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Research Article

Use of Response Surface Methodology for Optimising Raw Sorghum Proportion in Barley Malt Wort Production

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Abstract: A D-optimal design of response surface methodology (RSM) was used to develop a mathematical model for the optimisation of the proportion of raw sorghum adjunct in barley malt wort production with the use of ceremix (commercial enzyme). The responses investigated included filtration, dry weight extract, viscosity, total soluble nitrogen and free amino nitrogen. The results obtained ranged between 106.32-140.09m/s, 294.71-317.62 (1°/kg), 1.34-1.40cP, 288.06-542.28mg/l, and 57.15-101.30mg/l respectively. The variation in the levels of proportion of sorghum adjunct and barley malt affected the quality of the wort. The model for the filtration and viscosity were quadratic while others were lineal. The predicted solution for the optimisation is the use of 48.11g of barley malt and 50.00g of sorghum adjunct.
Keywords: response surface methodology, optimisation of proportion, commercial enzymes, viscosity, total soluble nitrogen, free amino nitrogen, dry weight, barley malt, ceremix, sorghum adjunct.

INTRODUCTION

Beer is the World's most widely consumed alcoholic beverage; it is the third most popular drink after water and tea [1]. Beer is an alcoholic beverage made from cereals like barley, corn, rice, oat, sorghum, and tuber crops like cassava. The process of beer production involved saccharification of starch by enzymes and fermentation of the resulting sugar. The starch and saccharification enzymes are usually derived from malting in which enzymes needed for the degradation of starch into fermentable sugars are produced [2].

Beers of European origin use barley as their major ingredient while in some other areas such as Africa barley malt is used with unmalted cereals such as rice, maize, sorghum, and millet as adjuncts mainly to lighten the flavour and to reduce cost of production. However the use of cassava for beer production has also been documented. The use of barley presents a major problem as barley is a temperate cereal and the African climate is unsuitable for its cultivation. Thus, barley grain or barley malt must be imported which requires the use of valuable foreign exchange and increases the price of the beer beyond the reach of most Africans [3].

Sorghum in Africa is processed into a very wide variety of attractive and nutritious traditional foods, such as semi-leavened bread, couscous, dumplings and fermented and non-fermented porridges. It is the grain of choice for brewing traditional African beers. The use of sorghum as brewing adjunct could be a major socio-economic advantage in the developing countries. Limitations for its use, however, include its low amylolytic potential, high gelatinization temperatures, and the presence of tannins [4].

Much interest has been generated in the use of sorghum in brewing. The use of malted sorghum in the production of kaffir beer, a traditional beverage has been well documented [5]. There have also been reports on the use of sorghum in the production of Western-type beer [6]. It has been the basis of traditional African beers such as the clear beers of West Africa (dolo and pito) and the opaque beers of southern Africa. These beers are part of the African tradition and remain very popular. Africans also enjoy lager and stout beers. These beers, of European origin, use barley as their major ingredient. This presents a major problem as barley is a temperate cereal and the African climate is unsuitable for cultivation [7].

Brewers who desire raw material cost savings or use of local raw materials may source under-modified barley malts or increase the ratio of adjunct. The limiting factor is to ensure an adequate complex of enzymatic activities for high-quality wort. The traditional source of enzymes used for the conversion of cereals into beer is barley malt [8]. If too little enzyme activity is present in the mash, there will be several undesirable consequences; the extract yield will be too low; wort separation will take too long; the fermentation process will be too slow; too little alcohol will be produced; the beer filtration rate will be reduced; and the flavour and stability of the beer will be inferior. Exogenous enzymes are used to supplement the malt's own enzymes in order to prevent these problems. Furthermore, commercial industrial enzymes are used to ensure better adjunct liquefaction, to produce low-carbohydrate beer ('light beer'), to shorten the beer maturation time, and to produce beer from cheaper raw materials. The substitution of a varying proportion of malt with sorghum introduces new dimensions to the grist composition [9]. It is not however clear how far mashing enzyme supplements in sorghum cultivar mashes could help alleviate the levels of fermentable sugars in its worts [10].

Response surface methodology has an effective track-record of helping researchers improve products and services. To determine the factor levels that will simultaneously satisfy a set of desired specifications. The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response. To determine the optimum combination of factors that yields a desired response and describes the response near the optimum [11].

Therefore, the objective of this study is to optimise the substitution of sorghum proportion (variety SK 5912) in barley wort as adjunct with the addition of industrial enzymes. The results of this study will supply relevant technical data available for the present and prospective investors that would have relied on "trial and error" method in sorghum substitution in barley wort as adjunct in beer production.

MATERIALS AND METHODS

The sorghum variety **SK 5912** was sourced from seed production unit of the Institute Agricultural Research, Zaria, Nigeria. The commercial enzymes Ceremix, was purchased from Novo chemicals. Ceremix is a mixture of bacterial amylase, proteinase, and glucanase. The malt used was sourced from Heineken(Amsterdam) while the yeast was from Davenport's brewery (Lowa, United States). D-Optimal response surface methodology was used for the design of the experiment. The independent and levels of variables were decided mainly from literatures. The independent variables and levels were the barley malt and unmalted sorghum adjunct. The barley malt proportion was taken at 20-50 percent and while 50-80 percent was used for sorghum adjunct respectively.

Preparation of Hot Wort Extract

Institute of Brewery Analytical Methods was used for the preparation of the hot water extract for sorghum adjunct and barley malt proportions as provided in the experimental design table (presented in table 1). i.e. 20g malt; 65g Sorghum, 50g malt;50sorghum malt,35gmalt;65g sorghum, 35g malt; 85 sorghum e.t.c.

For 50 g sorghum and 50 g malt, the sorghum grains (raw) was milled (coarse) with Buhler- Miag Universal laboratory disc mill at position 7. At 50 % replacement, 25 g of sorghum milled and 5 g of malt is placed in a mortal and brought to evenly dispersed slurry by gradual addition of 50 ml water during grinding. 150 ml of water was used to wash the mortal content into a preweighed stainless steel bearer and agitated using a magnetic stirrer. The content was placed in a water bath for 60 minutes. The content was latter removed, cooled to 65°C and addition 20 g of milled malt plus 5 g of malt and 150 ml of water (68°C). Enzymes was added at this stage and kept in water bath at 65°C for 2 hours followed by rapid cooling to 20°C.After cooling the stainless steel was carefully weighed and the content made up to 450 g. The content was thoroughly mixed and filtered through Watmann 113V and the volume of the wort collected in 30 min was determined. For 60 % sorghum to 40 % malt, 30 g of sorghum plus 5 g of malt (milled) was boiled with 200 ml of water before 15 g of mat (milled) was added. For 70 % sorghum and 30 % malt, 35 g of sorghum and 5 g of malt was boiled with 200ml of water and 10 g of malt added for mashing for 2 hours. For 80 % of sorghum, 40 g of sorghum and 5 g of malt was boiled with 200 ml of water and 5 g of malt added for mashing for 2 hours.

Analysis of Wort

The wort filtration and viscosity were determined as described by Bajomo and Young [9]. The wort filtration volume represents the volume of wort collected in 30 minutes through a Whatman 113V folded filter-paper, following recirculation of 50 ml turbid wort. Wort viscosities (cP) were calculated as the kinematic viscosities multiplied by the SG at 20°C, using a Class B Ostwald viscometer. The dry weight extract, total soluble nitrogen, and free alpha amino nitrogen, were determined as described by methods of analysis of the Institute of Brewery Analytical Methods [12].

The nitrogen content was determined using micro Kjedhal method for protein analysis as described by AOAC (2000). Approximately 1ml of the sample was weighed into the digestion tube of Kjeltec 2200 FOSS Tector Digestion unit (Foss Tecator Analytical AB Hoganas, Sweden). Two tablets of catalyst (containing 5g of K_2SO_4 and 5mg of Se) were added and also 12ml of concentrated H_2SO_4 added. Digestion was done for one hour at 420^0C. The distillation was done using 2200 FOSS distillation unit with addition of 80mls of water, 40mls NaOH (40%). The distillate was collected in 4% boric acid prepared with bromocresol green and melthyl red indicators. Titration was done using 0.1 M HCl using following formula:

$$\% N = \frac{(\text{Titre} - \text{Blank}) \times 14.007 \times 0.1 \times 100}{1000 \times \text{Sample weight}}$$

Statistical Analysis

All the experimental procedures were repeated in triplicate. The data collected were processed using a statistical package, Design Expert 6 (Stat ease Inc; Mineapolis USA, version). The software was used for the analysis of variance (ANOVA), mathematical modelling, regression analysis, predicted output and optimisation. The optimisation of the processing conditions was tailored toward assessing maximum responses of dry weight extract, viscosity, total soluble nitrogen, free amino nitrogen, and filtration.

RESULT AND DISCUSSION

Table 1: Experimental Design and Result of Responses

Run	Barley Malt	Sorghum	Filteration In 30min m/s	Dry Weight Extract 1°/kg	Viscosity cP	TSN mg/l	FAN mg/l
1	20	65	115.71	300.54	1.37	354.88	69.97
2	50	50	139	317.62	1.36	540.07	101.3
3	35	65	111.54	304.43	1.39	399.58	76.8
4	35	80	106.32	304.12	1.4	373.65	81.71
5	50	65	130.07	313.92	1.36	494.05	88.11
6	50	80	114.25	305.13	1.38	424.62	73.87
7	20	80	117.64	294.71	1.34	288.06	57.15
8	20	50	108.93	302.53	1.39	371.22	80.21
9	35	50	116.37	305.58	1.37	426.19	72.64
10	50	50	140.09	317.26	1.37	542.28	100.91
11	50	80	115.17	305.67	1.37	428.89	72.95
12	20	50	109.13	302.76	1.39	367.64	78.63

TSN-Total Soluble Nitrogen, FAN-Free Amino Nitrogen

Effect of Enzyme and Level of Sorghum Substitution on Wort Filteration

The result of the experiment on the wort filtration revealed highest volume of filtration 140.9 when 50g of sorghum adjunct and barley malt each were used in the production of wort beer while 35g and 65g combination of barley malt and sorghum adjunct recorded the lowest level of filtration (106.32). The trend of the result showed that increase in the substitution level of the adjunct increase the filtration to a peak before depreciation set in. According to Igyor [13], in its investigation on effect of processing method on sorghum wort filtration, it was discovered that sorghum worts had faster filtration rate than barley in infusion at 65°C and that the inclusion of sorghum could eliminate filtration problem. Application of certain exogenous enzymes in beer production aids wort filtration. Xylanases produced by Disporotrichum dimorphosporum in an effective amount improve filterability of wort [13].

Quadratic equation is the model that best described the experiment. The R-Squared of 0.9962 showed that the equation is good for the prediction. The "R-Squared "of 0.9846 is in reasonable agreement with the "Adjusted R-Squared" of 0.9980. The equation for the actual prediction and the response surface graph were given below:

Filteration= $+46.242-0.111*M+2.139*S+0.043*M^2-8.670E-003*S^2-0.037*M*S$.... EQ.1

(M-Malted barley, S- Sorghum)

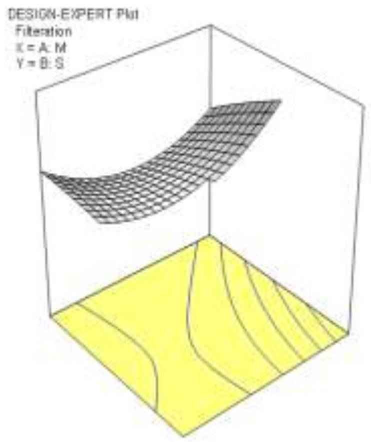

Fig. 1: Effect of Sorghum substitution on filteration of beer wort

Effect of Enzyme and Level of Sorghum Substitution on Dry Weight Extract

The highest dry extract weight 317.62(1°/kg) was reached at Sorghum: barley malt ratio at 50:50 while the lowest value 294.71(1°/kg) was recorded at the highest level of substitution of sorghum (80g). The dry weight extract is usually affected by the type of grain and how it is prepared. Malted barley yields at most about 80% of its dry weight in extract. Malomo et al., [14] revealed that the use of enzyme in wort does not increase the extract compared to control without commercial enzyme. Lack of fit test at 95% confidence level for the predicted model was non significant. The model satisfy lack of test and significant at $p<0.05$. The linear model best described the experiment with R-Squared and adjusted R-value at 0.9392 and 0.8885 respectively. The response surface graph is shown in Fig. 2 while the equation for the model is given below:

Dry Extract Weight= $+277.900 + 0.429*M + 0.660*S + 4.947E\text{-}003*M2 - 5.631E\text{-}003*S2 - 5.5E\text{-}003*M*S$............................ EQ 2.

(S- Sorghum adjunct, M-Barley Malt)

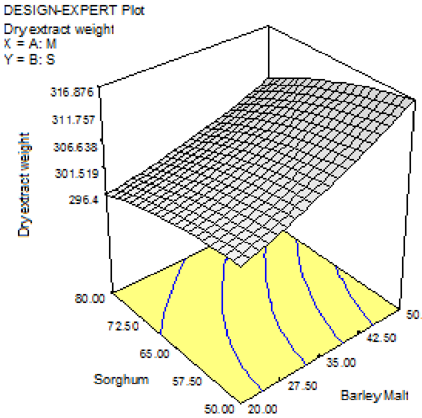

Fig. 2: Effect of Sorghum substitution on Wort Dry Extract Weight

Effect of Enzyme and Level of Sorghum Substitution on Viscosity

There was little effect of levels sorghum substitutions on the viscosity of the wort. The results ranged between 1.34 and 1.40Cp. The R- squared for the model is 0.7479 but with negative Predicted R- Squared which implied that the overall mean as the better predictor of the response. The mathematical equation was given in equation 3 and the response surface graph followed in Fig. 3:

Viscosity= $+1.48 + 2.98E\text{-}003*M\text{-}3.99E\text{-}003*\text{-}9.95E\text{-}005*M^2 + 1.16E\text{-}005*S^2 + 6.13E\text{-}005*M*S$-- EQ-3

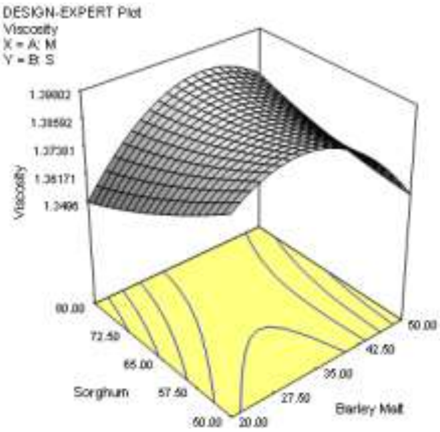

Fig. 3: Effect of Sorghum substitution on Wort Viscosity

Effect of Enzyme and Level of Sorghum Substitution on Total Soluble Nitrogen

The results of the twelve experiments conducted showed that the maximum Total Soluble Nitrogen of 542.28mg/l was recorded when equal ratio of barley malt and sorghum adjunct were used while the least recorded was 288.06mg/l at 20 percent barley malt and 80 percent adjunct. Observation of the result deduced that increase in sorghum proportion reduces the total soluble nitrogen of the wort. According to Malomo et al. [14], of the major applications of commercial enzymes in brewing is meant to improve the level of total soluble nitrogen in the wort.

The R- Squared recorded for the modelling was 0.9647 which showed that the model is fit. The predicted R-Squared and Adjusted R-Squared are reasonable for the prediction. The mathematical equation is presented as equation 4 below and the response surface graph in Fig. 4:

TSN= $422.75 + 5.0761*M - 2.96754*S$ ------ EQ.4

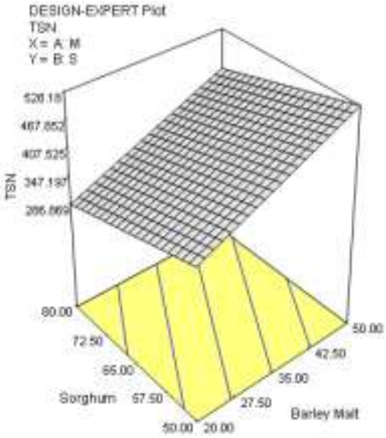

Fig. 4: Effect of Sorghum substitution on Wort TSN

Effect of Enzyme and Level of Sorghum Substitution on Wort Free Amino Nitrogen(FAN)

The result obtained on the effect of enzyme and level of sorghum substitution ranged between 72.64 and 101.30mg/l with equal weight (50g) of barley malt and sorghum giving the highest free amino nitrogen while combination of 35: 50 malt and sorghum produced the lowest amount of free amino nitrogen. According to Aastrup [15], FAN recommendations for malt brews were put between 120-220 mg/l(at 12°P). The use of linear equation best described the model.R-Squared of 0.7236 revealed that the model is significant. The predicted R squared of 0.5381 is in reasonable agreement with the Adjusted squared of 0.6622. The mathematical equation and the response surface graph wereprovided below:

FAN= $94.87 + 0.61*M - 0.59*S$-------EQ 5

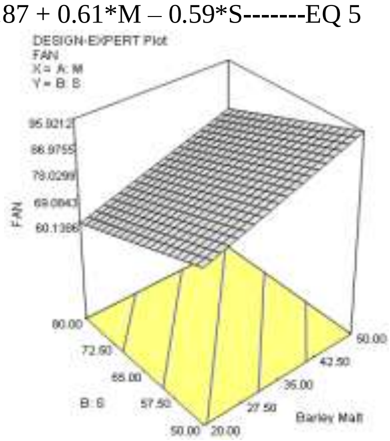

Fig. 5: Effect of Sorghum substitution on Wort FAN

CONCLUSION

The results of the experiment revealed that the levels of sorghum adjunct proportions in barley malt influenced the characteristic qualities (filtration, Dry Weight Extract, Viscosity, Total Soluble Nitrogen, and Free Amino Nitrogen) of the wort. The quadratic model best described the filtration and the viscosity while other parameters matched with linear models. The optimisation process required maximisation of the parameters investigated. The best solution predicted revealed the use of 48.11% of malted barley and 50.00% of sorghum as adjunct to produce filtration – 135.74m/s, Dry Weight Extract- 315.67(1°/kg), Viscosity- 1.37cP, Total Soluble Nitrogen- 518.59mg/l, Free Amino Nitrogen- 94.77mg/l with the desirability value of 0.768. The experiment might be a good predictor of the quality characteristics of barley malt and sorghum adjunct with the use of any suitable commercial enzyme such as ceremix.

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