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Research Article

Potential of β-Amylase from Sweet Potato (*Ipomoea batatas* Lam) Extract on the Mashing of *Safrari* Sorghum

Daruis Gayus Kameugne Limegne,[1] Steve Carly Zangue Desobgo,[2] and Emmanuel Jong Nso[1]

[1]*Department of Process Engineering, National School of Agro-Industrial Sciences (ENSAI), University of Ngaoundere, P.O. Box 455, Ngaoundere, Adamaoua, Cameroon*
[2]*Department of Food Process & Quality Control, Bioprocess Laboratory, University Institute of Technology (UIT), University of Ngaoundere, P.O. Box 455, Ngaoundere, Adamaoua, Cameroon*

Correspondence should be addressed to Steve Carly Zangue Desobgo; desobgo.zangue@gmail.com

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The aim of this study was to investigate the use of sweet potato as a local source of enzymatic extract for the saccharification of sorghum mash. Box-Behnken designs were employed to determine the optimal conditions for extracting crude enzymes and saccharifying *Safrari* sorghum mash. The optimal conditions for maximizing enzymatic activity were found to be a mass-to-volume ratio of 0.1, an extraction time of 210 min, and a temperature of 60°C. The theoretical and experimental enzymatic activities under these conditions were 23.83 U/mg and 23.49 U/mg, respectively. The extraction of enzymes under these optimal conditions resulted in wort with physicochemical parameters within the following ranges: turbidity (0.79 to 4.52 NTU), pH (5.40 to 8.85), brix (14.80 to 17.50°B), reducing sugars (0.17 to 0.2114 mg/mL), and titratable acidity (3.54 to 5.24 g/L). These findings demonstrate that the extract from Ipomoea batatas contains enzymes that can be effectively used in the mashing process of malted *Safrari* sorghum.

1. Introduction

In tropical regions like Africa, the production of lager beers often involves using a high proportion of cereals other than barley malt [1]. Among these cereals, sorghum is the primary one used as a substitute for barley in beer production [2]. Sorghum is rich in starch, with a content of approximately 69.5% [3], and has an amylopectin/amylose ratio of 75/25 [4], making it a valuable source of carbohydrates for efficient mashing and saccharification. However, brewing with sorghum presents challenges due to the complex malting process. Sorghum malt has a low level of β-amylases, which are responsible for breaking down starch into maltose. The deficiency of β-amylases in sorghum malt affects the saccharification of the mash [5], resulting in a low level of fermentable extract in the wort. Insufficient hydrolysis of starch also leads to difficulties during wort filtration and cloudiness of the beer [2, 3]. To overcome these challenges, researchers have explored various sources of enzymes to enrich sorghum mash. For example, Desobgo et al. [6] used commercial enzymes to increase the level of fermentable sugars in the wort when brewing the *Safrari* cultivar. However, Lyumugabe et al. [5] suggested that the β-amylase deficiency in sorghum malt can be addressed without using commercial enzymes. Other researchers have substituted a portion of sorghum malt with cereals, roots, and tubers to improve saccharification. Etim and EtokAkpan [7] found that substituting 20% of sorghum malt with sweet potato flour increased the level of β-amylases in sorghum mash and promoted saccharification. This is attributed to the presence of both β-amylases and β-glucanases in sweet potatoes [7, 8]. Studies have also explored the combination of sweet potato and sorghum for mash saccharification [9]. However, there is still a lack of optimization of enzymatic activity in these studies.

Therefore, this study is aimed at investigating the potential of an enzymatic extract from *Ipomoea batatas* (sweet potato) to improve the saccharification of sorghum mash. By utilizing sweet potato as a local source of β-amylases, this research seeks to facilitate the saccharification process during the production of sorghum beer.

2. Material and Methods

2.1. Material. *Marimar* sweet potatoes were bought in the Dang area of Ngaoundere, a city in Cameroon. The specific variety of sorghum (*Sorghum bicolor* L. Moench) used in the study is *Safrari*, sourced from the Institute of Agronomic Research for Development (IRAD) in Maroua, Cameroon.

For the statistical analysis, Minitab 2022 software was employed, while OriginLab 2022 was utilized to create the graphs.

2.2. Methods

2.2.1. Raw Material Treatment. The sweet potato was washed using tap water prior to being peeled and cut into strips. These strips were then dried at a temperature of 40°C for a duration of 72 hours. The dried strips were subsequently crushed and passed through a 500 μm sieve to obtain sweet potato powder. The powder was stored in kraft paper at a temperature of 25°C [10].

After obtaining the sorghum cultivar (*Safrari*), it was sifted to remove any impurities. To ensure its ability to germinate and produce malt suitable for brewing, the grains obtained through this method underwent a series of viability tests.

2.2.2. Characterization of Raw Material. The pH of *Ipomoea batatas* Lam powder was measured using the method described by Tamsen et al. [11]. After the samples were mineralized following the Kjeldahl method [12], the total nitrogen content was calculated using the colorimetric technique of Devani et al. [13]. The reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNSA) method, with a slight modification to the approach described by Krivorotova and Sereikaite [14]. The dry matter and ash content of the sample were determined using the AFNOR [15] method. The germination capacity, germination energy, thousand kernel weight, and moisture content of *Safrari* sorghum were determined according to the ASBC standard method [16].

2.2.3. Extraction of Amylases from Sweet Potato Powder and Activity Determination. After weighing the sweet potato, 100 mL of distilled water was added to it. The mixture was then homogenized and left to stand at a temperature of 25°C. Following this, the mixture was centrifuged at 4000 rpm for a duration of 20 minutes at a temperature of 4°C. Once the centrifugation process had separated the different phases, the less dense phase, known as the supernatant, was collected and stored at a temperature of 4°C. It is important to note that a slightly modified technique, as described by Rajagopal et al. [17] and Ramakrishnan and Rathnasamy [18], was employed for this purpose. To determine amylase activity, the methodology outlined by Raul et al. [19] was utilized.

The alpha amylase activity in the extract was quantified using the DNS method, as described in reference [20]. To summarize, the experimental procedure involved combining a reaction mixture consisting of 1% soluble starch, 20 mM phosphate buffer with a pH of 7, and fermented extract. This mixture was then incubated at a temperature of 37°C for 20 min. Following the incubation, 3,5-dinitrosalicylic acid (DNS) was added to the mixture. The amount of reducing sugar released during the assay was determined by measuring the intensity of color development at a wavelength of 540 nm using a UV-VIS spectrophotometer. One unit (1 U) of amylase activity is defined as the amount of enzyme that releases one micromole of maltose within a minute, under standardized assay conditions.

2.2.4. Modeling the Extraction of β-Amylase from Sweet Potatoes. The study employed response surface methodology (RSM) to investigate the impact of three variables, namely, the ratio of sample mass to solvent mass (g/mL), extraction time (min), and extraction temperature (°C), on α-amylase activity. RSM involves establishing regression equations that describe the relationships between the components (factors) of a product and its characteristics (responses), thereby reducing the number of experimental trials while maintaining the desired level of precision. Additionally, RSM helps identify the interaction effects among multiple variables [21]. In this study, a second-order polynomial model and the Box-Behnken RSM design were employed to assess the influence of these process variables on the desired outcome.

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum\sum_{i<j} \beta_{ij} x_i x_j, \quad (1)$$

where y is the measured response; x is the factor; β_0 is the constant; k is the number of factors; β_j is the coefficient of linear effects; β_{jj} is the coefficient of the quadratic effects; β_{ij} is the interaction coefficients.

Table 1 provides an overview of the range of factors that can impact enzyme activity. Equations (2) and (3) establish the relationship between coded values and their corresponding real values.

$$x_j = \frac{U_j - U_j^0}{\Delta U_j}, \quad (2)$$

with

$$U_j^0 = \frac{U_j^{\max} + U_j^{\min}}{2}, \quad (3)$$

where x_j is the value of the coded variable; U_j is the value of the actual variable; U_j^0 is the value of the real variable at the center of the domain; ΔU_j is the variation step; $U_j^{\min}$ is the

Table 1: Domain of the factors for enzyme activity.

Factors	Units	Low level (-1)	Center (0)	High level (+1)
Ratio (X_1)	m/v	0.050	0.075	0.100
Time (X_2)	min	30	120	210
Temperature (X_3)	°C	10	35	60

value of the real variable at the lower bound; $U_j^{\max}$ is the value of the real variable at the upper bound of the domain.

2.2.5. Validation of the Mathematical Model. The model underwent validation using four different methods: calculating the absolute average deviation (AAD), determining the accuracy factors (A_f) and bias factor (B_f) using Excel 2021, and calculating the coefficient of determination (R^2) and adjusted coefficient of determination (adjusted R^2) using Minitab 2022 software. These calculations were performed according to the formulas mentioned by Desobgo et al. [22]. The validation parameters for the models are listed in Table 2.

2.2.6. Optimization of Model Parameters. The Minitab 2022 optimization tool was utilized to maximize the enzymatic activity of the crude extract of *Ipomoea batatas* Lam.

2.2.7. Experimental Sorghum Malting Procedure. For three times, one kilogram of sorghum grain was washed with three liters of distilled water. The purpose of this process was to remove dust and other unnecessary substances. Additionally, the grains were soaked in three liters of distilled water for 48 h at room temperature (25°C), with the water being changed three times every 12 h. Throughout the four-day germination period, the grains were watered every six hours in a Heraeus D-63450 oven (Kendro Laboratory Product, Hanau, Germany) set at 25°C. The malt was then dried in a CKA 2000 AUF dryer for four days at 40°C. Prior to storage, the subsequent rootlets were separated from the malted sorghum.

2.2.8. Scanning Electron Microscopy. Malt from *Safrari* was immersed in liquid nitrogen at a temperature of -196°C. The frozen samples were then sliced through the germ using a sharp blade and attached to metal stubs. The samples, coated with a layer of gold, were examined using a Zeiss Evo LS15 scanning electron microscope (SEM) from Carl Zeiss in Oberkochen, Germany, operating at an acceleration voltage of 20 kV.

2.2.9. Mashing of Safrari Malt with Addition of Sweet Potato Enzyme Extract. A 600 mL beaker was filled with 250 mL of distilled water. Then, 50 g of *Safrari* (malted) flour (sieve passage, 1 mm) was added to the beaker and continuously homogenized until the mixture became uniform. The mixture was incubated at 45°C in a water bath (Memmert brand) for one hour, with intermittent stirring every five minutes. After the incubation, the mixture was decanted, and 100 mL of the liquid above the sediment was separated and set aside. The sorghum starch was gelatinized by boiling the corn for 40 min, stirring every 5 min, and then cooled to a temperature between 55°C and 65°C. The *Ipomoea batatas* Lam enzyme extracts were added to the separated liquid in a ratio ranging from 0 to 0.1 (v/v), based on the Box-Behnken matrix. After adding this mixture back into the mash, stirring continued for 30 to 90 min at a temperature range of 55 to 65°C, in 5-minute intervals. Once the mash had cooled to 25°C, it was filtered for 1 h 30 min using Whatman No. 4 paper (GE Healthcare Whatman, Fisher Scientific, France).

Table 2: Model validation parameters.

Validation parameters	Standard values	Acceptable values
R^2	1	≥92%
Adjusted R^2	1	≥80%
AAD	0	[0-0.3]
Bias factor (Bf)	1	[0.75-1.25]
Accuracy factor (Af)	1	[0.75-1.25]

2.2.10. Modeling. The criteria for selection were determined based on the existing literature. Therefore, the following factors are taken into consideration during the saccharification process of sorghum mash: the ratio of the volume of crude enzymatic extract to the volume of mash (v/v), the duration of saccharification (min), and the saccharification temperature (°C). A Box-Behnken design with three components and fifteen trials was used for modeling. Each trial was conducted twice, and the ranges for the factors are listed in Table 3. These models are presented in quadratic form, and the same validity criteria used for the previous extraction were applied.

2.2.11. Characterization of Sorghum Wort. According to the ASBC standards, measurements were taken for turbidity, pH, extract (°B), reducing sugar, and titratable acidity [16].

2.2.12. Statistical Analysis. Statistical analysis was carried out using ANOVA, and only factors with a probability P of less than 0.05 were considered to have a significant impact.

3. Results and Discussion

3.1. Characteristics of Raw Material

3.1.1. Sweet Potato Powder (Ipomoea batatas Lam). Table 4 provides important information about the quality and characteristics of the sweet potato flour being analyzed. The pH value of 5.85 falls within the range established by Tortoe et al. [23], which suggests that the flour is within an acceptable acidity level for storage and consumption. The moisture content of the sweet potato flour is measured at 5.49 ± 0.07%, indicating that it has a relatively low moisture content. This is a positive characteristic for storage,

Table 3: Domain of factors for *Safrari* malt mashing.

Factors	Units	Low level (-1)	Center (0)	High level (+1)
Ratio (X_1)	(v/v)	0	0.05	0.1
Time (X_2)	min	30	60	90
Temperature (X_3)	°C	55	60	65

Table 4: General chemical properties of *Ipomoea batatas* powder.

Features	Value
pH	5.85 ± 00
Moisture content (% DM)	5.49 ± 0.07
Ash content (% DM)	0.32 ± 0.01
Protein content (% DM)	2.51 ± 0.1
Reducing sugar content (% DM)	1.57 ± 0.04

Table 5: Physicochemical characteristics of unmalted *Safrari*.

Features	*Safrari*
Moisture content (%)	8.33
Germination capacity (%)	98.00
Germination energy (4 mL) (%)	96.00
Germination energy (8 mL) (%)	94.00
1000 grains' weight (g)	50.65

as lower moisture levels can help prevent microbial growth and extend the shelf life of the product. It is worth noting that this moisture content is lower than the range reported by Tortoe et al. [23], who found moisture content between 7 and 10%. The difference in moisture content could be attributed to variations in the sweet potato cultivar used, processing methods, or storage conditions. The ash content of the sweet potato powder is measured at 0.32 ± 0.01%. This indicates a relatively low mineral concentration in the flour. However, it is important to note that this value is lower than the ash content reported by Nogueira et al. [24], which was 1.64 ± 0.06%. The lower ash content in the current study could be attributed to the authors' utilization of the sweet potato harvest season and drying treatment to make the flour. The protein concentration of the sweet potato flour is measured at 2.51 ± 0.1%. This value is smaller than the protein concentration reported by Nogueira et al. [24], which was 2.91 ± 0.04%. The difference in protein concentration suggests that both studies used different sweet potato cultivars or processing methods that did significantly affect the protein content of the flour.

The sugar level in the sweet potato flour is measured at 1.57 ± 0.04%. This is significantly lower than the sugar level reported by Nogueira et al. [24], which was 6.23 ± 0.56%. The decrease in sugar level could be attributed to the specific sweet potato cultivar used in the current study, as different cultivars can have varying sugar content.

3.1.2. Unmalted Safrari Sorghum Grain. The results presented in Table 5 indicate that the moisture content of the *Safrari* cultivar sample is 8.34%, which falls within the recommended range for grain preservation (less than or equal to 13%). This information is supported by previous studies conducted by Briggs [25], Briggs et al. [26], [27], and Hough et al. [28]. Furthermore, the germinative capacity of the *Safrari* cultivar sample is 98%, indicating that a high percentage of the grains have the potential to germinate. The germinative energy, measured at 4 mL and 8 mL, is 96% and 94%, respectively, suggesting that the grains have a strong ability to initiate germination. These values are comparable to those reported by Desobgo et al. [29], further validating the potential of the *Safrari* cultivar for malting and brewing purposes. Additionally, the weight of one thousand kernels for the *Safrari* cultivar sample is determined to be 50.65 g. This value provides an indication of the size and density of the kernels, which is an important factor in the malting and brewing process.

3.1.3. Scanning Electron Microscopy of Unmalted and Malted Safrari. The results of the study showed that there was a network of starch-free cell walls in the endosperm along the endosperm-scutellum interface on day four of malting, as observed through scanning electron microscopy (SEM) of the grain's proximal sections (Figure 1(a)). This suggests that some changes occurred in the endosperm during the malting process. When comparing malted *Safrari* to unmalted *Safrari*, it was found that the malted variety exhibited starch degradation specifically at the endosperm-scutellum interface (Figure 1(b)). This indicates that the modification process began in this region and then progressed throughout the floury endosperm. The enzymatic hydrolysis of starch granules, protein bodies, and the protein matrix is a key mechanism underlying the endosperm transformation observed in malted grains. This process involves the action of enzymes that break down starch into smaller molecules, such as sugars, as well as the degradation of proteins. As a result of these enzymatic activities, air gaps are generated within the endosperm, leading to a decrease in hardness and density.

3.2. Modeling and Optimizing Beta-Amylase Activity Contained in Sweet Potato Powder Extract. The enzyme activity model can be deduced by utilizing the extraction matrix provided in Table 6. This model incorporates individual factors, interactions, and quadratic effects.

$$\begin{aligned} Y_{\mathrm{EA}}(\mathrm{U/mg}) = {} & 11.16 + 9.624x_1 + 1.044x_2 + 0.128x_3 \\ & + 0.69x_1^2 - 0.87x_2^2 + 0.59x_3^2 + 0.3x_1x_2 \\ & + 0.83x_1x_3 + 0.34x_2x_3, \end{aligned} \quad (4)$$

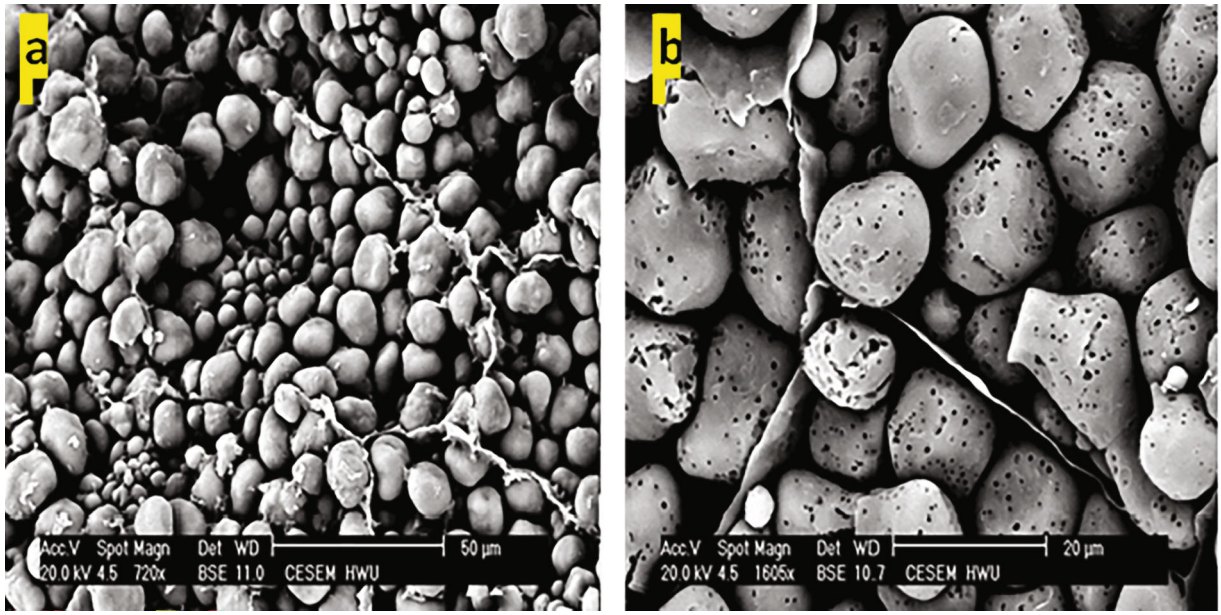

Figure 1: SEM for *Safrari* sorghum: (a) unmalted; (b) malted.

Table 6: Box-Behnken coded and transformed experimental values for *Ipomoea batatas* powder β-amylase extraction matrix with response.

Run	Coded values			Real values			Response
	x_1	x_2	x_3	Ratio (v/v) (X_1)	Time (min) (X_2)	Temperature (°C) (X_3)	β-Amylase activity (U/mg) (Y)
1	-1	-1	0	0.05	30	35	2.49
2	1	-1	0	0.10	30	35	20.43
3	-1	1	0	0.05	210	35	0.93
4	1	1	0	0.10	210	35	20.07
5	-1	0	-1	0.05	120	10	3.28
6	1	0	-1	0.10	120	10	21.57
7	-1	0	1	0.05	120	60	1.65
8	1	0	1	0.10	120	60	23.27
9	0	-1	-1	0.075	30	10	8.41
10	0	1	-1	0.075	210	10	12.87
11	0	-1	1	0.075	30	60	8.21
12	0	1	1	0.075	210	60	14.02
13	0	0	0	0.075	120	35	12.03
14	0	0	0	0.075	120	35	11.66
15	0	0	0	0.075	120	35	9.79

where Y_{EA} is the enzyme activity; x_1 is the sweet potato powder/water ratio; x_2 is the extraction time; x_3 is the extraction temperature.

The utilization of this model considers the validation of the model. Table 7 showcases the criteria for model validity.

According to the validation requirements of Table 7, it is confirmed that the model is valid. Therefore, it is suitable to conduct an exploratory analysis of the model's components. In this analysis, the impact of a factor will only be taken into consideration if its probability is less than 0.05. Table 8 presents the factors that are relevant to this condition and will be investigated.

3.2.1. Effect of the Ratio (x_1) on the Enzyme Activity. The results presented in Figure 2 show a significant increase in enzyme activity as the ratio of sweet potato mass to solvent volume increased from 0.05 to 0.1 during the extraction process. The enzyme activity increased from 2.22 U/mg to 21.47 U/mg, and this difference was found to be statistically significant ($p \leq 0.001$, Table 8). This increase in enzyme activity can be attributed to the addition of sweet potato powder during the extraction. The sweet potato powder acts as a source of enzymes, leading to a higher concentration of enzymes in the extract. As a result, the extract becomes more active and concentrated, leading to the observed increase in

Table 7: Validation of the model parameters.

Model	R^2 (>92%)	R^2 adjusted (>80%)	AAD [0-0.3]	Af [0.75-1.25]	Bf [0.75-1.25]
Y_{EA}	97.12%	91.94%	0.243	1.208	0.988

Table 8: Estimation of regression coefficients for enzyme activity of *Ipomoea batatas* powder extract.

Term	Coeff	Coef ErT	T value	p value
Constant	11.16	1.23	9.10	≤0.001
x_1	9.624	0.751	12.82	≤0.001
x_2	1.044	0.751	1.39	0.223
x_3	0.128	0.751	0.17	0.872
$x_1 * x_1$	0.69	1.11	0.63	0.558
$x_2 * x_2$	-0.87	1.11	-0.79	0.466
$x_3 * x_3$	0.59	1.11	0.53	0.616
$x_1 * x_2$	0.30	1.06	0.28	0.789
$x_1 * x_3$	0.83	1.06	0.78	0.469
$x_2 * x_3$	0.34	1.06	0.32	0.763

enzyme activity. This finding is consistent with a study conducted by Sun et al. [30], who also observed a similar increase in enzyme activity with the addition of sweet potato powder. This suggests that the mechanism behind this observation is likely to be the same in both studies.

3.2.2. Optimization of Beta-Amylase Extraction from Sweet Potato Powder. The text discusses the optimization of enzymatic activity by identifying the values of certain parameters. Table 9 presents the results, showing that a m/v ratio of 0.10, extraction period of 210 min, and extraction temperature of 60°C provide the optimal compromise among the various parameters. The predicted enzyme activity under these conditions is 23.83 U/mg, while the observed enzyme activity is 23.49 U/mg. This observed value is higher than that achieved by Hesam et al. [31], which was 16.37 U/mg. The underlying mechanism behind these results can be explained by the influence of environmental conditions, harvest period, and nutrient content in the soil on the protein content and beta-amylase activity. It is known that the total protein content in a sample can affect the enzymatic activity, and factors such as environmental conditions and nutrient availability can impact the protein synthesis in plants. Therefore, variations in these factors can lead to differences in enzyme activity [10].

3.3. Modeling the Impact of Ipomoea batatas Lam. Crude Extract on Safrari Wort. This study employed a Box-Behnken design, consisting of three components and three levels, to investigate the impact of process variables on turbidity, pH, brix, reducing sugars, and titratable acidity during the mashing of sorghum malt. Each batch involved 15 tests with three central points, following a statistically designed approach. The results are presented in Table 10.

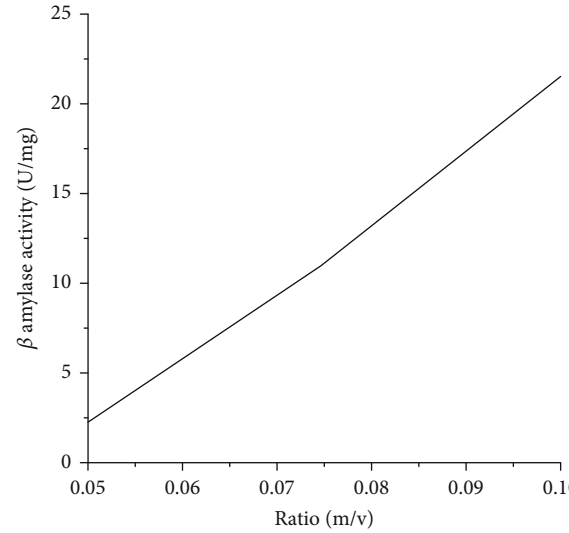

Figure 2: Evolution of the enzyme activity as a function of the ratio. Extraction time and temperature are fixed, respectively, at 120 min and 35°C.

Box-Behnken models establish relationships between individual factors, interactions, and quadratic effects with the response variables. These models comprise the following:

$$\begin{aligned} Y_{RS}(\text{mg/ml}) &= 0.1714 - 0.0051x_1 + 0.0046x_2 - 0.007x_3 \\ &\quad + 0.0166x_1^2 + 0.0143x_2^2 + 0.0019x_3^2 \\ &\quad + 0.0049x_1x_2 + 0.0115x_1x_3 + 0.0044x_2x_3, \end{aligned}$$

$$\begin{aligned} Y_{TA}(\text{g/L}) &= 4.315 - 0.287x_1 + 0.046x_2 + 0.158x_3 \\ &\quad - 0.158x_1^2 - 0.16x_2^2 + 0.164x_3^2 + 0.125x_1x_2 \\ &\quad - 0.367x_1x_3 + 0.047x_2x_3, \end{aligned}$$

$$\begin{aligned} Y_{pH} &= 6.07 + 0.021x_1 + 0.723x_2 - 0.169x_3 \\ &\quad - 0.056x_1^2 + 0.031x_2^2 + 1.624x_3^2 - 0.145x_1x_2 \\ &\quad - 0.162x_1x_3 + 0.2x_2x_3, \end{aligned}$$

$$\begin{aligned} Y_T(\text{NTU}) &= 1.455 - 0.098x_1 + 0.39x_2 - 0.249x_3 \\ &\quad - 0.503x_1^2 + 0.816x_2^2 + 0.786x_3^2 \\ &\quad - 0.157x_1x_2 + 0.75x_1x_3 - 1.051x_2x_3, \end{aligned}$$

$$\begin{aligned} Y_B(^\circ\text{B}) &= 16.55 - 0.606x_1 + 0.215x_2 - 0.031x_3 \\ &\quad - 0.331x_1^2 - 0.194x_2^2 + 0.694x_3^2 \\ &\quad + 0.175x_1x_2 + 0.037x_1x_3 - 0.05x_2x_3, \end{aligned} \tag{5}$$

Table 9: Optimal conditions for maximum β-amylase activity.

m/v ratio (g/ml)	Time (min)	Temperature (°C)	Theoretical enzyme activity (U/mg)	Experimental enzyme activity (U/mg)	Desirability
0.1	210	60	23.83	23.49	1

Table 10: Experimental matrix for Box-Behnken with responses.

Run	Coded values			Real values			Responses				
	x_1	x_2	x_3	Ratio (x_1)	Time (x_2)	Temperature (x_3)	Turbidity (NTU)	pH	Soluble solids (°B)	Reducing sugars (mg/mL)	Titratable acidity (g/L)
1	0	-1	0	0.05	30	60	1.1400	5.40	16.60	0.205801	4.37025
2	0.1	-1	0	0.10	30	60	1.4400	5.75	14.80	0.189150	3.53774
3	0	1	0	0.05	90	60	2.4100	6.63	16.90	0.205801	4.20561
4	0.1	1	0	0.10	90	60	2.0800	6.40	15.80	0.208755	3.87324
5	0	0	-1	0.05	60	55	3.0650	7.75	17.50	0.211441	4.03631
6	0.1	0	-1	0.10	60	55	1.1900	8.10	16.45	0.174916	4.20561
7	0	0	1	0.05	60	65	0.7870	7.50	17.30	0.182167	5.17099
8	0.1	0	1	0.10	60	65	1.9100	7.20	16.40	0.191567	3.87167
9	0.05	-1	-1	0.075	30	55	1.8100	7	16.90	0.198550	4.19942
10	0.05	1	-1	0.075	90	55	4.5200	8.55	17.20	0.198550	4.20561
11	0.05	-1	1	0.075	30	65	3.6955	6.50	17.00	0.167933	4.33769
12	0.05	1	1	0.075	90	65	2.2000	8.85	17.10	0.185659	4.53336
13	0.05	0	0	0.075	60	60	1.4340	6.03	16.65	0.170082	4.37025
14	0.05	0	0	0.075	60	60	1.4500	6.08	16.50	0.171693	4.37025
15	0.05	0	0	0.075	60	60	1.4800	6.10	16.50	0.172560	4.20561

where Y_{RS} is the reducing sugars; Y_{TA} is the titratable acidity; Y_{pH} is the pH; Y_T is the turbidity; Y_B is the brix; x_1 is the ratio; x_2 is the time; x_3 is the temperature.

The effectiveness of these models, all of which are interactive second-degree models, depends on the accuracy of a few input variables. Based on Table 11, all models are reliable and appropriate for conducting a thorough analysis of the components. Table 12's ANOVA only includes variables with a probability less than 0.05, indicating that these are the only variables that are relevant to this particular condition.

Table 11: Validation criteria of the different models from wort attributes.

Settings	R^2	R^2_{adj}	AADM	Bf	Af
Y_{RS}	0.9556	0.8757	0.014	1,001	1.014
Y_{TA}	0.9823	0.9503	0.009	1,000	1,009
Y_{pH}	0.9590	0.8852	0.025	1,000	1.025
Y_B	0.9617	0.8929	0.006	1,000	1.006
Y_{Tu}	0.9806	0.9457	0.050	1.007	1,050

3.3.1. Impact of Singular and Quadratic Factors on the Different Responses

(1) Impact of Ratio (x_1 and x_1^2). The results presented in Figure 3 show several changes in the parameters measured. The titratable acidity decreased from 4.44 g/L to 3.87 g/L, indicating a decrease in the overall acidity of the solution. This can be attributed to the transformation of potassium and sodium found in sweet potatoes into an alkaline solution when added during mashing. The presence of OH- ions in the solution can neutralize the acidity, resulting in a reduction in titratable acidity [32].

The brix, which is a measure of the sugar content, decreased from 16.82°B to 15.61°B (Figure 3). This decrease can be explained by the participation of sugars in Maillard reactions. Maillard reactions occur between reducing sugars and amino groups, leading to the formation of browning compounds [33]. In this case, the rate of sugar involvement in nonenzymatic browning is faster than the rate of starch hydrolysis into sugars. As a result, the overall sugar content decreases, leading to a decrease in brix [34].

The turbidity of the solution decreased from 1.05 NTU to 0.85 NTU (Figure 3). This decrease can be attributed to the presence of β-amylase in the sweet potato extract. β-Amylase is an enzyme that hydrolyzes starch into maltose. The hydrolysis of starch results in the production of linear and branched dextrins. These dextrins contribute to the turbidity of the solution. However, β-amylase also removes the nonreducible dextrin terminations, resulting in the production of maltose and a decrease in turbidity [35, 36].

The study conducted by Desobgo et al. [29] investigated the engagement of reducing sugars in Maillard reactions and its impact on the decrease in reducing sugar content. The authors proposed that the initial explanation for the decrease in reducing sugar content is the involvement of reducing

Table 12: ANOVA for the significance of the factors used during mashing of *Safrari* malt.

Term	*p* value				
	Reducing sugars	Titratable acidity	pH	Brix	Turbidity
Constant	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
x_1-ratio (v/v)	**0.044**	**≤0.001**	0.872	**0.001**	0.299
x_2-time (min)	0.058	0.162	**0.002**	**0.040**	**0.006**
x_3-temperature (°C)	**0.014**	**0.003**	0.235	0.701	**0.032**
x_1^2	**0.002**	**0.013**	0.772	**0.033**	**0.010**
x_2^2	**0.004**	**0.012**	0.872	0.148	**0.001**
x_3^2	0.521	**0.011**	**≤0.001**	**0.002**	**0.001**
x_1x_2	0.129	**0.027**	0.450	0.169	0.244
x_1x_3	**0.008**	**≤0.001**	0.401	0.744	**0.001**
x_2x_3	0.161	0.292	0.310	0.665	**≤0.001**

A factor has a significant impact on the response if its probability is $p < 0.05$ (data in bold).

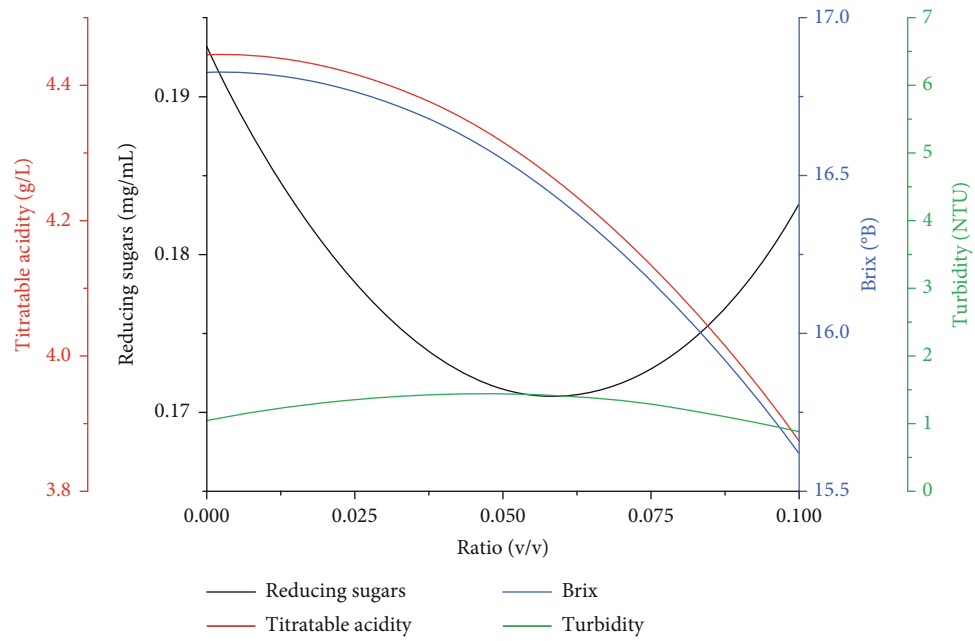

Figure 3: Evolution of titratable acidity, reducing sugars, brix, and turbidity as a function of ratio. Mashing time and temperature are fixed, respectively, at 60 min and 60°C.

sugars in Maillard reactions (Figure 3). Maillard reactions are complex chemical reactions that occur between reducing sugars and amino acids, leading to the formation of various compounds responsible for the browning and flavor development in food products. The reducing sugars, such as glucose and fructose, are known to participate in these reactions as reactive components [37–39]. According to Chevalier et al. [40], reducing sugars exhibit surprising reactivity during Maillard reactions. The variable rate of the reaction implies that the concentration of reducing sugars can either increase or decrease depending on the conditions and the presence of other reactants. In the context of the study, it was observed that as the ratio increased, the concentration of reducing sugars also increased. This observation can be explained by the variable rate of Maillard reactions. When the ratio is low, the rate of starch hydrolysis may surpass the rate of Maillard reactions, leading to an increase in reducing sugar content. However, as the ratio increases, the rate of Maillard reactions becomes more dominant, resulting in a decrease in reducing sugar content. The underlying mechanism of this phenomenon can be elucidated by considering the competition between starch hydrolysis and Maillard reactions. When the ratio of reducing sugars to starch is low, the breakdown of starch into reducing sugars occurs at a higher rate than the formation of Maillard reaction products. As a result, the concentration of reducing sugars increases. However, as the ratio increases, the rate of Maillard reactions becomes more significant, leading to a decrease in reducing sugar content.

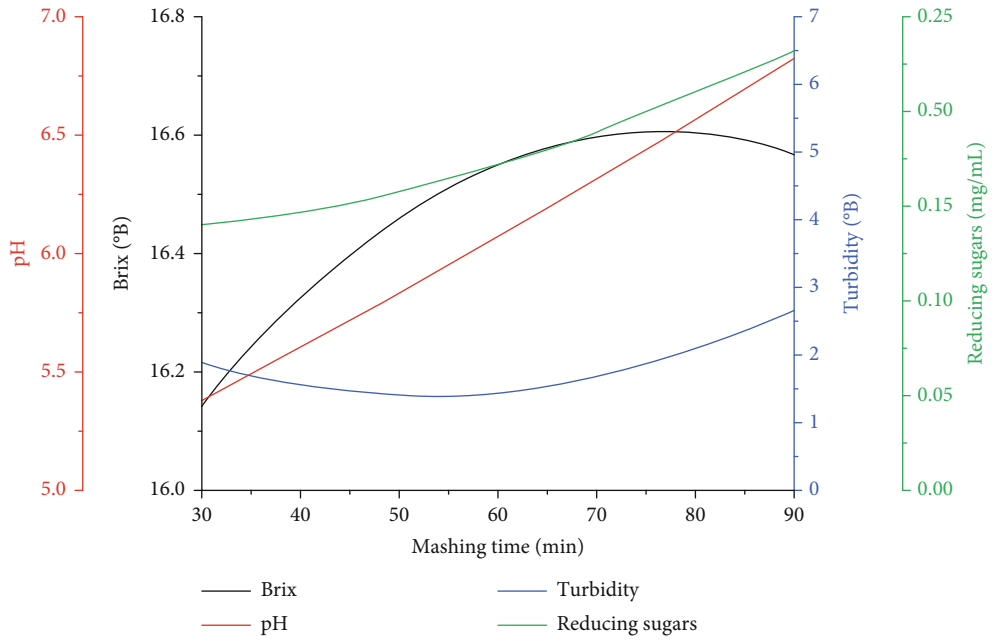

Figure 4: Evolution of pH, brix, turbidity, and reducing sugars as a function of mashing time. Ratio and mashing temperature are fixed, respectively, at 0.05 and 60°C.

(2) Impact of Mashing Time (x_2 and x_2^2). In this study, Figure 4 provides a graphical representation of the changes observed in various parameters as the mashing time is increased from 30 to 90 min. The turbidity of the mash, measured in nephelometric turbidity units (NTU), is seen to increase from 1.88 NTU to 2.66 NTU as the mashing time is prolonged. Similarly, the brix level, which indicates the sugar content of the mash, is observed to climb from 16.14°B to 15.59°B. The pH of the mash also shows an increase from 5.37 to 6.82, and the concentration of reducing sugars rises from 0.14 mg/mL to 0.23 mg/mL. These findings can be explained by the enzymatic hydrolysis of starch in the mash.

As the mashing time increases, the amylolytic enzymes have more time to act on the starch molecules, breaking them down into simpler sugars such as maltose and glucose. This enzymatic reaction becomes more efficient over time, leading to an increase in the concentration of reducing sugars in the medium. This is consistent with the peak activity of these enzymes during this particular time interval, as reported by Nso et al. [41]. The incomplete hydrolysis of starch, resulting in the release of insoluble residues (dextrin), and the interaction between polyphenols/proteins and protein/protein leading to cloudiness formation are two potential factors contributing to the increase in wort turbidity. During the hot mashing stage, high molecular weight proteins tend to lose water, coagulate, and form a stubborn material known as hot trub [42, 43]. It is worth mentioning that the addition of raw sweet potato extract, up to a concentration of 0.1%, may also contribute to the cloudiness of the mixture. The presence of sodium and potassium ions in the enzymatic extract of sweet potato can explain the increase in pH observed during the mashing process. These ions, by creating alkaline solutions in the medium, help neutralize a growing number of organic acids. Basílio et al. [44] also noted a similar pH elevation when cooking orange sweet potatoes.

(3) Impact of Mashing Temperature (x_3 and x_3^2). As depicted in Figure 5, an increase in mashing temperature resulted in a decrease in brix levels from 17.27°B to 17.21°B, a decrease in pH from 7.86 to 7.52, and a decrease in reducing sugars from 0.18 mg/mL to 0.16 mg/mL.

In this study, the increase in mashing temperature resulted in an increase in titratable acidity from 4.32 g/L to 4.64 g/L. Additionally, at a temperature of 60.8°C, the turbidity decreased from 2.49 NTU to 1.43 NTU before rising again to 1.99 NTU at 65°C (Figure 5). These changes in temperature can trigger Maillard reactions, where sugars combine with proteins, leading to a significant decrease in brix [34]. The appropriate temperature conditions allow for the release of specific *Safrari* malt components and extract acids, which are responsible for the decrease in pH. The decrease in pH is caused by the release of H^+ ions from the ionized latter alone. The decrease in reducing sugars may be a result of ongoing Maillard reactions, which require the presence of free amino acids, reducing sugars, pH, and temperature [34]. The increase in titratable acidity may be attributed to the components of malted *Safrari* and the organic acids present in the enzymatic extract, which are released into the medium and contribute to the increase in acidity [45]. The decrease in turbidity may be caused by the involvement of proteins in the medium in Maillard processes. However, the increase in turbidity in the wort, which is promoted by the formation

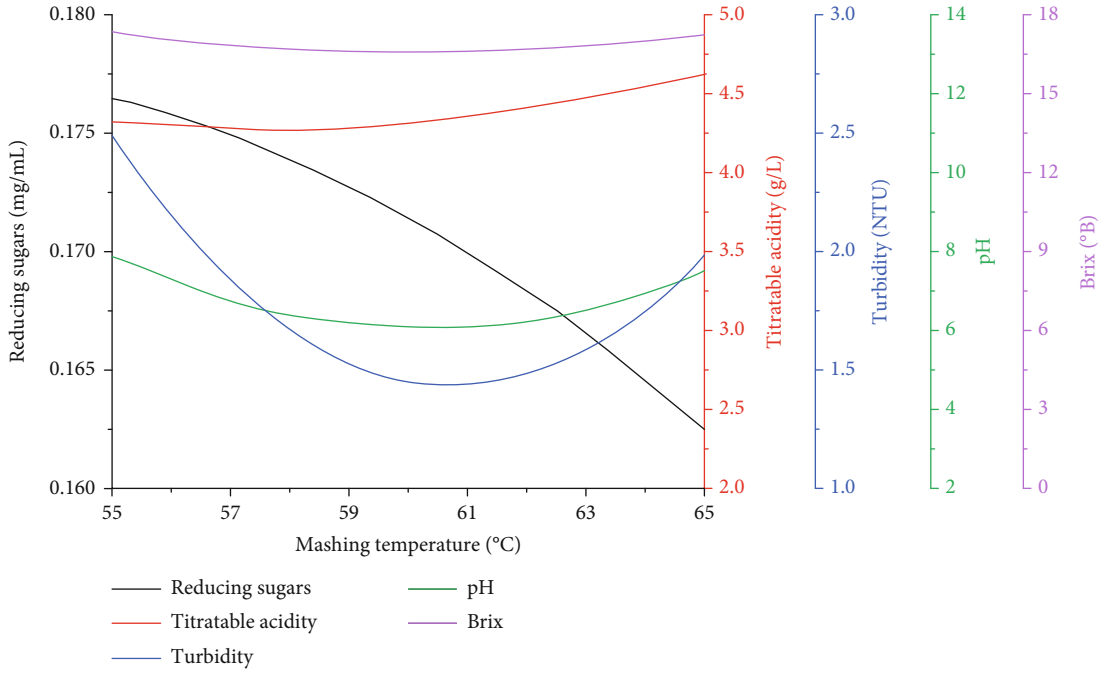

Figure 5: Evolution of reducing sugars, titratable acidity, turbidity pH, and brix, as a function of mashing temperature. Ratio and mashing time are fixed, respectively, at 0.05 and 60 min.

of a polyphenol-protein bond and partial precipitation of the complex, is not definitively explained by the significant release of polyphenols, insoluble proteins, and enzyme inactivation [46].

3.3.2. Impact of Interactions on the Different Responses

(1) Impact of Interaction Ratio/Mashing Time (x_1x_2). The study found that the interaction between x_1 and x_2 has a significant impact on the titratable acidity ($p = 0.027$; Table 12). Specifically, decreasing the ratio of x_1 and prolonging the saccharification time of x_2 lead to an increase in titratable acidity (Figure 6). This increase in titratable acidity can be attributed to the slower dissolution of K^+ and Na^+ ions in the extract as the ratio decreases over time. As a result, the production of bases in the medium is limited, ultimately leading to the rise in titratable acidity.

(2) Impact of Interaction Ratio/Mashing Temperature (x_1x_3). The interaction between the parameters x_1 and x_3 significantly increases the concentration of reducing sugars ($p = 0.008$; Table 12) and the turbidity ($p = 0.001$; Table 12), while significantly lowering the titratable acidity ($p \leq 0.001$; Table 12).

Reducing sugars increase as the ratio (x_1) and saccharification temperature (x_3) decrease. The decrease in ions that may affect the mash pH, caused by the smaller sweet potato extract, could help explain the rise in reducing sugars in the wort. Sweet potato β-amylase continues to function and assist in hydrolysis at its optimal pH. For a mashing time of 60 minutes, the optimal temperature for sweet potato β-amylase is between 55 and 59°C (Figure 7). This finding

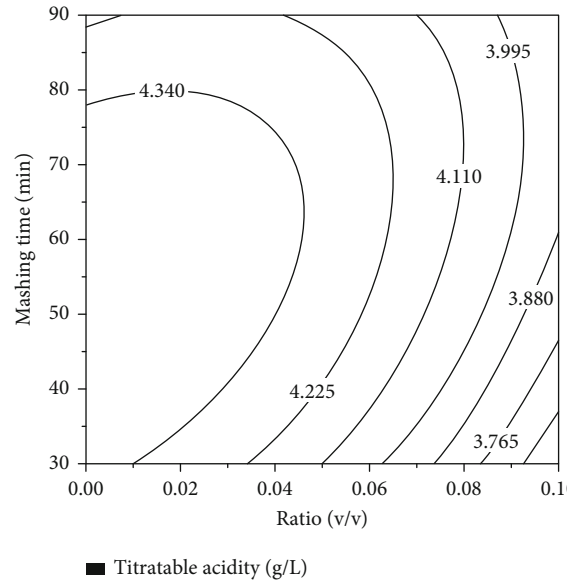

Figure 6: Contour plot showing the combined effect of ratio and mashing time on titratable acidity of wort.

aligns with previous studies that reported the maximum β-amylase activity of sweet potatoes at the optimal temperature [31, 47, 48].

When both the ratio (x_1) and saccharification temperature (x_3) are decreased, turbidity increases. This may be due to being within the optimal temperature range for extracting polyphenols from sorghum (<60°C) [49], which could explain the increase in turbidity. The combination of protein in the extract and malt also contributes to turbidity.

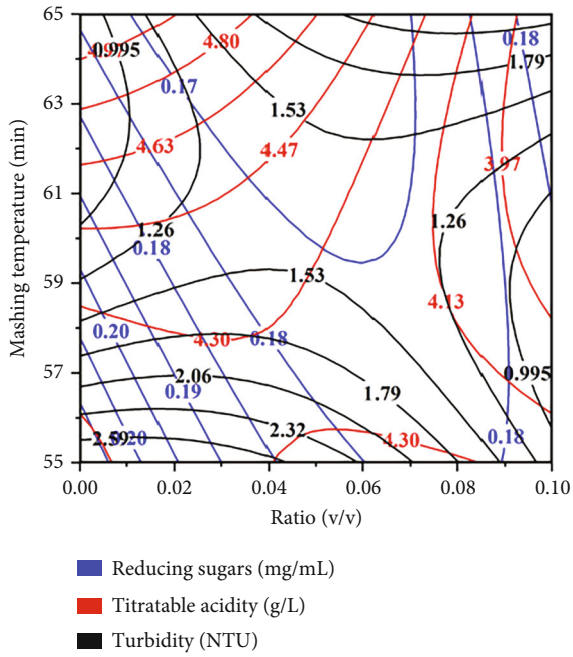

Figure 7: Contour plots showing the combined effect of ratio and mashing temperature on reducing sugars, titratable acidity, and turbidity of wort.

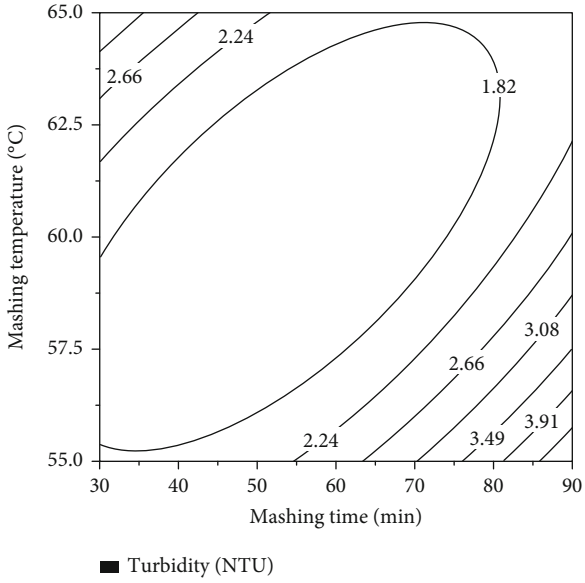

Figure 8: Contour plot showing the combined effect of mashing time and mashing temperature on the turbidity of wort.

On the other hand, when both the ratio (x_1) and saccharification temperature (x_3) are increased, turbidity also increases. Increasing the temperature can deactivate β-amylase [50–52] and disrupt the pH balance due to the presence of K^+ and Na^+ ions, potentially reducing the starch-hydrolyzing capacity of this enzyme as well as *Safrari* malt enzymes. These factors may lead to a significant amount of insoluble residue and a hazy wort.

When the ratio (x_1) is increased and the saccharification temperature (x_3) is decreased, the titratable acidity decreases. This decrease can be explained by the fact that as the ratio increases, more alkali ions dissolve and are neutralized by organic acids to form salts. Consequently, the titratable acidity declines.

(3) Impact of Interaction Mashing Time/Mashing Temperature (x_2x_3). The interaction between mashing time (x_2) and temperature (x_3) has a significant impact on turbidity, as indicated by a $p \leq 0.001$ in Table 12. Turbidity increases when mashing time is decreased and temperature is increased. This can be attributed to inadequate starch hydrolysis when the mashing time is shortened, leading to an increase in turbidity [53]. Additionally, as the temperature increases, the activity of β-amylase decreases gradually, resulting in poor hydrolysis and increased turbidity. Figure 8 illustrates that increasing mashing time and temperature leads to a decrease in turbidity. The formation of trub, a mixture of protein and other solids, can also contribute to turbidity. During mashing, proteins are released from the malt and can interact with polyphenols, forming complexes that can precipitate and contribute to trub formation and reduce turbidity.

4. Conclusion

This research is aimed at exploring the potential use of sweet potatoes as a source of β-amylase in the mashing process of malted *Safrari* sorghum. Through the development of mathematical models, optimal conditions were determined to achieve the highest β-amylase activity in the crude aqueous extract of *Ipomoea batatas* Lam. By incorporating this extract into the mashing of malted *Safrari*, the impact of *Ipomoea batatas* Lam's β-amylase on key wort characteristics such as brix, pH, titratable acidity, turbidity, and reducing sugar was assessed. It was found that each situation requires a specific combination of β-amylase utilization, mashing time, and temperature to achieve the desired wort characteristic.

Data Availability

Data are available within the manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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