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Review

Optimization of Red Sorghum (*Sorghum bicolor* (L.) Moench) Malt Mashing Using Response Surface Methodology

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Abstract: The Response Surface Methodology (RSM) was used to optimize the Released Glucose Equivalent during red sorghum malt mashing. Three parameters were tested: the β-amylase (EC 3.2.1.2) temperature level (min value = 62°C and max value = 63°C, TB), the α-amylase (EC 3.2.1.1) temperature level (min value = 72.5 and max value = 77.5°C, TA) and the duration of each stage (min value 15 min and max value 22.5 min, D). The optimal condition obtained during this study (TA = 79°C, TB = 63°C, and D = 18 min) would make it possible to obtain a wort of around 13° Plato. The Analysis of Variance shows that TB and D do not have a significant effect on Released Glucose Equivalent during mashing. This is probably due to the fact that in addition to the very low activity of β-amylase in sorghum malt, the starching temperature of sorghum starch is high and β-amylase is sensitive to heat. Therefore, this enzyme will be lost in larger quantities during mashing than α-amylase. However, the model obtained shows that the β-amylase Temperature (TB) can explain the release of simple sugars despite the fact that its effect is not statistically significant at the 0.1 threshold in the sense that the lack of adjustment of the model obtained is not significant at the same threshold. On the other hand, the temperature of α-amylase and the square of the duration significantly affect the release of simple sugars during mashing. The proposed model of the released Glucose Equivalent expressed as g of Glucose Equivalent per 100 g of red sorghum malt (GE) as affected by the β-amylase Temperature (TB), the α-Amylase Temperature level (TA), and the duration of each stage (D) obtained using RSM has presented a significant goodness of fit.

Keywords: *Sorghum bicolor*, Optimization, Mashing, Temperature, α-Amylase, β-Amylase

Introduction

Beer is a final product obtained after alcoholic fermentation, and its main raw materials are malt, hops, and yeast (*Saccharomyces cerevisiae*) (Chaichi *et al*., 2017). Since its discovery, beer has continued to develop, improve, and move from continent to continent (Chaichi *et al*., 2017; Anonymous, 2023 cited by Amisi *et al.*, 2024), with a considerable increase in its consumption around the world (Bwanganga *et al*., 2013a). Today, beer has become a lucrative source of taxation and a commodity on a global scale. For many experts, the future of the global beer industry now lies in Africa (ECOFIN, 2018).

Brewing companies strengthen their local raw material supplies from networks of contract farmers, thus allowing brewing companies to reduce the costs associated with the importation of conventional raw materials. This results in a market more inclined towards the consumption of beer produced with local raw materials. The most used cereals are, among others, wheat, rye, oats, maize, rice, as well as sorghum as substitutes for barley malt (Bwanganga *et al*., 2013a; ECOFIN, 2018; Amisi *et al*., 2020; Bwanganga *et al*., 2018).

Of all the cereals, sorghum has shown itself to be the most used and the most promising (given the figures

achieved by the companies that have embarked on this race) (ECOFIN, 2018). In developing countries, the technological interest in sorghum is due to its ability to generate a complex system of enzymes associated with the hydrolysis of starch. This is an important element for its use in modern brewing (Amisi *et al*., 2020; Bwanganga *et al*., 2018; Dillon, 1989; Larreta-Garde, 1997; Traoré *et al*., 2004). Also, this cereal allows having a gluten-free beer, which for people with celiac disease is a major advantage because they are allergic to gluten (Bwanganga *et al*., 2013a; ECOFIN, 2018; Amisi *et al*., 2020; Bwanganga *et al*., 2018; Dillon, 1989; Larreta-Garde, 1997; Traoré *et al*., 2004; Amisi *et al*., 2019). Sorghum meets both agronomic requirements and most requirements of the brewing industry. However, its use in brewing as a substitute for malting barley still poses a number of problems (Amisi *et al*., 2021). Apart from the constraints linked to the success of the saccharification, the brewing process poses several other problems to the biotechnologist that deserve a more specific look, these are: The presence of enormous quantities of polyphenols with a non-negligible effect on the work of enzymes, the low synthesis and low activity of sorghum malt wort amylases, the main brewing enzymes (Amisi *et al*., 2019).

The aim of this study is to move from laboratory scale to pilot scale production of sorghum-based beers. It is, therefore, a question of producing a mashing diagram that optimizes the release of simple sugars, or which allows better saccharification. One of the difficulties we have encountered in optimizing saccharification when using sorghum in brewing is finding a compromise for better starch hydrolysis by starch key hydrolytic enzymes during brewing. Indeed, the low level of activity of sorghum β-amylase coupled with its heat-sensitivity contrasts with the sorghum starch thermal properties. This difficulty of better reconciling these two objectives (starching and saccharification) can only be circumvented by seeking a compromise.

Thus, in this study, the Response Surface Method (RSM) was used not only to optimize the release of simple sugars (Released Glucose Equivalent: GE) but also and above all to refine the model of the released Glucose Equivalent expressed as g of Glucose Equivalent per 100 g of red sorghum malt (GE) as affected by the β-amylase temperature level (min value = 62°C and max value = 63°C, TB), the α-amylase temperature level (min value = 72.5 and max value = 77.5°C, TA) and the duration of each stage (min value 15 min and max value 22.5 min, D).

Materials and Methods

An ecotype of the Red Sorghum cultivar (*Sorghum bicolor* L. Moench) obtained from Goma in North Kivu (DR Congo) was used in this study. The homogeneity of the grains was obtained after a visual inspection and any gnawed grains, attacked by insects, and so forth were eliminated from the lot. The sorghum grains used in this study had 26 g of Thousand Grain Weight (TGW), 11% of moisture content (w.b), 96% of germinative capacity, and 95% of germinative energy.

Malting

The grains were sorted manually then the phenolic compounds were eliminated as described by Amisi *et al*. (2020); Bwanganga *et al*. (2018); Dillon (1989); Larreta-Garde (1997); Traoré *et al*. (2004); Amisi *et al*. (2019); Amisi *et al*. (2021); Bwanganga *et al*. (2013b), with some modifications. Red Sorghum grains (1 kg) were mixed with 1 L of acetone/distilled water (70/30: vol/vol); the mixture was left to stand for 20 min and then centrifuged at 5000 rpm for 5 min. After centrifugation, the grains were rinsed with distilled water until clear rinsing water was obtained to ensure that a large amount of the phenolic compounds was eliminated from the sorghum grains. Grains were steeped 48 h in distilled water and then germinated 72 h in the dark. The green malts obtained were dried at 40°C in an oven (type Memmert Models 30-1060) for 48 h and then ground and sieved (diameter 1 mm).

After the malting process, the resulting malt had 255 U/g of α-amylase, 50 U/g of β-amylase, 6 U/g of β-glucanase, 2.7 GAE/g of total phenolic (GAE), 0.07% C.E of condensed tannins, 42% of soluble nitrogen and 32% of soluble nitrogen after 2h boiling.

Brewing

The brewing was carried out with test values used by Amisi *et al*. (2023) with some minor modifications. To do this, sorghum malt flour (138 g) was placed in a 1,000 mL volumetric flask, containing 100 mL of 50 mM acetate buffer solution (mixture of CH_3COOH and CH_3COONa) at pH 5.

The temperature was brought to 50°C for 10 min in a thermostatically controlled water bath. The Response Surface Method was used to optimize the effect of the β-amylase temperature level (min value = 62°C and max value = 63°C, TB), the α-amylase temperature level ((min value = 72.5 and max value = 77.5°C, TA) and the duration of each stage (min value 15 min and max value 22.5 min, D). This experimental plan (Table 1) is used to optimize the response (Released Glucose Equivalent) and to refine the model after determining important factors.

After mashing, all worts were boiled (100°C boiling under reflux for 75 min), cooled, and centrifuged at 5000 rpm for 5 min.

Table 1: The experimental plan for response surface analysis

RunOrder	PtType	Blocks	TA	TB	D
1	0	1	75.0	62.5	19
2	1	1	72.5	62.0	15
3	1	1	77.5	62.0	23
4	0	1	75.0	62.5	19
5	-1	1	75.0	61.7	19
6	1	1	72.5	62.0	23
7	0	1	75.0	62.5	19
8	-1	1	70.8	62.5	19
9	-1	1	75.0	63.3	19
10	1	1	77.5	63.0	23
11	0	1	75.0	62.5	19
12	-1	1	79.2	62.5	19
13	-1	1	75.0	62.5	12
14	1	1	72.5	63.0	23
15	1	1	72.5	63.0	15
16	0	1	75.0	62.5	19
17	1	1	77.5	63.0	15
18	0	1	75.0	62.5	19
19	1	1	77.5	62.0	15
20	-1	1	75.0	62.5	25

The model is GE = $\beta_0+\beta_1TA+\beta_2TB+\beta_3D+\beta_{11}TA^2+\beta_{22}TB^2+\beta_{33}D^2+\beta_{12}TA\times TB+\beta_{13}TA\times D+\beta_{23}TA\times D+\beta_{123}TA\times TB\times D$.

Where GE is the released Glucose equivalent expressed as g of Glucose Equivalent per 100 g of red sorghum malt, *TB* is the β-amylase temperature level (min value = 62°C and max value = 63°C), *TA* is the α-amylase temperature level (min value = 72.5 and max value = 77.5°C), *D* is the duration of each stage (min value 15 min and max value 22.5 min), β_0 is constant, β_1, β_2 and β_3 are linear coefficients, β_{11}, β_{22} and β_{33} are quadratic coefficients and β_{12}, β_{13}, β_{23} and β_{123} the interaction coefficients.

Dosage of Reducing Sugars

Reducing sugars were assayed using the method described by Miller, (1959) with a few minor modifications, a method testing for the presence of free carbonyl groups of sugars by an oxidation-reduction reaction between the free carbonyls of sugars which oxidize and the carboxylic acid function of dinitrosalicylic acid.

The Dinitrosalicylic (DNS) reagent was prepared by mixing 0.75 g of 3,5-dinitrosalicylic acid, 22.5 g of sodium potassium tartrate, and 1.2 g of NaOH in that order in 75 ml of distilled water (this reagent has been stored at 4°C and should not be used after 15 days). The calibration was prepared with glucose (0-0.5-1-1.5 and 2 g/L). The different worts obtained were diluted (1/10 dilution). DNS solution (1 mL) was added to each sample, then the mixture was stirred and boiled for 5 min. The flasks were then cooled in an ice bath and the Optic Density (DO) at 540 nm was measured. The quantity of sugar released was then calculated and expressed as g of glucose equivalent per 100 g of wort.

Results and Discussion

Results

The Analysis of Variance of the Response Surface Regression: G of Released Glucose Equivalent per 100 g of red sorghum malt wort versus TA; TB and D using Stepwide selection of terms are presented in Table (2).

TB is the β-amylase temperature level (min value = 62°C and max value = 63°C), TA is the α-amylase temperature level (min value = 72.5 and max value = 77.5°C) and D is the duration of each stage (min value 15 min and max value 22.5 min).

The equation of the model is:

$$GE = -58.0 + 0.3226TA + 0.577TB + 0.917D - 0.0249D^2 \quad (1)$$

Where *GE* is expressed in g of Released Glucose Equivalent per 100 g of Red sorghum malt wort, TB is the β-amylase temperature level (min value = 62°C and max value = 63°C), TA is the α-amylase temperature level (min value = 72.5 and max value = 77.5°C) and D is the duration of each stage (min value 15 min and max value 22.5 min).

The optimization plot of glucose equivalent/100 g of red sorghum malt wort is presented in Fig. (1) and the surface plots are presented in Fig. (2a-c).

Table 2: Analysis of variance of the response surface regression (α = 1)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	11.8755	2.96886	7.72	0.001
Linear	3	10.0752	3.35840	8.74	0.001
TA	1	8.8842	8.88418	23.11	0.000
TB	1	1.1380	1.13799	2.96	0.106
D	1	0.0530	0.05301	0.14	0.716
Square	1	1.8003	1.80027	4.68	0.047
D*D	1	1.8003	1.80027	4.68	0.047
Error	15	5.7671	0.38447		
Lack of fit	10	2.4476	0.24476	0.37	0.916
Pure error	5	3.3195	0.66390		
Total	19	17.6425			

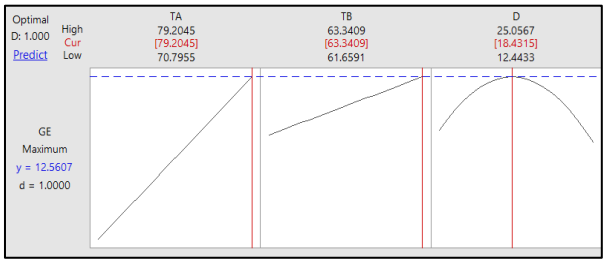
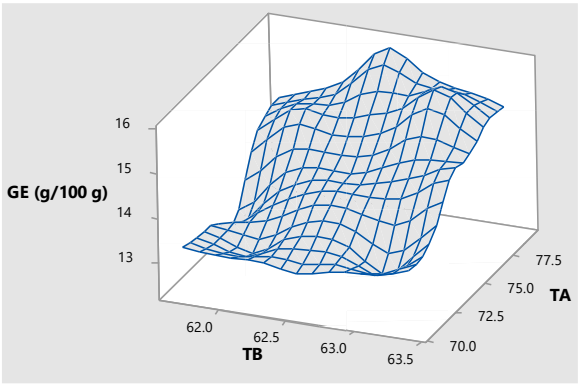
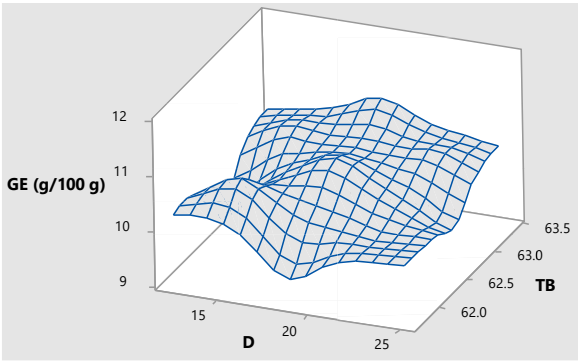


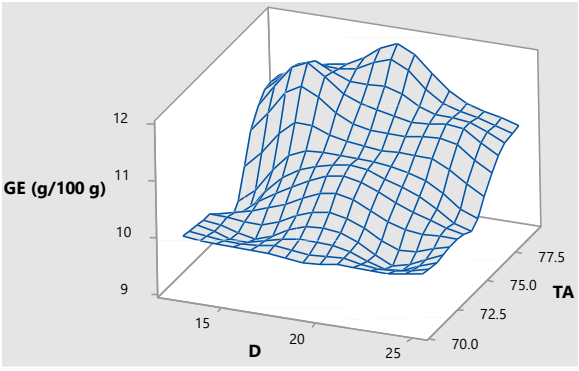
Fig. 1: Optimization plot of g of glucose equivalent /100 g of red sorghum malt wort (GE) as affected by the β-amylase temperature level (min value = 62°C and max value = 63°C, TB), the α-amylase temperature level (min value = 72.5 and max value = 77.5°C, TA) and the duration of each stage (min value 15 min and max value 22.5 min, D)



(a)



(b)



(c)

Fig. 2: (A, B and C) Surface plots of g of glucose equivalent /100 g of red sorghum malt wort (GE) vs the β-amylase temperature level (min value = 62°C and max value = 63°C, TB), the α-amylase temperature level (min value = 72.5 and max value = 77.5°C, TA) and the duration of each stage (min value 15 min and max value 22.5 min, D)

According to the model obtained using the Response Surface Method, the optimal brewing program solution for a high release of reducing sugar expressed as g of Glucose Equivalent (GE) per 100 g of red sorghum wort is TA = 79.2°C, TB = 63.3°C and D = 18.4 min.

According to the model obtained using the Response Surface Method, the optimal brewing program solution for a high release of reducing sugar expressed as g of Glucose Equivalent (GE) per 100 g of red sorghum wort is TA=79.2°C, TB=63.3°C, and D=18.4 min.

Discussion

The brewing conditions used in this study made it possible to obtain quantities of released sugars from 9.3 to 11.9 g of Glucose Equivalent /to 100 g of red sorghum malt wort. These results show that it is possible to achieve better wort gravity necessary for a better fermentation process. The quantity of released glucose equivalents increases with the increase in the temperature of the stages of both α and β-amylases, even if this increase is less great with the increase in the temperature of the stage of β-amylase. As far as duration is concerned, a maximum is clearly obtained at the inflection point of 18.4 min (Fig. 1). Results of this study show that the β-amylase temperature level (min value = 62°C and max value = 63°C, *TB*) and the duration of each stage (min value 15 min and max value 22.5 min, *D*) haven't significant effect on released glucose equivalent during mashing (Table 2). On the other hand, the temperature of α-amylase and the square of the duration significantly affect the release of simple sugars. This would possibly be due to the fact that β-amylase activity is very low in sorghum malt (Bwanganga *et al*., 2013b). Also, the fact that the starching temperature of sorghum starch is high and that β-amylase is heat-sensitive, this enzyme would be lost in larger quantities during mashing than α-amylase. The release of simple sugars cannot be attributed to a single enzyme exclusively because the brewing conditions do not allow distinguishing between enzymes that hydrolyze starch. The model obtained (Eq. 1) shows that the *TB* parameter can explain the release of simple sugars despite the fact that its effect is not statistically significant at the 0.1 threshold in the sense that the Lack-of-Fit of the model obtained is not significant at the same threshold (Table 2). In any case, the 72.5-77.5°C dedicated to the hydrolysis of the starch by α-amylase had a significant effect on released sugars during the brewing of sorghum malts. The optimal conditions obtained during this study (*TA* = 79°C, *TB* = 63°C, and *D* =18 min) would make it possible to obtain a wort of around 13 Plato.

Contrary to the results of Amisi *et al*. (2021) where the optimal temperature for α-amylase was 75°C much higher than that of 70°C obtained by Egwim and Oloyede (2010), the optimal temperature in this study for the α-amylase activity was 79°C. It should be noted that the temperature of 70°C obtained by Egwim and Oloyede (2010) was obtained during which the pH-fixed process, was not indicated. In this study, the scale chosen for the activity of α-amylase was chosen by looking at compromise around the values obtained by Amisi *et al*. (2021); Egwim and Oloyede (2010),

following a common response of all starch hydrolytic enzymes (α- and β-amylases, α-glucanase and limit-dextrinase) under brewing conditions. The difference between the value found by Egwim and Oloyede (2010) and that obtained in this study would also be due to the pH set at 5 in this study, whereas the optimum pH for α-amylase obtained by Egwim and Oloyede (2010) was 5.8.

On purified extracts of Sorghum Malt α-amylase, obtained under optimized malting temperature and pH conditions, Adefila *et al.* (2012); and Ogbonna *et al*. (2004) found optimum temperature and pH values to be 60°C and 6.5 respectively. The difficulty for a study like this one is to find a compromise between the response of α-amylase and that of β-amylase for a cereal such as Sorghum, whose synthesis of β-amylase does not take place in large quantities (Bwanganga *et al*., 2015). According to Amisi *et al*. (2021), the optimum pH for both α and β-amylase is pH = 5, a value lower than that of sorghum α-amylase. The pH was fixed while the pH of the wort was the sum of several interactions: Dissociation and association of weak acids and bases, amino acids, phosphoric acid, carboxylic acids from malt and bicarbonate, and calcium ions from water, and so forth, is difficult to fix in advance. Thus, during a classic brew, it will sometimes be a question of correcting the pH of the wort by adding acids or bases, which is sometimes made difficult by the fact that the wort is generally well-buffered.

Conclusion

This study follows a series of studies conducted in our laboratory relating to the optimization of red sorghum malt mashing conditions (temperatures and durations of each stage) so as to obtain high starch hydrolyzation. Its overall objective is to develop a mashing scale which going to allow the implementation of large-scale red sorghum malt brewing. The need to optimize these parameters which from far or near can influence the proper conduct of the rest of the brewing stages of Sorghum malt wort (otherwise its use in brewing will be blocked) is more than recommended. The RSM was used to optimize the hydrolysis of starch during the mashing of sorghum malt. The mashing process used leads to efficient extraction of reducing sugars. The optimum procedure was TA = 79.2°C, TB = 63.3°C, and D = 18.4 min leading to a wort gravity of 13°Plato. The response of hydrolytic enzyme activities was by measuring as common response of all starch hydrolyzing enzymes (α- and β-amylases, α-glucosidase (EC 3.2.1.20) and limit-dextrinase (EC 3.2.1.41)) in the brewing conditions (temperature and pH).

This preliminary study has shown that the focus of further studies should be on the one taking into account the interactions that exist in the wort. Additionally, it must also be ensured that this study be supplemented by the screening of wort properties and the optimization of the fermentation conditions in order to provide brewers with the information necessary to properly integrate Sorghum into their processes on both artisanal and industrial scale.

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Author's Contributions

Arthur Kapepa Amisi: Contributed to conception and design, and/or acquisition of data, and/or Analysis and interpretation of data, and in drafting the article.

Robby T. Kasonga: Contributed to the material preparation, acquisition of data and analysis.

Bernard Kiwa Mbwanganga: Contributed in reviewing critically for significant intellectual content.

Jean-Claude T. Bwanganga: Supervised the study and gave the final approval of the version to be submitted and any revised version.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and also this research did not involve human or animal subjects. Therefore, no ethical approval is required.

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