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Full Length Research Paper

Optimisation of malting of Cameroonian rice (*Oryza sativa*) cultivars for beer production

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The aim of this study was to determine the optimum malting conditions, for rice beer production, of seven cultivars of local rice (*Oryza sativa* L.) cultivated in the North and Far North Regions of Cameroon. Characteristic tests prior to malting (thousand corn weight, germinative energy, germinative capacity and starch content) of the cultivars (NL36, NL56, ITA306, ITA300, BKN, IR46, NL60) were determined using analytical methods described by The American Society of Brewing Chemists (ASBC). The best cultivars used for the experimental design were NL36 and ITA300, having starch contents greater than 60% DM, germinative energies and capacities greater than or equal to 96%. A Box-Behnken Design was used to investigate the influence of the steeping duration (24-48 h), germination duration (4-7 days), and kilning temperature (45-50°C) on the diastatic power and reducing sugar content of NL36 and ITA300 malt. The optimum malting conditions for maximum diastatic power of NL36 malt (38.47 WK) and reducing sugar content (16.79 g/100 g DM) were: steeping duration of 40 h, germination duration of 4 days, and kilning temperature of 50°C with a desirability value of 0.94. The optimal conditions for a maximum diastatic power of ITA300 malt (39.097 WK) and reducing sugar content (10.34 g/100 g DM) were: steeping duration of 43 h, the germination duration of 5 days, and kilning temperature of 50°C with a desirability value of 0.97. Although rice malt has a low diastatic power compared to barley and sorghum malt, it contains limit dextrinase and α-glucosidase, which can act synergistically with α and β-amylases. These rice cultivars can be used as the main cereal for beer production, or as adjuncts to enhance the enzyme potential of sorghum or maize malt.

Key words: Steeping, kilning, diastatic power, reducing sugar, germination, amylases.

INTRODUCTION

Beer can be defined as a beverage obtained essentially by yeast fermentation of an aqueous extract of pre-germinated cereals like barley, sorghum, wheat, rice, etc., to which hops or hop extracts are added (De Clerck, 1982). The body of beer is provided by the cereal, specifically cereal malt. The malt may be partly substituted by starch-rich adjuncts, such as maize (corn), sorghum, wheat or rice.

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(Howe, 2020). Barley or wheat in general is the basic cereal for beer. This can be problematic for individuals who suffer from celiac disease, an intolerance to the gluten proteins found in barley and wheat, and who should follow a gluten-free diet which would also exclude beer (Hager et al., 2014, Ceccaroni et al., 2019; Cela et al., 2020). It may be useful to substitute the conventional barley or wheat ingredients in beer production with gluten-free rice or sorghum, which are readily available in Cameroon. More so, barley is not readily cultivated in Cameroon and therefore a possibility to produce beer with alternative raw materials (rice, sorghum). In Cameroon, there is an increase in rice production with an estimated 313084 tons produced in 2019, the Far North and North West Regions being the major production areas (MINADER, 2020). Local rice is mostly consumed in the form of fufu or milled into flour. In some areas, it is used in the processing of local alcoholic spirits. The high starch content of rice (about 70% of dry matter), makes this cereal perfectly suitable for brewing (Howe, 2020). Traditionally, it is used for the treatment of skin and hair (Marto et al., 2018) and the husk can be used as fuel (Quispe et al., 2016, 2019; Lantasi and Syafrudin, 2020) or aid during the filtration of the mash.

The production of beer involves three major stages: malting, brewing and fermentation (Owuama, 1999; Aroh, 2019). In this study, emphasis was laid on malting, which is the first step for beer production. Malting involves controlled germination of cereal grains during which enzymes are synthesised and food reserves are modified for ease of transformation. It is a process involving steeping, germination and drying (kilning) of cereal grain to obtain the malt (De Meo et al., 2011; Yorke et al., 2021).

Several studies (Usansa et al., 2009; Adebowale et al., 2010; Ceppi and Brenna, 2010; Owusu-Mensah et al., 2011; Usansa et al., 2011; Agu et al., 2012; Taylor et al., 2013; Mayer et al., 2014; Ceccaroni et al., 2019; Ofoedu et al., 2021) showed that rice malt could be a possible raw material in brewing, but none of the studies achieved complete saccharification of the rice malt during mashing apparently due to non-mastery of the malting process and the high gelatinisation temperature of rice. Similarly, sorghum, which is Africa's fourth most important crop in terms of tonnage after maize, rice and wheat (FAOSTAT, 2015), presents an enzyme deficit in malt (Palmer, 1989; Disharoon, 2020) leading to poor quality of wort during mashing (Desobgo, 2013). The enzyme deficit in sorghum malt can be compensated with the use of rice malt, which is rich in limit dextrinase and α-glucosidase and can act synergistically with α- and β-amylases (Taylor et al., 2013) for proper saccharification during mashing.

The main objective of this study is to optimize the malting process of Cameroonian rice cultivars for beer production. Specifically, this study seeks to determine the malting potential of Cameroonian rice cultivars and to determine the optimum conditions of malting of Cameroonian rice for maximum enzyme potential and grain modification.

MATERIALS AND METHODS

To carry out this study, seven Cameroon paddy rice (NL36 (NL36), NL56 (NL56), ITA306, ITA300, BKN, IR46, NL60 (NL60)) cultivars were used. The synoptic methodology adapted is as shown in Figure 1.

Proximate analysis of the rice and or rice malt

The quality parameters considered prior to malting were the moisture content, germinative energy, germinative capacity and thousand-corn-weight, while the parameters after malting were diastatic power and starch content. Each analysis was modified from Analytica-EBC (1998), and each experiment was done in triplicates.

Determination of the moisture content

Each cultivar was separately milled to form fine grits. The mass of an empty weighing dish M_0 was measured and a given quantity of the sample was added to the dish to give a resulting mass M_1. The dish containing the sample was then placed in an oven which had been preheated to 105°C. After 24 h, the sample was removed from the oven and allowed to cool to room temperature in a desiccator. The mass, M_2, of the dried sample was recorded. The moisture content was expressed as a percentage using the formula:

$$\%Moisture\ content = \frac{(M1-M2)}{(M1-M0)} \times 100 \quad (1)$$

where M_0: Mass of the empty weighing dish, M_1: Mass of weighing dish and sample before drying, and M_2: Mass of weighing dish and sample after drying.

Germinative energy: BRF method

The scope was to determine the percentage of grains which could be expected to germinate fully if the sample is malted normally at the time of the test.

For each rice sample to be tested, four Petri dishes were prepared by placing a filter paper (Whatmann No. 1) on the bottom of each dish. Exactly 4 and 8 mL of distilled water were introduced in the first two (set 1) and last two (set 2) dishes, respectively. Thereafter, 100 grains were evenly placed on each paper, ensuring good contact. All the dishes were covered with lids then sealed, in order to prevent moisture loss, and placed in a dark cabinet. Chitted grains were recovered after 24, 48 and 72 h of steeping, thus avoiding excessive moisture uptake by those grains which germinated early. The average cumulative counts for set 1 and 2 were considered as germinative energy 4 and 8 mL, respectively. The difference between the 4 and 8 mL tests was considered water sensitivity. The results were expressed as percentages rounded to the nearest whole number.

Germinative capacity

The aim was to determine the percentage of viable rice grains using

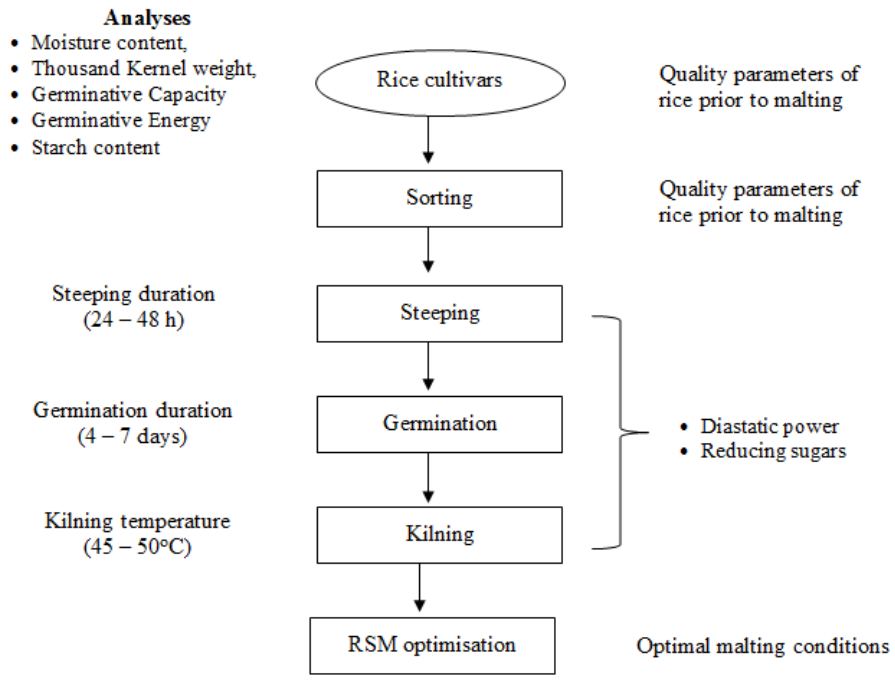

Figure 1. Synoptic diagram of the experimental methodology.
Source: Author

a hydrogen peroxide-assisted growth test. A freshly prepared hydrogen peroxide solution (30%) was prepared by diluting 5 mL of 300 g/L H_2O_2 to 200 mL with distilled water. Two lots of 200 whole grains of each cultivar were counted and each lot was steeped in 200 mL of a freshly prepared H_2O_2 solution at ambient temperature. The solution was strained off after 24 h and replaced with a fresh one. After 48 h, the number of grains that have developed both root and shoot were recorded, and the germinative capacity, expressed as a percentage to the nearest whole number, was calculated using the formula.

$$\text{Germinative capacity} = \frac{200-n}{2} \times 100 \quad (2)$$

Determination of thousand Kernel weight

To determine the average grain weight as a qualitative parameter during brewing two lots of 40 g of rice were weighed, then half grains and foreign matter were removed and the weight subtracted. The number of grains in each lot was then counted. The thousand corn weight, expressed in gram to 1 decimal place, was then calculated using the formula:

$$G = \frac{1000M(100-H)}{100N} \quad (3)$$

where G: weight (g) of 1000 grains, M: weight (g) of the sample of counted grains, H: moisture content of grains (%), and N: number of grains counted in the sample taken.

Diastatic power

The diastatic power is a measure of the amylolytic potential of malt, that is, the ability of the malt to enzymatically convert starch into fermentable sugars.

Preparation of enzyme extract

The enzyme extract was prepared by mixing 10 g of malt flour with 480 mL of distilled water in a water bath at 40°C for 1 h. The mixture was then adjusted to 510 mL with distilled water and then filtered. The supernatant was collected as an enzyme extract (ASBC, 2004).

Test

A 5 mL acetate buffer was added to a 100 mL starch solution (20 g/L) in a 200 mL beaker and the resulting mixture was homogenised at 20°C. After about 20 min, 5 mL of malt enzyme extract was added, homogenised and placed at 20°C for 30 min. In order to deactivate the enzymes present, 4 mL of NaOH (1 mol/L) was added and the mixture was adjusted to 200 mL with distilled water. A few drops of thymolphthalein were added to the mixture causing a blue colouration.

Blank

A 2.35 mL NaOH (1 mol/L) and 5 mL malt enzyme extract were successively added to a 100 mL starch solution (20 g/l) and the volume was adjusted to 200 ml with distilled water. From the resulting solution, 50 mL was removed and placed in a 250 mL conical flask. 25 mL of iodine solution and 3 mL of NaOH were added to the conical flask and well homogenised. The conical flask was then closed to avoid losses in iodine and left to stand for 15 min. Thereafter, 4.5 mL of 0.5 mol/L sulphuric acid was added and

Table 1. Calibrating method and reducing sugars assay by DNS method.

No. of tubes	1 (Blank)	2 (S_1)	3 (S_2)	4 (S_3)	5 (S_4)	6 (S_5)	Test
0.5 mL /ml Standard maltose (ml)	0	0.1	0.2	0.3	0.4	0.5	0
Sample to be tested (ml)	/	/	/	/	/	/	0.1
Distilled water (ml)	0.5	0.4	0.3	0.2	0.1	0	0.4
DNS Reagent (DNS, NaOH, double Na and K tartrate) (mL)	1	1	1	1	1	1	1
Incubate the tubes in a boiling water bath for 05 min, cool immediately in cold water							
Concentration of maltose	0	0.033	0.067	0.1	0.133	0.167	

Measure the absorbance at 540 nm.
Source: Author

unreacted iodine was titrated with thiosulfate solution up to the disappearance of the blue colour. The diastatic power was then evaluated using the formulae:

$$a)\ DP_1 = F(V_B - V_T) \quad (4)$$

$$b)\ DP_2 = \frac{100DP_1}{100 - H_1} \quad (5)$$

Where DP_1= Diastatic power (WKU) on the sample, DP_2= Diastatic power (WKU) on the malt, VB= Volume (mL) of thiosulfate solution upon titration with the blank, VT= Volume (mL) of thiosulfate solution upon titration with the test sample, F= Correction factor to obtain the result by 100 g, F (10g) = 68.4, and H= Moisture content (%m/m) of the malt.

Determination of starch contents

The starch content was determined using the method described by Dicko et al. (2006).

Determination of reducing sugar

The reducing sugar concentration was measured using the 3,5 dinitrosalicylic (DNS) colourimetric method of Fisher and Stein (1961).

Sample preparation

Exactly 2.4 g of sample was introduced in 25 mL of distilled water and the mixture was boiled in a water bath for 15 min. It was then clarified through centrifugation at 3500G for 10 min at a temperature of 25°C. The supernatant was removed and 5 mL of distilled water added to the centrifugation tube containing the residue was again centrifuged. 1 mL of zinc acetate (2 g/100 mL) and 1 mL of potassium iron cyanide (10.6 g/100 mL) were added to the supernatant and then filtered. The supernatant was recovered, completed to a 50 mL volumetric flask using distilled water and followed an assay procedure.

Preparation of DNS solution

The method described by Alejandro et al. (2020) for the determination of reducing sugar content was modified and used. In a 100 mL flask, 1 g of DNS was weighed and dissolved in 20 mL of 10% NaOH. Then 30 g of Na and K double tartrate were weighed and dissolved in 50 mL of distilled water. The two solutions were mixed and made up to 100 mL of distilled water.

A standard solution S1 of maltose with a concentration of 2 mg/mL was prepared by mixing 0.2 g of maltose in 100 mL of distilled water. The respective standard solutions S2, S3, S4, and S5, of respective concentration 0.25, 0.5, 1, and 1.5 mg/mL were prepared by diluting solution S1. Using standard solutions S1, S2, S3, S4, S5 of maltose, the calibration range was prepared and the assay of the samples was carried out as indicated in Table 1.

Expression of results

The quantity of reducing sugar in each trial was determined based on the calibration curve equation:

$$OD = aq + b$$

Where OD is optical density; q is the quantity of reducing sugar, and a, b is constants to be determined

The quantity of sugar in g/100 g DM is given by the relation:

$$Q = \frac{f \times q \times V_T \times 100}{m \times Vi \times DM} \quad (6)$$

where Q is the quantity of sugar in the trial, V_T is the total volume of the extract, m is mass of the trial in g, Vi is volume of sample analysed, DM is dry matter, and F is dilution factor.

Experimental design modelling

The factors evaluated were those which are critical for the malting process. These factors (variables) were steeping duration (X_1), germination duration (X_2) and kilning temperature (X_3). The effects of these variables were studied by means of response surface methodology (RSM). RSM is based on relating product properties to regression equations that describe inter-relations between input parameters and product properties (Dangat et al., 2021; Ferreira et al., 2007). It reduces the number of experimental runs but maintains the expected accuracy and also determines responses to the interactive effect of different variables (Savic et al., 2015). In this study, a second-order polynomial model was used to determine the effect of these process variables on their responses and the RSM design used was the Box-Behnken Design (BBD).

Table 2. Coded levels and real values of independent variables for the Box-Behnken design.

Factor	Unit	Symbol	Level	
			-1	1
Steeping duration	h	X_1	24	48
Germination duration	Days	X_2	4	7
Kilning temperature	°C	X_3	45	50

Source: Author

Table 3. Design matrix for the malting process.

Run	Steeping duration (X_1)	Germination duration (X_2)	Kilning temperature (X_3)
1	0	-1	1
2	-1	1	0
3	1	0	1
4	-1	0	-1
5	0	0	0
6	0	0	0
7	-1	0	1
8	0	0	0
9	1	-1	0
10	1	1	0
11	0	-1	-1
12	-1	-1	0
13	1	0	-1
14	0	1	1
15	0	1	-1

Source: Author

$$Y = \beta 0 + \sum_{i=1}^{k} \beta iX + \sum_{i=1}^{k} \beta ii{x_i}^2 + \sum \sum_{i=1}^{k} \beta ijX_iX_j + \varepsilon \quad (7)$$

where Y and X_i were, respectively the response (dependent variable) and the factors (independent variables), $\beta 0$, βi, βii and βij are, respectively the value of the coefficient at the centre level, linear terms, quadratic terms and interactive terms.

The coded levels and real values of the independent variables steeping duration, germination duration and kilning temperature are presented in Table 2, while Table 3 presents the design matrix for the malting process.

The two measured responses were diastatic power and reducing sugar content. The coefficients of the models were obtained using Minitab version 16 software (Minitab, Coventry, UK.). This software was also used to carry out statistical analysis (ANOVA) of the models, and the curves were plotted using Sigmaplot software version 12.5 (Systat Software, Software, San Jose, CA, U.S.A).

The validation of the models was obtained by calculating the average absolute deviation (AAD) of a set of data, the bias factor (B_f), the accuracy factor (A_f) and R^2. For the model to be valid the AAD should be close to zero while the bias factor and accuracy factor should be between 0.75 and 1.25 and R^2 should be closed to 1 which was expressed thus:

$$AAD = \frac{\left[\sum_{i=1}^{N}\left(\frac{Y_{i,exp}-Y_{i,cal}}{Y_{i,cal}}\right)\right]}{N} \quad (8)$$

$$B_f = 10^{\frac{1}{N}\sum_{i=1}^{N}\left|\log\left(\frac{Y_{i,observed}}{Y_{i,predicted}}\right)\right|} \quad (9)$$

$$A_{f1} = 10^{\frac{1}{N}\sum_{i=1}^{N}\left|\log\left(\frac{Y_{i,cal}}{Y_{i,exp}}\right)\right|} \quad (10)$$

where $Y_{i,exp}$ and $Y_{i,cal}$ were, respectively experimental and calculated responses and N was the number of experiments used in the calculation.

Optimization of the malting process

During the numerical optimization, the constraint and the objective function of these variables were chosen. The objective function for the diastatic power was maximized since the higher the diastatic power, the greater the enzyme potential in starch saccharification during mashing. Also, the reducing sugar content was maximized

Table 4. Recapitulative of the characteristics of 07 cultivars of local rice.

Characteristics	Cultivars						
	NL36	IR46	NL60	ITA300	BKN	I306	NL35
Moisture content (%)	12.4±0.1	17.6±0.01	14±0.0	11.3±0.1	9.5±0.0	11.1±0.1	12.3±0.0
Germinative Energy (4ml) (%)	97±0	91±1	92±0	97±1	92±0	95±0	93±1
Germinative Energy (8ml) (%)	98±0	93±2	95±2	97±0	94±1	95±1	93±0
Germinative Capacity (%)	97±0	65±1	77±2	95±1	75±2	75±1	77±0
Water sensitivity (%)	-1	-2	-3	0	-2	0	0
Thousand kernel weight (g)	22.7±0.1	18.3±0.0	21.4±0.0	23.2±0.0	24.3±0.0	21.4±0.3	19.6±0.0
Starch content	69.3±0.0			67.6±0.0			

Source: Author

since they are useful substrates of many chemical reactions, particularly during fermentation.

RESULTS AND DISCUSSION

Proximate characteristics of rice cultivars prior to malting

The analyses carried out were: moisture content, germinative energy, germinative capacity, water sensitivity, thousand kernel weight and starch content. Table 4 presents the characteristics of the seven cultivars of rice.

The moisture content of the 05 cultivars NL36, I306, BKN, ITA300 and NL35 were, respectively 12.4±0.1, 11.3±0.1, 9.5±0.0, 11.1±0.1 and 12.3±0.0%, implying the cereal grains can be stored for a long period; the critical moisture content being 13% (Briggs, 1998). The cultivars IR46 and NL60, with relatively high moisture content, were dried at 30°C for 12 h before storage; otherwise, they could be used immediately.

The germinative energy gives information about the proportion of grains that will germinate under the conditions of a specified test. The germinative energy, both 4 and 8 mL tests, of the cultivars IR46, NL60, BKN, I306 and NL35 were lower than the recommended 96% for barley (Analytica-EBC, 1998). For the NL36 and I-TA300 cultivars, their values obtained were greater than 96% 97±0 (4 mL) and 98±0 (8 mL) for NL36 and 97±1 (4 mL) and 97±0 (8 mL). This implies that these cultivars, NL36 and ITA300, can fully germinate during malting under standard conditions. However, from the outcome of water sensitivity, the seven rice cultivars used were not sensitive to water, thus the need to consider steeping duration as a variable to be modelled and optimised for proper malting.

Germinative capacity measures the capacity of the grains to germinate, with the critical value being 95%. Just as in the case with germinative energy, the resulting values of germinative capacities (GCs) for the rice cultivars IR46, NL60, BKN, I306 and NL35 were below 95%. NL36 and ITA300 cultivars had values higher than the recommended minimum of 95%.

From the germinative energies and capacities of the 07 rice cultivars, the NL36 and ITA300 cultivars were used for malting optimisation.

The Thousand Kennel Weight (TKW) gives an indication of the starch content of a cereal. All tested rice cultivars showed TKW values lower (18-24 g) than typical TKW values of barley (38-40 g) (MEBAK, 2011) and the “Madjeru” sorghum cultivar (41.2 g) according to Desobgo et al. (2012). However, the results obtained were similar to those of Mayer et al. (2014), which were 19.1-20.2 g for rice subspecies “indica” and 21.2-36.3 g for “japonica” species. Despite these lower values for rice, it still has a reasonable portion of starch which can be modified during malting and hydrolysed during mashing.

The starch content of NL36 and ITA300 rice were respectively 69.26±0.03 and 67.57±0.03%, values approximate to the 70% of Marconi et al. (2017). This high starch content could be hydrolysed during mashing to produce sweet wort rich in fermentable sugars.

Mathematical model development

Three factors with three levels of BBD were used to evaluate the effect of process variables (steeping duration, germination duration and kilning temperature) on the responses to diastatic power and reducing sugar content. This was done on the two rice cultivars NL36 and ITA300 based on their proximate characteristics carried out prior to malting. A total of 15 batch experiments including three centre points were carried out in duplicates using statistically designed experiments, and the results are shown in Table 5. The BBD experimental data was analysed by multi-regression analysis, and the results showed that linear and interactive (2FI) terms exhibited lower F-values but high p-values when compared with the quadratic term. The cubic model was found to be aliased.

The quadratic model was chosen to navigate the

Table 5. Box Behnken experimental design with results.

Run	St (X_1)	Gt (X_2)	KT (X_3)	DP for NL36 (Y1)	RS for NL36 (Y2)	DP for ITA300 (Y3)	RS for ITA300 (Y4)
1	36	4	50	39.1	17.87	33	9.24
2	24	7	47.5	28.9	11.99	28	5.72
3	48	5.5	50	31	5.45	40	10.34
4	24	5.5	45	26	2.83	30.1	5.72
5	36	5.5	47.5	32.8	2.40	33.1	8.14
6	36	5.5	47.5	33.1	2.40	32.8	8.14
7	24	5.5	50	29.3	9.15	34.2	10.34
8	36	5.5	47.5	29.9	2.40	33	8.14
9	48	4	47.5	30.2	9.15	23.1	6.60
10	48	7	47.5	29.5	4.36	25.6	3.39
11	36	4	45	26.5	1.37	23.5	5.19
12	24	4	47.5	28.4	5.88	24	3.10
13	48	5.5	45	32.5	0.39	25.8	9.02
14	36	7	50	28	7.41	31	4.18
15	36	7	45	36	6.97	27.2	4.18

St: Steeping duration; Gt: germination duration; KT: kilning temperature; DP: diastatic power; RS: reducing sugar.
Source: Author

Table 6. Validity criteria for the different models.

Parameter	R^2 (%)	AAD	BF	AF
DP for NL36 malt	93.21	0.024	1.000	1.025
RS for NL36 malt	97.04	0.147	0.886	1.227
DP for ITA300 malt	97.27	0.018	1.000	1.018
RS for ITA300 malt	94.41	0.084	1.002	1.088

DP: Diastatic power; RS: reducing sugar content.
Source: Author

malting of two cultivars of local rice (NL36 and ITA300). A second-order polynomial equation (quadratic) was developed to understand the interactive correlation between the responses and the process variables. The final models obtained in terms of actual factors are represented in the given equations:

$$Y_1 = 31{,}933 + 1{,}325X_1 - 0{,}225X_2 + 0{,}800X_3 - 0{,}300X_1X_2 - 1{,}200X_1X_3 - 5{,}150X_2X_3 - 2{,}692X_1^2 + 0{,}008X_2^2 + 0{,}458X_3^2$$

$$Y_2 = 2{,}397 - 1.313X_1 - 0{,}444X_2 + 3.539X_3 - 2.724X_1X_2 - 0.316X_1X_3 - 4.015X_2X_3 + 0.749X_1^2 + 4.699X_2^2 + 1.310X_3^2$$

$$Y_3 = 32{,}967 - 0{,}225X_1 + 1{,}025X_2 + 3{,}950X_3 - 0{,}375X_1X_2 + 2{,}525X_1X_3 - 1{,}425X_2X_3 - 1{,}971X_1^2 - 5{,}821X_2^2 + 1{,}529X_3^2$$

$$Y_4 = 8.140 + 0.558X_1 - 0.833X_2 + 1.248X_3 - 1.457X_1X_2 - 0.825X_1X_3 - 1.012X_2X_3 - 0.140X_1^2 - 3.297X_2^2 + 0.855X_3^2$$

where Y_1, Y_2, Y_3 and Y_4 are, respectively the diastatic power content for NL36, reducing sugar content for NL36, diastatic power content for ITA300 and then reducing sugar content for ITA300, X_1, X_2, and X_3 are steeping duration, germination duration, and kilning temperature, respectively.

All the models are valid and can then be used for exploratory analysis of the factors (Table 6). This analysis will consider a statistically significant effect of a factor only if the *p*-value is less than 0.05, and only such factors were considered of interest.

Analysis of variance (ANOVA) for the regression models

The significance and adequacy of the models were tested using ANOVA. Both regression models have p-values less than 0.05. The F test values F 7.63 for NL36 diastatic power, F 18.20 for NL36 reducing sugar content, F 19.77 for ITA300 diastatic power and F 9.39 for ITA300

Table 7. ANOVA for NL36 malt Quadratic model of diastatic power content.

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	160.281	9	17.809	7.63	0.019	Significant
X_1-Steeping duration	14.045	1	14.045	6.01	0.058	
X_2-Germination duration	0.405	1	0.405	0.17	0.694	
X_3-Kilning temperature	5.120	1	5.120	2.19	0.199	
X_1X_2	0.360	1	0.360	0.15	0.711	
X_1X_3	5.760	1	5.760	2.47	0.177	
X_2X_3	106.090	1	106.090	45.43	**0.001**	Significant
X_1^2	26.751	1	26.751	11.45	**0.020**	Significant
X_2^2	0.000	1	0.000	0.00	0.992	
X_3^2	0.776	1	0.776	0.33	0.589	
Residual	11.677	5	2.335			
Lack of fit	5.430	3	1.810	0.58	0.683	Not significant
Pure error	6.247	2	3.123			
Cor total	171.957	14				

Source: Author

reducing sugar content indicating that the quadratic models could explain most of the variation in the responses. The accuracy of the reduced quadratic models was assessed by the coefficient of determinations R^2 and adjusted R^2. The responses of the diastatic power content for NL36 and ITA300 had R^2 of 0.93 and 0.97, respectively and adjusted R^2 of 0.81 and 0.92, respectively, while the responses of reducing sugar content for NL36 and ITA300 had R^2 of 0.97 and 0.94, respectively and adjusted R^2 of 0.92 and 0.84, respectively. This implies that 93.21 and 97.27% of the variation in the diastatic power content for NL36 and ITA300, respectively, and 97.04 and 94.41% of the variation in the reducing sugar content were explained by the generated quadratic models. The adjusted R^2 value is a corrected value of R^2 after excluding the insignificant terms from the models. Both models displayed high adjusted R^2 values (>0.80). The lack of fit values for the diastatic power content and reducing sugar content for both NL36 and ITA300 were insignificant (p>0.05), suggesting that the models generated were adequately fitted to the experimental data.

Effects of malting parameters on the diastatic power of NL36 and ITA300 malts

The effect of three parameters namely steeping duration, germination duration, and kilning temperature on the diastatic power of NL36 and ITA300 were investigated. Among these parameters, diastatic power content for NL36 was affected by the steeping duration (X_1), followed by the kilning temperature (X_3) and finally the germination duration (X_2). From the ANOVA (Table 7), the interaction terms of germination duration and kilning temperature (X_2X_3) and the second-order term of steeping duration (X_1^2) had significant effects ($p < 0.05$) on the diastatic power of NL36. Meanwhile, the linear terms of steeping duration (X_1), germination duration (X_2), kilning temperature (X_3), interaction terms of steeping duration and kilning temperature (X_1X_3), steeping duration and the germination duration (X_1X_2) and the second-order terms of germination duration and the kilning temperature had no significant effects ($p >0.05$) on the diastatic power of NL36 malt.

On the other hand, diastatic power content for ITA300 was affected by the kilning temperature (X_3), followed by the steeping duration (X_1) and finally the germination duration (X_2). As seen in Table 8, the kilning temperature (X_3), the interaction terms of steeping duration and kilning temperature (X_1X_3), the second-order term of steeping duration (X_1^2), and germination duration (X_2^2) all had significant effects ($p < 0.05$) on the diastatic power of ITA300. Meanwhile, the linear terms of steeping duration (X_1), germination duration (X_2), kilning temperature (X_3), interaction terms of germination duration and kilning temperature (X_2X_3), steeping duration and the germination duration (X_1X_2) and the second-order term of kilning temperature had no significant effects ($p >0.05$) on the diastatic power of ITA300 malt.

Germination duration and kilning temperature for diastatic power for malt NL36

Figure 2 presents the effect of germination duration and kilning temperature on the diastatic power of NL36 malt. For the NL36 malt, its diastatic power increases when the

Table 8. ANOVA for ITA300 malt Quadratic model of diastatic power content.

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	317.778	9	35.309	19.77	0.002	Significant
X_1-Steeping duration	0.405	1	0.405	0.23	0.654	
X_2-Germination duration	8.405	1	8.405	4.71	0.082	
X_3-Kilning temperature	124.820	1	124.820	69.87	0.000	Significant
X_1X_2	0.562	1	0.562	0.31	0.599	
X_1X_3	25.502	1	25.502	14.28	0.013	Significant
X_2X_3	8.123	1	8.123	4.55	0.086	
X_1^2	14.342	1	14.342	8.03	0.037	Significant
X_2^2	125.103	1	125.103	70.03	0.000	Significant
X_3^2	8.634	1	8.634	4.83	0.079	
Residual	8.932	5	1.786			
Lack of fit	8.885	3	2.962	126.93	0.008	Significant
Pure error	0.047	2	0.023			
Cor total	326.709	14				

Source: Author

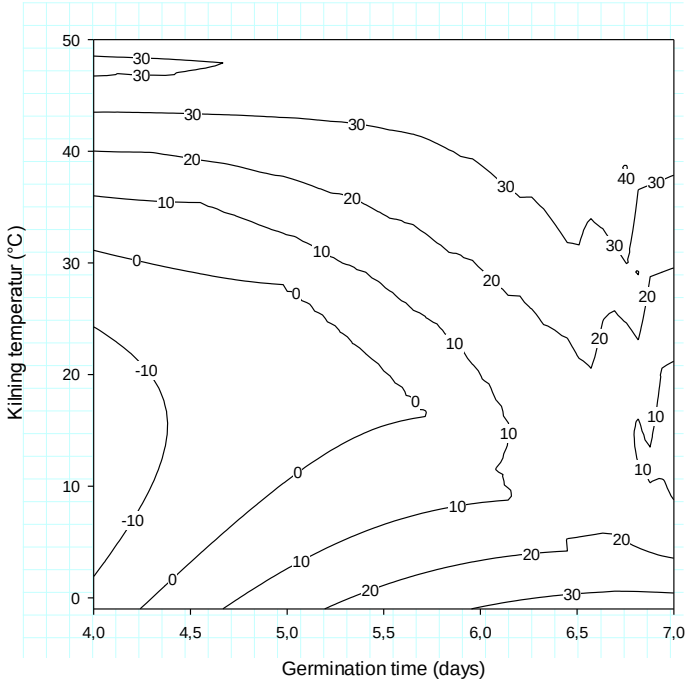

Figure 2. Effect of germination duration and kilning temperature on the diastatic power of NL36 malt.
Source: Author

germination duration increases and is constant during the first 20°C increase in temperature during kilning and starts increasing at temperatures above 30 to 50°C where it becomes constant. Also, the combined effect of germination duration and kilning temperature for this malt reduces its diastatic power with time. During the first 20°C increase, the activity of alpha and beta amylases in the malt is low. This might be because at that temperature, those enzymes are still inactive and an increase from 30 to 50°C activates them.

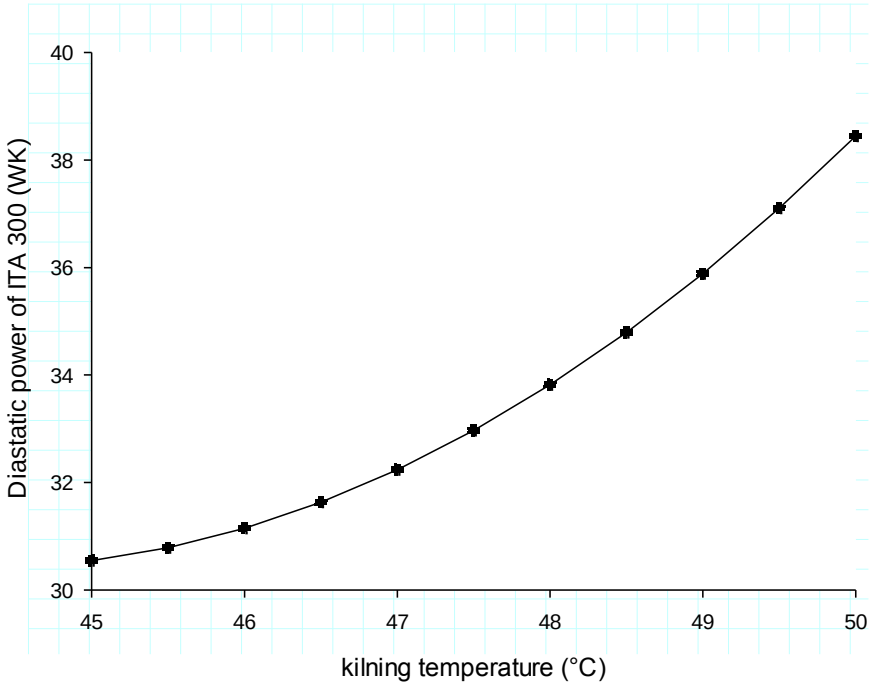

Figure 3. Effect of kilning temperature on the diastatic power of ITA00 malt.
Source: Author

Kilning temperature for diastatic power for malt ITA300

From Figure 3, the diastatic power for ITA300 malt increases with an increase in temperature. For this malt variety, this increase can be justified by the fact that, as temperature increases, there is a loss in water and this loss increases the dry matter content and conversely the diastatic power content. Also, an increase in temperature from 45 to 50°C can be favourable for amylases present in rice malt for it to be produced.

Steeping duration and kilning temperature for diastatic power for malt ITA300

Steeping is critical in malting because the production of enzymes in grains depends on the steeping period (Marconi, 2017). The effect of steeping duration and kilning temperature on the diastatic power of ITA300 malt is as shown in Figure 4. The diastatic power decreases as steeping duration increases since during steeping there is a loss in nutritious substances in the steep liquor, which might inhibit amylase activity. On the other hand, diastatic power for ITA300 increases as kilning temperature increases. This might be due to increase activity of α-glucosidase, which shows high activity in rice malt has an optimum temperature of 55°C (Kebler et al., 2005). Also, the combined effect of steeping duration and kilning temperature increases the diastatic power.

Effects of malting parameters on the reducing sugar content of NL36 and ITA300

The effect of steeping duration, germination duration, and kilning temperature on the reducing sugar of NL36 and ITA300 were investigated. Among these parameters, reducing sugar content for NL36 was affected by the kilning temperature (X_3), then the steeping duration (X_1) and finally the germination duration (X_2). From the ANOVA (Table 9), the kilning temperature (X_3), the interaction terms of steeping duration and kilning temperature (X_1X_3), the interaction terms of germination duration and kilning temperature (X_1X_3) and the second-order term of germination duration (X_2^2) had significant effects ($p < 0.05$) on the reducing sugar content of NL36. Meanwhile, the linear term of germination duration (X_2), interaction terms of steeping and germination durations (X_1X_2), and the second-order terms of steeping duration and the kilning temperature had no significant effects ($p > 0.05$) on the reducing sugar content of NL36 malt.

On the other hand, reducing sugar content for ITA300 was affected by the kilning temperature (X_3), followed by the germination duration (X_2) and finally the steeping duration (X_1). From Table 10, the steeping duration (X_1), kilning temperature (X_3), the interaction terms of steeping duration and kilning temperature (X_1X_3), the second-order

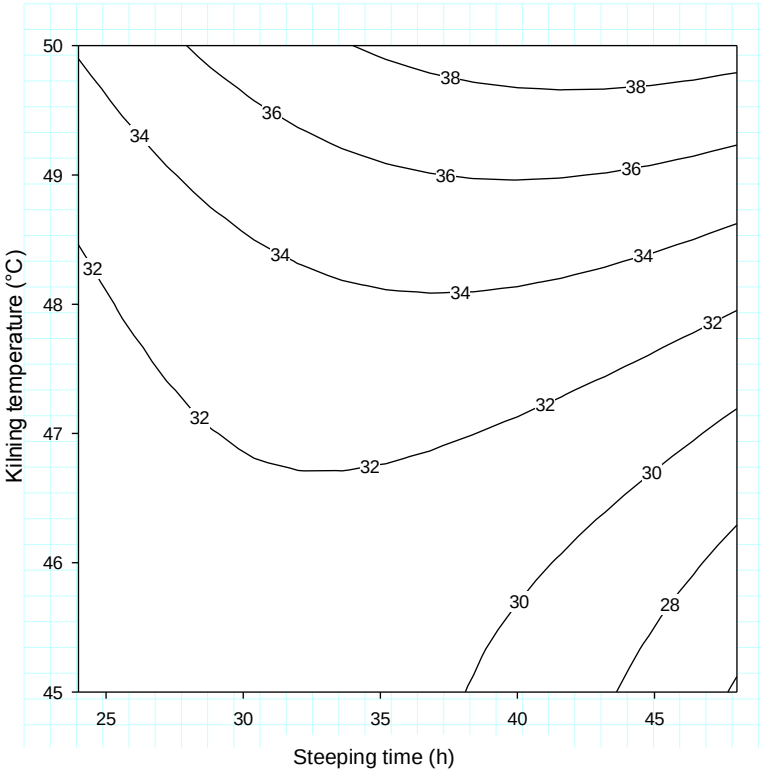

Figure 4. Effect of steeping duration and kilning temperature on the diastatic power of ITA300 malt
Source: Author

Table 9. ANOVA for NL36 malt Quadratic model of the reducing sugar content.

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	295.357	9	32.817	18.20	0.003	Significant
X_1-Steeping duration	13.791	1	13.791	7.65	0.040	Significant
X_2-Germination duration	1.577	1	1.577	0.87	0.393	
X_3-Kilning temperature	100.168	1	100.168	16.46	0.010	Significant
X_1X_2	29.681	1	29.681	0.22	0.658	
X_1X_3	0.399	1	0.399	35.77	0.002	Significant
X_2X_3	64.488	1	64.488	55.57	0.001	Significant
X_1^2	2.072	1	2.072	1.15	0.333	
X_2^2	81.526	1	81.526	45.22	0.001	Significant
X_3^2	6.339	1	6.339	3.52	0.120	
Residual	9.013	5	1.803			
Lack of fit	9.013	3	3.004	*	*	
Pure error	0.000	2	0.000			
Cor total	304.371	14				

Source: Author

term of steeping duration (X_1^2) and germination duration (X_2^2) had significant effects ($p < 0.05$) on the reducing sugar content of ITA300. Meanwhile, the linear terms of steeping duration (X_1), germination duration (X_2), interaction terms of germination duration and kilning temperature (X_2X_3), steeping duration and the kilning

Table 10. ANOVA for ITA300 malt Quadratic model of the reducing sugar content.

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	80.5425	9	8.9492	9.39	0.012	Significant
X_1-Steeping duration	2.4930	1	2.4930	2.61	0.167	
X_2-Germination duration	5.5541	1	5.5541	5.83	0.061	
X_3-Kilning temperature	12.4692	1	12.4692	13.08	0.015	Significant
X_1X_2	8.4967	1	8.4967	8.91	0.031	Significant
X_1X_3	2.7223	1	2.7223	2.86	0.152	
X_2X_3	4.0963	1	4.0963	4.30	0.093	
X_1^2	0.0726	1	0.0726	0.08	0.794	
X_2^2	40.1397	1	40.1397	42.10	0.001	Significant
X_3^2	2.7006	1	2.7006	2.83	0.153	
Residual	4.7675	5	0.9535			
Lack of fit	4.7675	3	1.5892	*	*	
Pure error	0.0000	2	0.0000			
Cor total	85.3100	14				

Source: Author

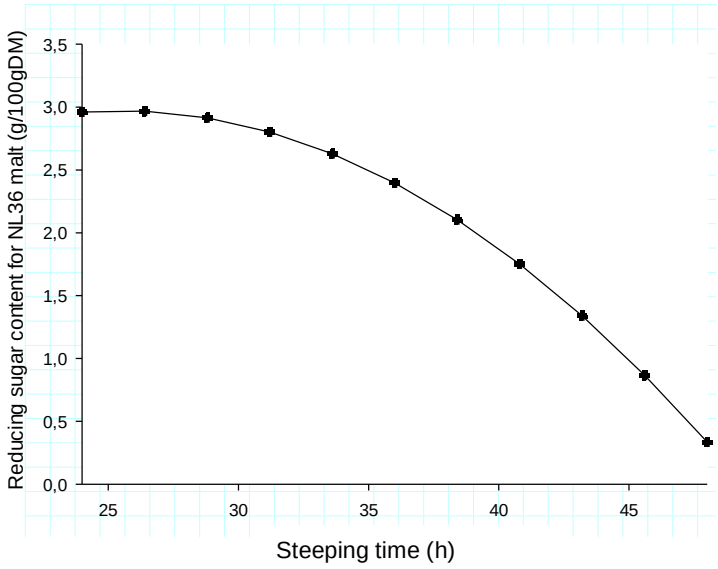

Figure 5. Effect of steeping duration on the reducing sugar content of NL36 malt.
Source: Author

temperature (X_1X_3) and the second-order terms of the steeping duration and the kilning temperature had no significant effects ($p > 0.05$) on the reducing sugar content of ITA300 malt.

Effect of steeping duration for NL36 malt on reducing sugar content

The extreme values for steeping duration were 24 and 48 h. The reducing sugar content of NL36 malt decreases with an increase in steeping duration (Figure 5). During steeping, starch molecules in rice absorb water and might cause hydrolysis leading to reducing sugars. Also, specific enzymes are synthesized and the protein matrix along with cell walls were hydrolyzed, permitting starch-degrading enzymes access to the starch granules (Stewart, 2013; Fox, 2018).

As the steeping duration is prolonged, soluble substances, reducing sugars inclusive, may leak out into

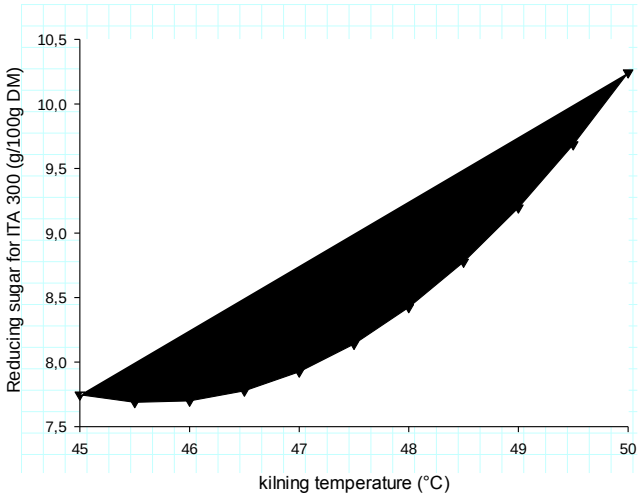

Figure 6. Effect of kilning temperature on the reducing sugar content of NL36 malt.
Source: Author

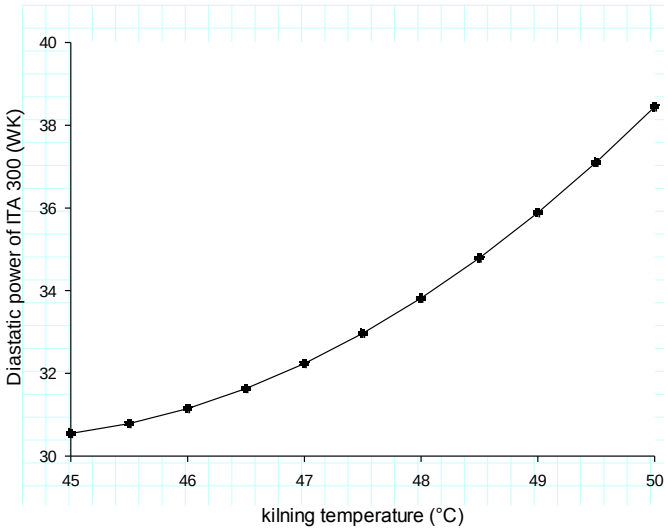

Figure 7. Effect of kilning temperature on the reducing sugar content of ITA300 malt.
Source: Author

the steep liquor, thereby leading to losses.

Effect of kilning temperature of NL36 and ITA300 malt on reducing sugar

The reducing sugar content increases with an increase in kilning temperature. During kilning, there is a loss of water which increases the dry matter content thereby increasing the net reducing sugar concentration. Also, at the initial stage of kilning when the water content is relatively high, the kilning temperature (45 to 50°C), though not optimum, favours the actions of amylases present in rice malt (NL36 and ITA300) initiating the hydrolysis of starch to reducing sugars (Kebler et al., 2005). As kilning continues, the water content of the malt drops as well as starch hydrolysis, but the relative percentage of reducing sugar increases as there is an increase in dry matter. These variations are as shown in Figures 6 and 7 for NL36 and ITA300, respectively.

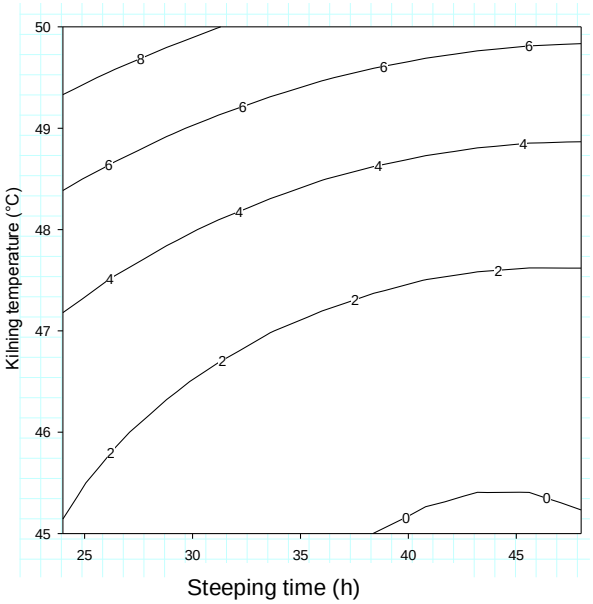

Figure 8. Effect of steeping time and kilning temperature on the reducing sugar content of NL 36 malt.
Source: Author

Effect of the interactions steeping duration/kilning temperature and germination duration/kilning temperature, respectively on reducing sugar content of NL36 malt

When the steeping duration increases, the reducing sugar content decreases, while the reducing sugar is not affected by kilning temperature (Figure 8). A combination of the effect of the two factors leads to a drop in the reducing sugar content. Therefore, considering this interaction for NL36 malt, a reduction in the steeping duration will favour an increase in reducing sugar.

The effect of germination duration and kilning temperature on the reducing sugar content of NL36 malt is as shown in Figure 9. As kilning temperature increases the reducing sugar remains constant. At the initial stages of germination, there is a rapid increase in reducing sugar as starch hydrolysis is favoured by the increasing amylolytic enzyme synthesis (Steward, 2013). The reducing sugar produced is being used up in the later stages of germination in the development of shoots and roots, causing a net drop in the reducing sugar content. The combined effect of the germination duration and kilning temperature reduces the reducing sugar content of NL36 malt.

Effect of the interaction of steeping duration/germination duration on reducing sugar content of malt ITA300

Figure 10 shows the effect of the steeping and germination durations on the reducing sugar content of ITA300. When steeping proceeds, the reducing sugar content increases and also as germination proceeds, the reducing sugar increases from the fourth to the fifth day and then reduces from the sixth to the seventh day. An initial increase in reducing sugar during germination is due to the partial hydrolysis of starch. As germination duration increases, the reducing sugars produced are being used up in the development of shoots and roots, leading to a drop in reducing sugar content (Briggs et al., 2004; Steward, 2013).

Optimization and validation of the malting conditions

The Box-Behnken Design (BBD) was used to achieve the optimal conditions for the malting of the two local rice cultivars NL36 and ITA300. According to the numerical optimization step of the program, the desired goal for each independent variable (steeping duration, germination duration, kilning temperature) and response (diastatic power and reducing sugar content) were chosen. For the optimization, the desired goal for the responses of diastatic power and its reducing sugar content were chosen as “maximum,” whereas the independent process variables were selected to be “within range”. The optimum malting conditions for maximizing the diastatic power of NL36 malt (38.47 WK) and its reducing sugar content (16.79 g/100 g DM) were found to be a steeping duration of 39.51 h, the germination duration of 4 days, and the kilning temperature of 50°C with a desirability value of 0.94. The

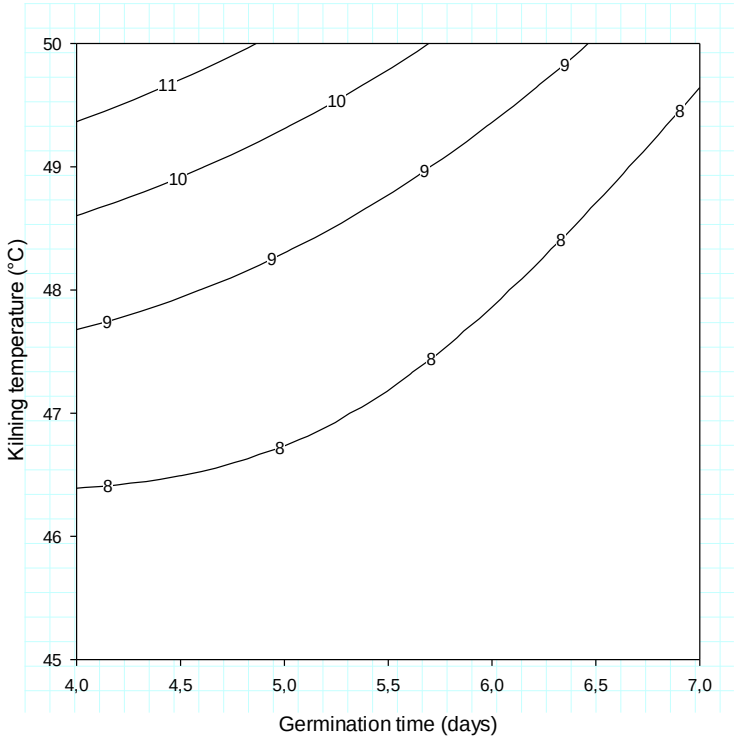

Figure 9. Effect of germination time and kilning temperature on the reducing sugar content of NL 36 malt.
Source: Author

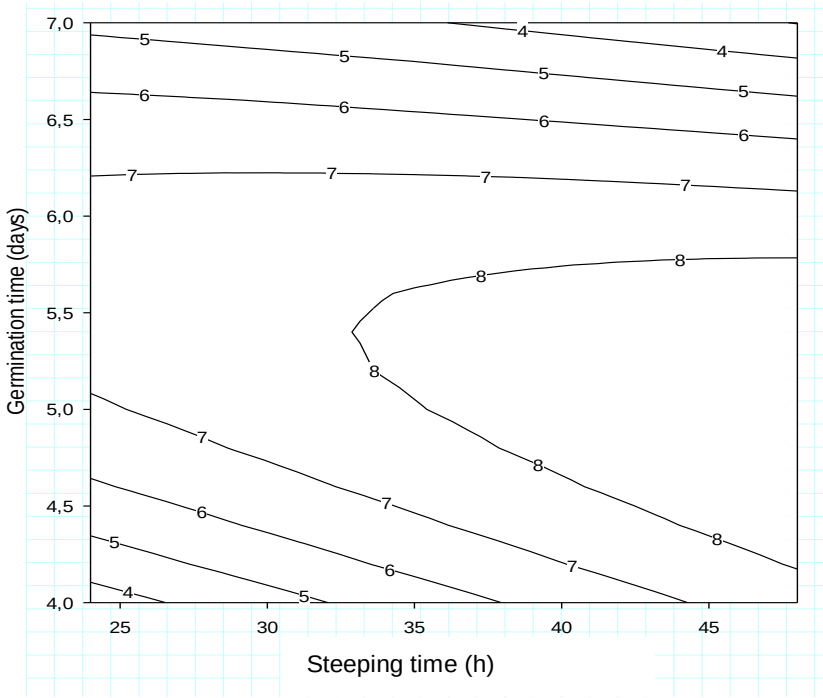

Figure 10. Effect of Steeping duration and germination duration on reducing sugar content of ITA300 malt.
Source: Author

suitability of optimum conditions for predicting optimum response values was tested under modified optimized conditions; steeping duration of 40 h, the germination duration of 4 days, and the kilning temperature of 50°C. Triplicate experiments were performed under the modified optimized conditions, and the mean values (51.6%) obtained from real experiments demonstrated the validation of the optimized conditions.

On the other hand, the optimum malting conditions for maximizing the diastatic power of ITA300 malt (39.097 WK) and its reducing sugar content (10.34 g/100 g DM) were found to be a steeping duration of 42.91 h, germination duration of 5.3 days, and the kilning temperature of 50°C with a desirability value of 0.97. The suitability of optimum conditions for predicting optimum response values was tested under modified optimized conditions; the steeping duration of 43 h, the germination duration of 5 days, and the kilning temperature of 50°C. Triplicate experiments were performed under the modified optimized conditions, and the mean values (52.04%) obtained from real experiments demonstrated the validation of the optimized conditions.

Conclusion

The aim of this study was to determine the optimum conditions of malting for beer production of seven cultivars of local rice (*Oryza sativa* L.) cultivated in the North and Far North Regions of Cameroon. Of the seven, two rice cultivars (NL36 and ITA300) showed good quality attributes prior to malting concerning germinative energy, germinative capacity and the starch content, an indication for proper modification during malting. Modelling of the malting process for NL36 malt showed that the interactive effect of germination duration and kilning temperature had a significant effect on its diastatic power. Also, the steeping duration, the interactive effect of steeping duration and kilning temperature and the interactive effect of germination duration and the kilning temperature had a significant effect on its reducing sugar content. On the other hand, for ITA300, the kilning temperature and the interactive effect of steeping duration and kilning temperature had a significant effect on its diastatic power. Also, the kilning temperature and the interactive effect of steeping and germination duration had a significant effect on its reducing sugar content.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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