn and kept aside. The temperature of the mash was then raised to boiling so as to gelatinise sorghum starch during 40 min with intermittent stirring at intervals of 5 min before cooling to 65°C. The 50 ml of supernatant, to which commercial enzymes are added according to the Doehlert experimental design of three factors, were added to the mash and mashing continued for 1 h and 30 min with intermittent stirring at intervals of 10 min. The mash was cooled and filtered at 25°C for 1 h and 30 min using Whatmann paper no. 42.

G. Determination of turbidity

After mashing, filtration and cooling, the wort is placed inside the HACH 2100N turbidimeter (Hach company

headquarters, Loveland CO, USA). The turbidity value is obtained after stabilization of the value showed in NTU.

H. Optimization of Models

Models were optimized as previously described [30]. The optimal zone of intersection of the curves was highlighted.

III. RESULTS AND DISCUSSION

A. Modeling

Turbidity is a measurement which translates the quantity of suspended matter in wort. The follow-up of this data makes it possible to have an idea on the clearness of wort and thus of beer. Mathematical modeling of the action of the brewery enzymes during mashing would make it possible to understand the impact of each enzyme on this response.

The mathematical models obtained took account of the coded values of factors and arise as follows respectively for unmalted and malted *Madjeru*:

$$Y_{MadTU}(X_1, X_2, X_3) = 7{,}548 + 3{,}412X_1 - 2{,}389X_2 - 0{,}465X_3 - 5{,}056X_1X_2 + 0{,}774X_1X_3 + 1{,}567X_2X_3 - 2{,}036X_1^2 + 2{,}202X_2^2 - 4{,}449X_3^2 \quad (1)$$

$$Y_{MadMTU}(X_1, X_2, X_3) = 6{,}112 + 0{,}290X_1 + 0{,}730X_2 + 1{,}298X_3 + 0{,}765X_1X_2 - 2{,}407X_1X_3 + 0{,}133X_2X_3 + 2{,}164X_1^2 - 0{,}060X_2^2 - 2{,}350X_3^2 \quad (2)$$

With: Y_{MadTU} (X_1, X_2, X_3) representing the mathematical model for unmalted *Madjeru*; Y_{MadMTU} (X_1, X_2, X_3) representing the mathematical model for malted *Madjeru*; X_1, Hitempase 2XL; X_2 , Bioglucanase TX and X_3 Brewers protease.

These mathematical models are polynomials having several variables with correlation coefficients R^2 = 0.966 for unmalted *Madjeru* and R^2 = 0.912 for malted *Madjeru*. These coefficients, coupled to AAD values of 0.093 and 0.050 for unmalted and malted *Madjeru*, respectively, allowed for the validation of the models for the wort turbidity. In addition, bias factors of 0.8 and 1.02, coupled to exactitude factors of 1.13 and 1.20 for both unmalted and malted *Madjeru*, respectively, also allowed for validation of the models according to the method described [32-34].

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 interacting form (X_1X_2, X_1X_3 and X_2X_3). They were statistically considered significant or not if the probability (P) of increasing or decreasing turbidity was $\leqslant 0.05$ or $\geqslant 0.05$, respectively (Table 3).

TABLE II.   MATRICES OF DOEHLERT, CODED AND EXPERIMENTAL VALUES

Coded values			Experimental values			*Madjeru*					
						Turbidity					
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[a]	Theo[b]	Res[c]
0.000	0.000	0.000	1500	468.75	50	7.680	7.548	0.132	6.317	6.112	0.205
1.000	0.000	0.000	3000	468.75	50	8.600	8.924	-0.324	8.337	8.566	-0.229
0.500	0.866	0.000	2250	937.50	50	7.300	6.138	1.162	7.953	7.716	0.237
-0.500	-0.866	0.000	750	0.00	50	6.000	6.864	-0.864	5,270	6.162	-0.892
0.500	-0.866	0.000	2250	0.00	50	15.333	14.655	0.678	6.293	5.790	0.503
-0.500	0.866	0.000	750	937.50	50	6.400	7.105	-0.705	6.320	6.764	-0.444
0.500	0.289	0.816	2250	625.18	100	4.200	4.851	-0.651	8.370	5.658	2.712
-0.500	-0.289	-0.816	750	312.32	0	4.677	3.579	1.098	5.390	2.828	2.562
0.500	-0.289	-0.816	2250	312.32	0	7.450	7.821	-0.371	7.753	4.861	2.892
0.000	0.577	-0.816	1500	781.07	0	3.100	3.582	-0.482	7.223	3.827	3.396
-0.500	0.289	0.816	750	625.18	100	2.600	2.269	0.331	10.567	7.111	3.456
0.000	-0.577	0.816	1500	156.43	100	5.900	5.580	0.320	8.323	5.103	3.220
0.000	0.000	0.000	1500	468.75	50	7.168	7.548	-0.380	6.270	6.112	0.158
-1.000	0.000	0.000	0	468.75	50	2.500	2.100	0.400	8.050	7.986	0.064
-1.000	-0.866	-0.816	0	0.00	0	0.354	0.598	-0.244	7.143	3.477	3.666
0.000	0.000	0.000	1500	468.75	50	7.800	7.548	0.252	6.317	6.112	0.205
0.000	0.000	0.000	1500	468.75	50	7.200	7.548	-0.348	6.303	6.112	0.191

a Experimental result values.
b Theoretical values (values coming from mathematical models).

c Residue.

TABLE III.   ESTIMATION OF REGRESSION COEFFICIENTS FOR THE TURBIDITY OF UNMALTED AND MALTED *MADJERU*.

| Effect	Coefficient		Std. deviation		t-statistics		P-value	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
CONSTANTE	7.548	6.112	0.436	0.285	17.320	21.477	0.000	0.000
X_1	3.412	0.290	0.448	0.293	7.610	0.992	0.000	0.354
X_2	-2.389	0.730	0.391	0.255	-5.292	2.477	0.001	0.042
X_3	-0.465	1.298	0.369	0.241	-1.028	4.392	0.338	0.003
X_1^2	-2.036	2.164	0.769	0.502	-2.648	4.312	0.033	0.004
X_2^2	2.202	-0.060	0.559	0.365	2.952	-0.122	0.021	0.906
X_3^2	-4.449	2.350	0.482	0.315	-6.140	4.966	0.000	0.002
X_1*X_2	-5.056	0.765	0.877	0.573	-4.991	1.157	0.002	0.285
X_2*X_3	1.567	0.133	0.772	0.504	1.434	0.186	0.195	0.858
X_1*X_3	0.774	-2.407	0.914	0.597	0.690	-3.290	0.512	0.013
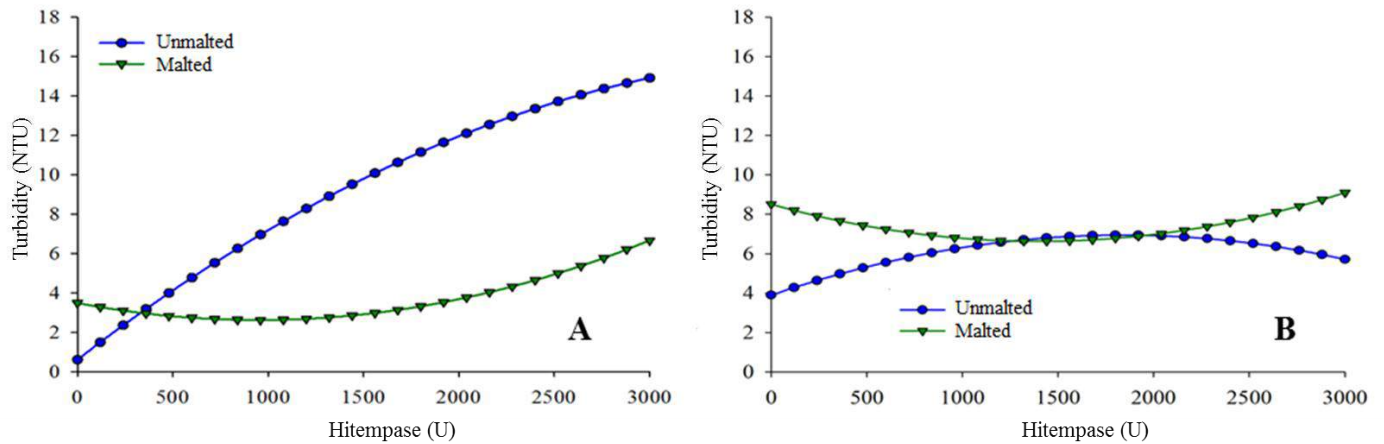
Figure 1. **(A)** : Effect of Hitempase (α-Amylase) on the evolution of *Madjeru* worts turbidity (NTU). **(B)** : Effect of Hitempase (α-Amylase) on the evolution of *Madjeru* worts turbidity (NTU) in the presence of Bioglucanase TX (750 BGU) and Brewers Protease (60 mg).
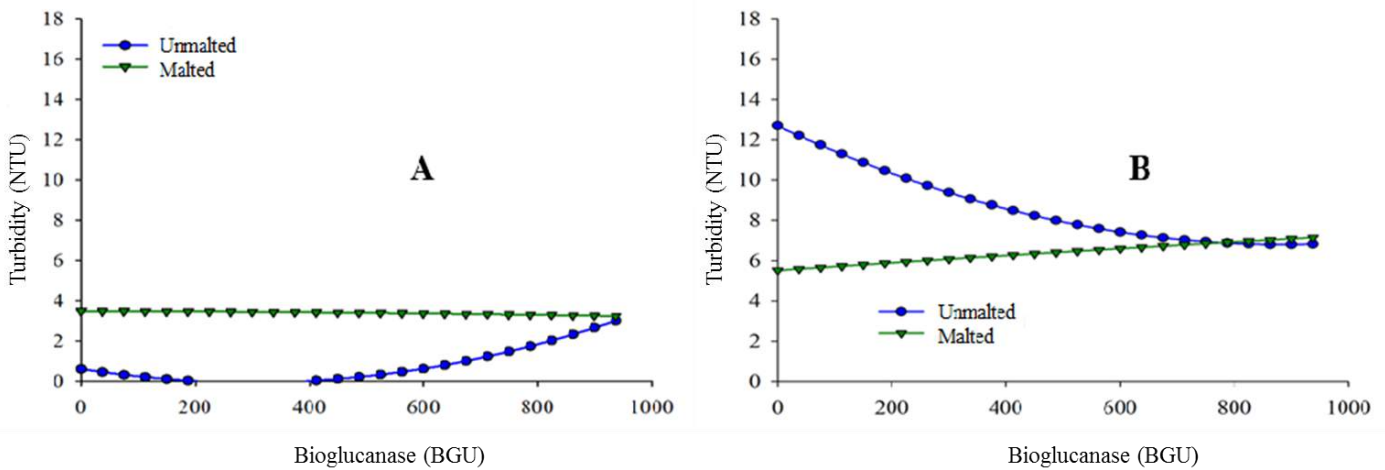

Figure 2. **(A) :** Effect of Bioglucanase (β-Glucanase) on the evolution of *Madjeru* worts turbidity (NTU). **(B)** : Effect of Bioglucanase (β-Glucanase) on the evolution of *Madjeru* worts turbidity (NTU) in the presence of Hitempase 2XL (1875 U) and Brewers Protease (60 mg).

Figure 1A represents the influence of Hitempase 2XL on the turbidity of unmalted and malted *Madjeru* worts. In the absence of this enzyme, turbidity was 0.59 NTU and 3.47 NTU respectively for unmalted and malted *Madjeru*. In the case of unmalted *Madjeru*, after addition of Hitempase 2XL, one observes an increase in turbidity up to a maximum value of 14.91 NTU corresponding to 3000 U. In the case of malted *Madjeru*, there was a drop of turbidity up to a minimal value of 2.61 NTU at 948.40 U; this turbidity increases thereafter up to 6.66 NTU at 3000 U. An addition of 331.24 U of Hitempase 2XL leads to obtaining worts having same turbidity as well for unmalted as for malted *Madjeru*, at 2.97 NTU.

Figure 1B represents the influence of Hitempase 2XL on the turbidity of unmalted and malted *Madjeru* worts, in the presence of Bioglucanase TX (750 BGU) and Brewers Protease (60 mg). In absence of Hitempase 2XL, the initial turbidity was 3.89 NTU and 8.50 NTU respectively for unmalted and malted *Madjeru*. In the case of unmalted *Madjeru*, after addition of Hitempase 2XL, one observes an increase in turbidity up to a maximum value of 6.94 NTU at 1835.70 U. A reduction thereafter is observed and, one reaches a minimal value of 5.71 NTU at 3000 U. In the case of malted *Madjeru*, turbidity drops up to the minimal value of 6.62 NTU at 1397.90 U; then, it increases up to 9.09 NTU at 3000 U. Additions of 1261.90 U and 1958.40 U of Hitempase 2XL leads to worts having same the turbidity both for unmalted and malted *Madjeru*, at 6.67 NTU and 6.92 NTU respectively.

The results show that the effect of Hitempase 2XL on wort turbidity would be more important for unmalted *Madjeru* than for malted *Madjeru*. This would be explained by the fact this enzyme alone (case of unmalted *Madjeru*), would be less effective in the solubilization of the major compound of the grain. It is especially the starch which would be solubilized by Hitempase 2XL and in addition, not only into simple sugars but also into oligosaccharides and dextrins (colloidal materials). Dextrins and significant amounts of proteins and other sugars
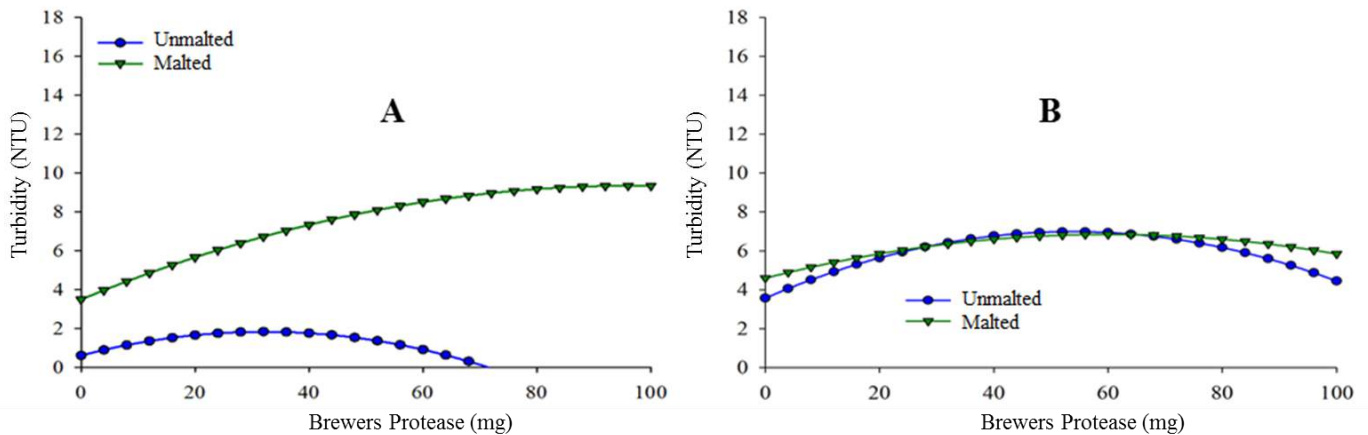

Figure 3. (A) : Effect of Brewers Protease on the evolution of *Madjeru* worts turbidity (NTU). (B) : Effect of Brewers Protease on the evolution of *Madjeru* worts turbidity (NTU) in the presence of Hitempase 2XL (1875 U) and Bioglucanase TX (750 BGU).
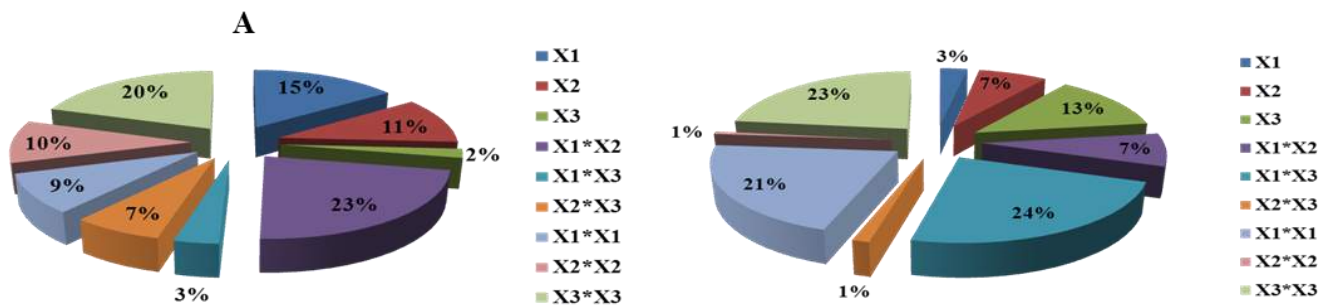

Figure 4. (A) : Contribution of each factor on the model for unmalted *Madjeru* wort turbidity (NTU). (B) : Contribution of each factor on the model for malted *Madjeru* wort turbidity (NTU).

such as the β-glucanes remain insoluble. However, during malting, (case of malted *Madjeru*) the grain is subjected to the action of a naturally balanced and variable quantity of hydrolytic enzymes which were synthetized in order to ensure

partial solubilization of the macromolecules of any nature [36, 37]. These enzymes continue to solubilize the molecules during mashing, decreasing therefore the turbidity of worts. It is nevertheless important to note that under the conditions described for Figure 1A, an increase of Hitempase 2XL in excess ($\geqslant$ 948.40 U), led to an increase in turbidity. The low turbidity observed in the absence or the presence of Hitempase 2XL (fig. 1A) at weak concentrations ($\leqslant$ 331.24 U) could be explained by the inefficiency of the enzyme at this concentration to release sufficient quantities of colloidal material by hydrolyzing starch (oligosaccharides and dextrins).

Increase in turbidity of wort up to 1835.70 U (Fig. 1B) in the case of unmalted *Madjeru* showed that at the beginning, the action of the enzyme led much more to the formation of the colloidal material (oligosaccharides and dextrins) than soluble material (monosaccharide). Beyond this concentration, the drop in turbidity would explain subsequent solubilization in particular oligosaccharides by the enzyme. The quantities of natural enzymes developed during malting, in addition to the quantities of added brewery enzymes, are sufficient to continue the solubilization of the macromolecules up to a Hitempase 2XL concentration of 1397.90 U. Any additional quantity (Hitempase 2XL) beyond this could increase the turbidity of worts.

According to the mathematical models, in its linear form (X_1), the impact of Hitempase 2XL on turbidity is significant (P = 0.000; Table 3) for unmalted *Madjeru* wort, and non-significant (P = 0.354) for malted *Madjeru* wort (Table 3). This action contributes to 15% and 3% for unmalted and malted *Madjeru* worts respectively (Fig. 4A and Fig. 4B). In its quadratic form (X_1^2), the action of Hitempase 2XL is statistically significant for the two types of wort (P = 0.033 and P = 0.004 respectively; Table 3). Its contribution to turbidity in this quadratic form (X_1^2) is indeed 9% and 21% for unmalted and malted *Madjeru* worts respectively (Fig. 4A and Fig. 4B).

Figure 2A represents the influence of Bioglucanase TX on the turbidity of unmalted and malted *Madjeru* worts. In the absence of this enzyme, the initial turbidity was 0.59 NTU and 3.47 NTU respectively. In the case of unmalted *Madjeru*, after addition of Bioglucanase TX, one observed a reduction of wort turbidity up to a value of 0.00 NTU at 201.47 BGU. After that, the curve increased to reach 3 NTU at 937.50 BGU. In the case of malted *Madjeru*, turbidity decreased slightly to reach 3.22 NTU at 937.5 BGU.

Figure 2B represents the influence of Bioglucanase TX on the turbidity of unmalted and malted *Madjeru* worts in the presence of Hitempase 2XL (1875 U) and Brewers Protease (60 mg). In the absence of this enzyme, initial turbidity was 12.70 NTU and 5.50 NTU respectively for unmalted and malted *Madjeru* worts. In the case of unmalted *Madjeru*, after addition of Bioglucanase TX, one observes a reduction of wort turbidity up to the minimal value of 6.82 NTU at 937.5 BGU. In the case of malted *Madjeru*, turbidity increases up to 7.14 NTU at 937.5 BGU. Another observation shows that an addition of 777.52 BGU of Bioglucanase TX led to the same turbidity of 6.88 NTU both for unmalted *Madjeru* and malted *Madjeru*.

A reduction in turbidity, followed an increase in the case of unmalted *Madjeru* worts, translated the fact that until the quantities in Bioglucanase TX of 201.47 BGU, this enzyme managed to solubilize β-glucanes responsible for the turbidity of worts. Beyond this concentration (as for Hitempase 2XL), an additional enzyme in the mash would constitute additional source of colloidal particles and thus increase in the turbidity of worts. In fact Bioglucanase TX hydrolyzed the β (1→3; 1→4) – glucanes link by the non-reducing ends in units of glucose, disaccharide, cellobiose and laminaribiose [37] which, would be solubilized thereafter [25]. In the case of malted *Madjeru*, the turbidity was quasi constant with increase in enzyme concentration (a light reduction of 0.23 NTU is approximately of 6%). These results showed that the effect of Bioglucanase TX on the modification of the wort turbidity profile during mashing was not important compared to that of Hitempase 2XL.

The results in the case of malted *Madjeru* show that malting mobilizes sufficient enzymes to weaken the grain and make these macromolecules more soluble so that there is no need for additional Bioglucanase TX during mashing. A contribution moreover in this enzyme did nothing but increased turbidity. On the other hand, the requirement in this enzyme during mashing of unmalted *Madjeru* is important to reduce turbidity.

The mathematical models showed, in its linear form (X_1) that, the impact of Bioglucanase TX on turbidity is significant both for unmalted *Madjeru* wort (P = 0.001; Table 3) and malted *Madjeru* wort (P = 0.042; Table 3). The increase in turbidity would be allotted to the enzymes synthesized during malting. This action contributes to 11% and 7% for unmalted and malted *Madjeru* respectively (Fig. 4A and Fig. 4B). In its quadratic form (X_1^2), the action of Bioglucanase TX (Table 3) is statistically significant for unmalted *Madjeru* (P = 0.021), and non-significant for malted *Madjeru* (P = 0.906). Its contribution to turbidity in this quadratic form (X_1^2) is indeed of 10% and 1% for unmalted and malted *Madjeru* respectively (Fig. 4A and Fig. 4B).

Figure 3A represents the influence of Brewers Protease on the turbidity of unmalted and malted *Madjeru* worts. In the absence of this enzyme, initial turbidity was 0.59 NTU and 3.47 NTU respectively. In the case of unmalted *Madjeru*, after addition of Brewers Protease, one observes an increase in turbidity up to 1.82 NTU at 32.12 mg. Thereafter turbidity decreased to reach 0.00 NTU at 71.31 mg. The case of malted *Madjeru* led to an increase in turbidity after addition of Brewers Protease, up to a maximum value of 9.34 NTU at 96.80 mg. On the other hand, at 100 mg of added enzyme, one has a turbidity of 9.33 NTU.

Figure 3B represents the influence of Brewers Protease on the turbidity of unmalted and malted *Madjeru* worts in the presence of Hitempase 2XL (1875 U) and Bioglucanase TX (750 BGU). In the absence of this enzyme, turbidity was 3.56 NTU and 4.59 NTU respectively for unmalted and malted *Madjeru* worts. After addition of Brewers Protease, one noted an increase in turbidity for both unmalted *Madjeru* worts and malted *Madjeru* worts up to respective maximum values of 6.98 NTU and 6.84 NTU at 53.73 mg and 59.98 mg

respectively. Thereafter turbidity decreased respectively to reach 4.44 NTU and 5.84 NTU at 100 mg. By adding 28.14 mg and 65.34 mg of Brewers Protease in the mash, one obtained both for unmalted and malted *Madjeru*, respective turbidity of 6.21 NTU and 6.82 NTU.

In the case of unmalted *Madjeru*, low turbidity could be explained by the colloidal matter residues which passed through the filter paper. On the Figure 3B, obtaining more important turbidity in absence of Brewers Protease, could be explained by the incomplete hydrolysis of the mash by α-amylase and protease. The action of protease on the mash of unmalted cereal was initially, the release of the starch granules while proceeding by a solubilization of protein film surrounding them. The first products are some amino-acids and especially peptides. The latter initially will increase turbidity before undergoing thereafter a solubilization under the action of Brewers Protease.

In the case of malted *Madjeru*, considering that the components of cereal already underwent a partial solubilization during malting [36-39], one had from the start higher turbidity. Additional protease only could not increase the turbidity of worts resulting from mashing.

The mathematical models showed that, in its linear form (X_1), the impact of Brewers Protease on wort turbidity was non-significant for unmalted *Madjeru* (P = 0.338; Table 3), but significant for malted Madjeru wort (P = 0.003; Table 3). The increase in turbidity would be allotted to the multitude of enzymes synthesized during malting. This action contributes to 2% and 13% for unmalted and malted *Madjeru* respectively (Fig. 4A and Fig. 4B). In its quadratic form (X_1^2), the action of Brewers Protease (Table 3) was statistically significant both for unmalted *Madjeru* (P = 0.000) and malted *Madjeru* (P = 0.002). Its contribution to turbidity in this quadratic form (X_1^2) was indeed of 20% and 23% for unmalted and malted *Madjeru* respectively (Fig. 4A and Fig. 4B).

The statistical analyzed (Table 4) present the impacts of interactions $(X_1X_2, X_1X_3$ and $X_2X_3)$ between these enzymes on the turbidity of worts. The contributions were presented in the Figures 4A and 4B. They was globally statistically significant for unmalted *Madjeru* (P = 0.007), but not for malted *Madjeru* (P = 0.069).

Interaction X_1X_2 (Hitempase 2XL/Bioglucanase TX) had a significant impact on unmalted *Madjeru* wort but not-

significant for that of malted *Madjeru* wort (P = 0.002 and 0.285 respectively; Table 3). It contributed to a total value of 23% for wort turbidity of unmalted *Madjeru* and to a total value of 7% for wort turbidity of malted *Madjeru* (Fig. 4A and Fig. 4B).

Interaction X_1X_3 (Hitempase 2XL/Brewers Protease) was not significant on the turbidity of unmalted *Madjeru* worts (P = 0.512; Table 3), but was significant on the turbidity of malted *Madjeru* worts (P = 0.013; Table 3). It contribution was of 3% and 24% respectively (Fig. 4A and Fig. 4B).

Interaction X_2X_3, corresponding to the couple Bioglucanase TX/Brewers Protease did not had significant impact on the turbidity both for unmalted *Madjeru* worts (P = 0.195) and malted *Madjeru* worts (P = 0.858; Table 3). It contribution was of 7% and 1% respectively (Fig. 4A and Fig. 4B).

A comparison made in Table 4 between turbidity of unmalted and malted *Madjeru* worts, showed that there is no significant difference (P = 0.232).

B. Optimization

The mathematical models obtained for the follow-up of unmalted and malted worts turbidity are represented by equations 5.1 and 5.2.

An optimization of these equations permitted to minimize unmalted and malted worts turbidity. These minimal characteristics are shown in Table 5. One could for unmalted and malted *Madjeru*, obtain turbidity close to 0.00 NTU and 5.27 NTU respectively.

To obtain wort whose turbidity was lower than 15 NTU both for unmalted *Madjeru* and malted *Madjeru*, it was consequently necessary to superimpose the contour plots. By maintaining the contribution in Bioglucanase at 0 BGU (-0,866), the expressions of equations 5.1 and 5.2 became:

$$Y_{MadTU}(X_1, X_3) = 11,268 + 7,790X_1 - 1,822X_3 + 0,774X_1X_3 - 2,036X_1^2 - 4,449X_3^2 \qquad (6.1)$$

$$Y_{MadMTU}(X_1, X_3) = 5,434 - 0,372X_1 + 1,182X_3 - 2,407X_1X_3 + 2,164X_1^2 - 2,35X_3^2 \qquad (6.2)$$

The layout of the contour plots after transformation of the coded values into real values permitted to obtain wort of turbidity lower than 15 NTU (Fig. 5)

TABLE IV.   ANALYSE DE VARIANCE POUR COMPARAISON ENTRE LES TURBIDITES DES MOUTS DE MADJERU NON MALTE ET MALTE.

Source	Ddl	Sum square	Mean square	F	Probability
Inter-groups	1	9.462	9.462	1.48	0.232
Intra-groups	32	204.164	6.380		
Total	33	213.627			

TABLE V. OPTIMAL CONCENTRATION OF ENZYMES FOR MINIMAL CHARACTERISTICS OF UNMALTED AND MALTED *MADJERU* WORT.

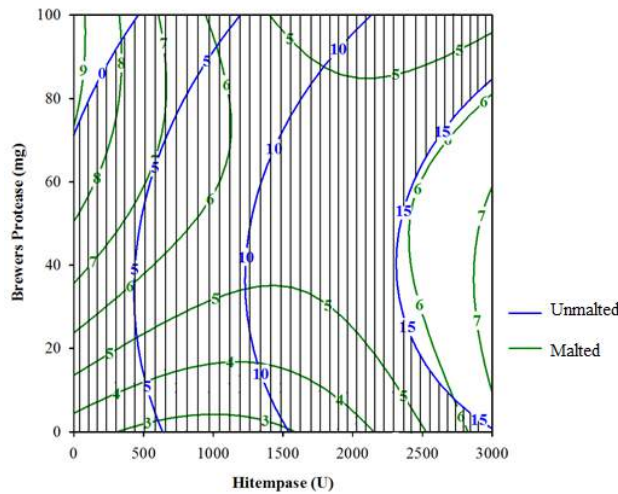| Sorghum	Factors	Optimal Combinaisons		Response
		Coded values	Real values	Turbidity (NTU)
unmalted *Madjeru*	Hitempase 2XL	-1	0 U	0
	Bioglucanase TX	-0.315	298.25 BGU	
	Brewers Protease	-0.816	0 mg	
Malted *Madjeru*	Hitempase 2XL	-0.075	1387.50 U	5.27
	Bioglucanase TX	-0.866	0 BGU	
	Brewers Protease	-0.290	32.23 mg	

Figure 5. Aire de combinaisons enzymatiques induisant une turbidité (NTU) optimale des moûts du cultivar *Madjeru*.

IV. CONCLUSION

The effects of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers protease) on the turbidity were studied during the mashing of unmalted and malted *Madjeru*. Hitempase 2XL was the principal enzyme responsible for the increase of unmalted *Madjeru* wort turbidity. Optimization of mashing properties through models clearly describing the actions of individual commercial mashing 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 of wort obtained after such studies should be the control of that turbidity after fermentation.

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