

# The Fermentability of Worts Produced from Some Sorghum Varieties and their Combinations with Malted Barley using *Saccharomyces uvarum*.

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DOI: <https://doi.org/10.51584/IJRIAS.2025.10020016>

Received: 27 January 2025; Accepted: 01 February 2025; Published: 06 March 2025

## ABSTRACT

This study was carried out to investigate the fermentability of worts produced from some sorghum varieties and their combinations with malted barley using *Saccharomyces uvarum*. The infusion method of mashing was used with the addition of external enzymes (amylase, protease and glucanase) for the sorghum wort production. The yeast (*Saccharomyces uvarum*) was pitched in worts from three varieties (CSR-01, CSR-02 and Samsorgh-17) of sorghum (*Sorghum bicolor*), barley and their combinations. Changes in some process parameters like pH, apparent extract, free alpha amino nitrogen (FAN), yeast concentration and yeast viability were investigated over a period of six-day fermentation. Results showed that the pH value of the samples reduced from 5.6 to 4.6, the apparent extract of the samples reduced from 12.50 to 3.00oP, free alpha alpha amino nitrogen (FAN) value of the samples also reduced drastically from 150.00 to 34.00 mg/l in the course of the fermentation process from the first day to the six day. However, there were increases in some other parameters checked during the fermentation process. Total acidity increased from 5.6 to 4.6 on the pH scale, yeast viability evaluation showed a slight increase from 96 to 99 % and the yeast concentration also increased from 216 x 10<sup>4</sup> to 280 x 10<sup>4</sup> cells/ml. The apparent fermentability of the samples which is an experiment that gave the overall information on the rate at which the fermenting yeast cells were able to assimilate the available nutrients in the wort and consequently convert the available fermentable sugars into alcohol and carbon dioxide showed that barley malt, CSR-01 malt, CSR-02 malt, 50 % barley & 50 % CSR-01, 50 % barley & 50 % CSR-02, 50 % barley & 50 % samsorgh-17, 25 % barley & 75 % CSR-01, 25 % barley & 75 % CSR-02, 25 % barley & 75 % samsorgh-17 wort samples had apparent fermentability of 72.00, 37.50, 44.00, 36.00, 50.00, 62.00, 48.00, 42.00, 53.00, and 42.00 % respectively. 50 % barley & 50 % CSR-02 wort sample had the highest apparent fermentability (62 %) compared with other samples used in the study in relation to barley (72 %). 50 % barley & 50 % CSR-02 wort sample had better brewing qualities than CSR-01 and Samsorgh-17 with their combinations, as the yeast (*Saccharomyces uvarum*) proliferates well in its wort during the fermentation process.

**Keywords:** Fermentability, Wort, Sorghum, Malted barley and *Saccharomyces uvarum*.

## INTRODUCTION

### Wort fermentability

Wort fermentability describes the capacity of malt to produce fermentable sugars that are utilized by yeast to produce alcohol during fermentation and is an important parameter when determining malt quality. Wort fermentability or Apparent Attenuation Limit (AAL) is an important quality attribute to consider when

determining the malting performance of barley/sorghum varieties. The fermentability of the wort is the proportion of the wort dissolved solids (extract) which can be fermented, and is expressed as a percentage [3]. Variation in wort fermentability or AAL may be influenced by the equipment used in the assay, the effect of different yeast strain and incubation conditions. Utilizing a dried yeast as an alternative to fresh yeast has perceivable advantages including not requiring equipment to culture and propagate yeast, convenience of use, long shelf life and certified purity, allowing comparisons between different laboratories. The storage and rehydration conditions of the dried yeast are especially important for preserving cell viability. Slow rehydration kinetics in the low to mid water activity range and higher rehydration temperatures have increased cell viability in *Saccharomyces cerevisiae*. It was previously reported that a dried yeast strain (Mauribrew lager 497), was tested as being suitable for the determination of fermentability and had similar performance to fresh yeast strains obtained from commercial Australian breweries [6]. Fermentability of malt wort depends on an adequate supply of the essential nutrients required by yeasts. The amino acid content is an important malt parameter to the yeast growth and metabolism in malt wort. To increase brewing fermentability and efficiency, malts with high levels of free amino nitrogen and amino acid are essential [4].

Malt has been widely used for brewing, in which the first process is malting of barley/sorghum is a complex process depending on numerous factors, such as the variety of barley/sorghum, steeping, germination and kilning stages. These steps influence the malt quality parameters such as kolbach index, wort viscosity, fine coarse extract difference and free amino nitrogen [15]. The free Amino Nitrogen (FAN) content of wort prescribes efficient yeast cell growth and fermentation performance. Free amino nitrogen consist of the individual amino acids, small peptides and ammonia ions formed during malting, the relative amount varies. Free Amino Nitrogen (FAN) is an essential component of yeast nutrition in brewing as it promotes proper yeast growth and efficiency [7], [10]. It also plays a role in the maintenance of foam stability [7]. Wort is a highly complex medium consisting of fermentable sugars (fructose, sucrose, glucose, maltose and maltotriose), dextrins, nitrogenous materials, vitamins, ions, mineral salts, trace elements, and many other constituents [1]. In wort, the main nitrogen sources for yeast metabolism are individual amino acids, small peptides and ammonium ions formed from the proteolysis of barley malt proteins during malting and mashing, collectively known and measured as Free Amino Nitrogen (FAN) [1].

During fermentation, brewing yeasts are required to adapt rapidly to this rich environment, using the available nitrogen for the synthesis of cellular proteins and other cell compounds. Adequate levels of FAN in wort ensure efficient yeast cell growth and hence, an appropriate fermentation performance [9]. Free Amino Nitrogen is believed to be a good index for potential yeast growth and efficiency, with most yeast strains consuming peptides no longer than tripeptides. It is believed that only 40% of the total oligopeptides available are used for nitrogen metabolic activity and that the rest may contribute to haze development (polypeptide-polyphenol complexes) or foam stability [9]. FAN measurement in wort has been used within the brewing industry for historical reasons such as the ease and availability of the analytical methods. Free Amino Nitrogen (FAN) has long been regarded as a general index for the prediction of healthy yeast growth, viability and fermentation efficiency, and consequently beer quality and stability. Sufficient levels of the individual wort amino acids, ammonium ions, and small peptides which constitute FAN, have been shown to ensure efficient yeast cell growth and consequently improved wort fermentation performance. Proteins contained in beer are for the most part derived from water soluble proteins contained in the grains used in the malting and brewing process. These proteins are important determinants of beer quality. Yet most proteins are modified or lost during the malting process. Various proteins and polypeptide fragments from malt extracts have been identified by mass spectroscopy [2]. Proteins in beer that are introduced from malt cereal adjuncts such as proteases, hordeins, serpins and others, are to a large extent processed to peptides and free amino acids during mashing. Added to the total nitrogen content of beer are nucleic acids and other non-protein derived nitrogen sources. The values obtained for the protein content of beers are almost exclusively based on Kjeldah assays which are complicated, require specifically specialized equipment, use relatively large quantities of beer and measure total nitrogen are not specifically nitrogen derived from protein peptides or amino acids [5].

## **Sorghum**

Sorghum is used in lager beer brewing as malt and/or raw sorghum [11]. The successful research that led to the development of commercial sorghum lager beer brewing focused on enzymes in sorghum malting, sorghum malting technology, and sorghum brewing technology [13]. However, identification of sorghum types with specific grain characteristics suitable for lager brewing remains a major area of concern [14]. Currently, sorghum types that differ substantially in chemical composition are used for lager beer brewing in Africa which includes; white pericarp type II tannin sorghum in Nigeria and white pericarp type 1 non-tannin sorghums in Uganda [12],[11]. Mashing with malted sorghum and enzymes in lager beer brewing yields a high level of free amino nitrogen (FAN) needed to ensure efficient buffering capacity and optimum yeast performance during fermentation [12].

However, a low level of fermentable sugars is produced in sorghum malt mashing, attributed to the high starch gelatinization temperature and low  $\beta$ -amylase activity in sorghum compared with barley [14]. In practice, sorghum malt mashing requires the addition of exogenous enzymes in order to produce fermentable sugars. It has been proposed that sorghum in form of raw grain rather than malt with exogenous enzymes is a more logical and cost-effective approach [11], [14].

## **Sample Collection**

### **Sorghum Samples**

Three varieties of sorghum grains (CSR-01, CSR-02 and Samsorgh - 17) were obtained from the Institute of Agricultural Research (IAR), Ahmadu Bello University, Zaria. The samples were collected under sterile conditions with clean sacks and brought to the laboratory for immediate use. Both CSR-01 and CSR-02 sorghum varieties lack tannin and appear white in color. Samsorgh-17 variety contains copious amounts of polyphenolic compounds such as tannin in the seed coat which combine with anthocyanin to give it its yellow colour.

### **Barley Malt Samples**

The barley malt samples were obtained from Intafact Beverages Limited, SAB Miller Drive, Harbour Industrial Layout, Bridge Head, Off Atani Road, Onitsha, Anambra State. They were collected under sterile condition with clean container and brought to the laboratory for immediate use.

### **Yeast Sample**

The yeast sample was also obtained from Intafact Beverages Limited, SABMiller Drive, Harbour Industrial Layout, Bridge Head, Off Atani Road, Onitsha, Anamrbra State. The yeast sample was collected under sterile condition with a clean cooler filled with ice to avoid autolysis of the yeast cells and it was then brought to the Laboratory of Microbiology Department, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, for immediate inoculation.

## **Equipment Sterilization**

All rubber and glass wares were washed with water, detergent and hypochlorite. The glass wares were afterwards sterilized in the autoclave at 121 °C temperature with pressure of 15 Psi for 15 minutes. The autoclave was then allowed to cool before the glass wares were removed aseptically.

## **Mashing**

According to the Recommended Method of Analysis of the Institute of Brewing [8]. The barley malt sample and three sorghum varieties Samsorgh -17, CSR-01 and CSR-02 were mashed using infusion method. 110 g of the ground malt of the three varieties of sorghum and barley were weighed into 1000 ml of distilled water, each which was heated up from 45 - 50°C using a water bath and allowed for 30 – 45 mins followed by an increment of temperature to 76°C. it was continuously stirred at 10 min intervals and the saccharification

timing for each of the samples was checked every 10 seconds starting from the temperature of 68°C for barley. Under this particular method, the sorghum malts were mashed with exogenous enzyme. The complete conversion of the mash was determined by iodine test. After mashing, the mash was cooled and filtered using Whatman No. 1 filter paper to produce wort. The wort was boiled for 10 min and cooled in a refrigerator at 4°C prior to further wort analysis.

### Wort analysis

Determination of Free Amino Nitrogen (FAN), Original Gravity, Hot Water Extract (HWE), apparent colour and pH were carried out according to the Recommended Methods of Analysis of the Institute of Brewing [8].

### Wort colour determination

According to the Recommended Method of Analysis of the Institute of Brewing [8]. The spectrophotometer was standardized by ensuring that the cells were matched, two cells were filled with distilled water and the wavelength was set at 530nm. The two cells read zero when placed in the spectrophotometer's light path. One of the matched cells was removed and refilled with the standard dichromate solution. The reading was checked with distilled water blank. The next reading with the standard dichromate solution was observed, and it read 0.18. The wort was filtered through the Whatman No 1 filter paper. The results were taken directly from the scale using 10 mm matched cells. The readings were rounded off to the nearest whole number or the nearest 0.5 as required.

### pH Measurement

The pH of the wort sample was determined using a standard digital laboratory pH meter.

### Determination of free alpha amino nitrogen of worts

The samples were diluted with distilled water to contain 1-3 mg amino nitrogen per litre. It is recommended that wort be diluted 1-100 ml and beer 1-50 ml. 2 ml of the diluted sample was taken and 1ml of colour reagent were added in a test tube. Stopper with a glass ball and placed in a boiling water both for exactly 16 minutes and then it was cooled in a water bath at 20°C for 20 minutes. After this time, 5ml of diluting solution was added, mixed and the absorbance was measured at 570nm in a 10mm cell against a reference sample prepared from the reagents plus 2ml of distilled water in place of the diluted wort. With each set of determinations, three replicate of glycine standard checks were made using 2ml of diluted glycine solution.

$$\begin{aligned} & \text{Free alpha amino nitrogen in mg /1litre} \\ & = \frac{\text{Absorbance 570nm of test solution} \times 2 \times \text{dilution}}{\text{Mean absorbance of standards}} \end{aligned}$$

The results were reported to the nearest whole number.

### Determination of original gravity (OG)

500ml of wort sample at 18-22°C was measured using a measuring cylinder and a standard sccharometer was dipped into the wort sample and the readings were taken as well as the temperature values.

### Determination of hot water extract (HWE)

According to the Recommended Method of Analysis of the Institute of Brewing [8]. 50 g of the ground sample was weighed into the mashing beaker and placed in a hot water bath for 15 min. 360 ml of 65°C distilled water was added, stirred at 30 min interval for 1h to eliminate all lumps. The mash was later cooled and transferred to a 515 ml measuring flask. The beaker was rinsed inside into the flask and made up to 515 ml and mixed by inversion.

Thereafter, the mash was filtered off and the specific gravity was obtained. The extract yield was obtained from the relation:

$$\text{Extract (as is)} = \text{Excess gravity} \times 10.310 \text{ kg}$$

$$\text{Extract (d\Dry)} = \frac{\text{Extract (as is)} \times 100}{1000 - m}$$

Where,  $m$  = moisture content of grain.

### Determination of apparent fermentability

The specific gravity of the prepared sample was determined at 15.5°C and then, 300-400ml of the sample was stirred with 30g of pressed yeast at 100-150 rpm in a 500ml flask with the neck covered with aluminum foil. The specific gravity of the filtered solution was determined at 15.5°C after 6 h.

$$\text{Apparent fermentability \%} = \frac{(O.G - F.G)}{O.G} \times 100$$

Where  $O.G$  = original gravity - 1000

$F.G$  = final gravity (attenuation limit) - 1000, taking water at 15.50C as 1000.

The above formula gives apparent fermentability. The real fermentability is obtained by multiplying the apparent fermentability by a factor which is 0.819 for wort.

### Yeast viability determination

Yeast cells were mixed with a solution of methylene blue. Viable yeast cells will reduce methylene blue enzymatically to a colourless stage. Dead cells with no enzymatic activity stains blue and the percentage of unstained cell is a measure of the viability of the yeast. This was determined by diluting equal volume of methylene blue solution and yeast sample in a test tube until a suspension is obtained that will have approximately 100 yeast cells in a microscopic field. After mixing, a small drop of well mixed suspension was placed onto a microscopic slide, covered with a cover slip and examined microscopically using a magnification of X40 after 5 to 10min. Time is necessary because when the contact is too short or too long, a too low or respectively too high percentage of dead cells will be found. Total count of unstained cells were noted.

### Yeast concentration determination

This was determined using method of cell count with methylene blue.

The haemocytometer and glass cover slip were cleaned with 70% ethanol. Samples were prepared and haemocytometer loaded with the glass cover -slip placed on the counting chambers. The cells suspension were diluted 1:1 with 0.2% methylene blue, 10 microliters of cell sample was transferred into the haemocytometer using a pipette and timing 30 seconds for the cells to settle. The haemocytometer was placed under a microscope with a typical magnification of 100 for manual cell count. Focus was on the grid pattern and the cell particles, the total number of cells found were counted in the 4 large corner squares. Counts of live cells (without methylene blue) and dead cells (with methylene blue).

$$\text{Total cells/ml} = \frac{\text{Total cells counted} \times \text{dilution factor}}{\# \text{ of Squares}} \times 10,000 \text{ cells/ml}$$



## RESULTS

Table 1: Saccharification temperature and time of worts from barley malt, malted sorghum varieties and their combinations.

Sample	Temperature (°C)	Time(min)
Barley malt	68	68
CSR-01 malt	74	85
CSR-02 malt	72	80
Samsorgh-17 malt	76	87
50% Barley malt & 50% CSR-01 malt	71	77
50% Barley malt & 50% CSR-02 malt	70	75
50% Barley malt & 50% Samsorgh-17 malt	74	81
25% Barley malt & 75% CSR-01 malt	72	76
25% Barley malt & 75% CSR-02 malt	71	74
25% Barley malt & 75% Samsorgh-17 malt	73	78

Table 2: Rate of filtration of whole mash of barley malt, malted sorghum varieties and combinations.

Sample	Time (min)
Barley malt	65
CSR-01 malt	78
CSR-02 malt	71
Samsorgh-17 malt	82
50% Barley malt & 50% CSR-01 malt	75
50% Barley malt & 50% CSR-02 malt	69
50% Barley malt & 50% Samsorgh-17 malt	79
25% Barley malt & 75% CSR-01 malt	76
25% Barley malt & 75% CSR-02 malt	70
25% Barley malt & 75% Samsorgh-17 malt	80

Table 3: Colour of worts from barley malt, malted sorghum varieties and their combinations.

Sample	ABSX100(°10B)
Distilled water	1.00
Standard dichromate solution	18.00
Barley malt	20.80
CSR-01 malt	22.80
CSR-02 malt	28.20
Samsorgh-17 malt	26.20
50% Barley malt & 50% CSR-01 malt	20.70
50% Barley malt & 50% CSR-02 malt	26.90
50% Barley & 50% Samsorgh-17 malt	25.10
25% Barley malt & 75% CSR-01 malt	19.60
25% Barley malt & 75% CSR-02 malt	24.10
25% Barley malt & 75% Samsorgh-17 malt	28.10

Table 4: Hot water extract of worts from barley malt, malted sorghum varieties and their combinations.

Sample	Extract value ( <sup>0</sup> L/kg)
Barley malt	506.50
CSR-01 malt	324.16
CSR-02 malt	364.68
Samsorgh-17 malt	445.72
50% Barley malt & 50% CSR-01 malt	405.20
50% Barley malt & 50% CSR-02 malt	425.46
50% Barley malt & 50% Samsorgh-17 malt	465.98
25% Barley malt & 75% CSR-01 malt	384.94
25% Barley malt & 75% CSR-02 malt	384.94
25% Barley malt & 75% Samsorgh-17 malt	455.85

Table 5: Apparent extract of worts from barley malt, malted sorghum varieties and their combinations during fermentation at 15.5<sup>0</sup>C.

Apparent extract value ( <sup>0</sup> P)							
Sample	0 h	24 h	48 h	72 h	96 h	120 h	144 h
Barley	12.50	11.50	10.00	8.50	6.50	4.50	3.00
CSR-01	8.00	7.50	7.00	6.50	6.00	5.50	5.00
CSR-02	9.00	8.00	7.50	7.00	6.50	6.00	5.00
Samsorgh-17	11.00	10.50	10.00	9.00	8.00	7.50	7.00
50% Barley & 50% CSR-01	10.00	9.50	9.00	8.00	7.00	5.50	5.00
50% Barley & 50% CSR-02	10.50	9.50	8.00	7.50	6.50	5.00	4.00
50% Barley & 50% Samsorgh-17	11.50	10.50	9.00	8.00	7.00	6.50	6.00
25% Barley & 75% CSR-01	9.50	9.00	8.50	8.00	7.50	6.50	5.50
25% Barley & 75% CSR-02	9.50	8.00	8.50	7.00	6.00	5.00	4.50
25% Barley & 75% Samsorgh-17	11.20	10.00	9.00	8.50	7.50	7.00	6.50

Table 6: Free Alpha Amino Nitrogen of worts from barley malt, malted sorghum varieties and their combinations during fermentation at 15.5<sup>0</sup>C

Sample	FAN(mg/l)						
	0 h	24 h	48 h	72 h	96 h	120 h	144 h
Barley	150.00	147.00	132.00	129.00	121.00	110.00	105.00
CSR-01	63.00	62.00	60.00	57.00	56.00	53.00	50.00
CSR-02	70.00	69.00	66.00	61.00	58.00	55.00	54.00
Samsorgh-17	48.00	47.00	43.00	41.00	39.00	34.00	31.00
50% Barley & 50% CSR-01	90.00	88.00	82.00	76.00	72.00	66.00	64.00
50% Barley & 50% CSR-02	99.00	97.00	92.00	88.00	82.00	79.00	77.00
50% Barley & 50% Samsorgh-17	83.00	81.00	76.00	71.00	69.00	66.00	64.00
25% Barley & 75% CSR-01	69.00	67.00	62.00	59.00	54.00	51.00	49.00
25% Barley & 75% CSR-02	82.00	80.00	73.00	69.00	63.00	60.00	57.00
25% Barley & 75% Samsorgh-17	56.00	54.00	47.00	41.00	38.00	35.00	34.00

Table 7: pH values during the fermentation

	pH						
Sample	0 h	24 h	48 h	72 h	96 h	120 h	144 h
Barley	5.2	5.2	5.0	4.9	4.8	4.8	4.6
CSR-01	5.6	5.4	5.2	5.0	4.9	4.9	4.9
CSR-02	5.4	5.4	5.2	5.0	4.9	4.8	4.8
Samsorgh-17	5.3	5.3	5.2	5.1	5.0	4.9	4.9
50% Barley & 50% CSR-01	5.6	5.6	5.5	5.4	5.3	5.3	5.2
50% Barley & 50% CSR-02	5.3	5.3	5.2	5.1	4.9	4.8	4.8
50% Barley & 50% Samsorgh-17	5.3	5.3	5.2	5.1	5.0	4.9	4.8
25% Barley & 75% CSR-01	5.6	5.6	5.4	5.2	5.1	5.1	5.0
25% Barley & 75% CSR-02	5.4	5.4	5.2	5.1	5.0	5.0	4.9
25% Barley & 75% Samsorgh-17	5.3	5.3	5.2	5.1	5.0	5.0	4.9

Table 8: Concentration of yeast during the fermentation process. Yeast concentration (cells/ml)

Sample	0 h	24 h	48 h	72 h	96 h	120 h	144 h
Barley	216x10 <sup>4</sup>	220 x10 <sup>4</sup>	229 x10 <sup>4</sup>	240 x10 <sup>4</sup>	258 x10 <sup>4</sup>	272 x10 <sup>4</sup>	280 x10 <sup>4</sup>
CSR-01	216x10 <sup>4</sup>	218 x10 <sup>4</sup>	224 x10 <sup>4</sup>	228 x10 <sup>4</sup>	231 x10 <sup>4</sup>	239 x10 <sup>4</sup>	241 x10 <sup>4</sup>
CSR-02	216x10 <sup>4</sup>	219 x10 <sup>4</sup>	228 x10 <sup>4</sup>	233 x10 <sup>4</sup>	242 x10 <sup>4</sup>	252 x10 <sup>4</sup>	256 x10 <sup>4</sup>
Samsorgh-17	216x10 <sup>4</sup>	217 x10 <sup>4</sup>	220 x10 <sup>4</sup>	225 x10 <sup>4</sup>	228 x10 <sup>4</sup>	231 x10 <sup>4</sup>	233 x10 <sup>4</sup>
50-50% Barley & CSR-01	216x10 <sup>4</sup>	219 x10 <sup>4</sup>	226 x10 <sup>4</sup>	230 x10 <sup>4</sup>	236 x10 <sup>4</sup>	244x10 <sup>4</sup>	248 x10 <sup>4</sup>
50-50% Barley & CSR-02	216x10 <sup>4</sup>	220 x10 <sup>4</sup>	226 x10 <sup>4</sup>	238 x10 <sup>4</sup>	251 x10 <sup>4</sup>	260 x10 <sup>4</sup>	266 x10 <sup>4</sup>
50-50% Barley & Samsorgh-17	216x10 <sup>4</sup>	218 x10 <sup>4</sup>	220 x10 <sup>4</sup>	223 x10 <sup>4</sup>	227 x10 <sup>4</sup>	231 x10 <sup>4</sup>	238 x10 <sup>4</sup>
25-75% Barley & CSR-01	216x10 <sup>4</sup>	219 x10 <sup>4</sup>	224 x10 <sup>4</sup>	228 x10 <sup>4</sup>	235 x10 <sup>4</sup>	239 x10 <sup>4</sup>	244 x10 <sup>4</sup>
25-75% Barley & CSR-02	216x10 <sup>4</sup>	220 x10 <sup>4</sup>	228 x10 <sup>4</sup>	233 x10 <sup>4</sup>	241 x10 <sup>4</sup>	251 x10 <sup>4</sup>	258 x10 <sup>4</sup>
25-75% Barley & Samsorgh-17	216x10 <sup>4</sup>	218 x10 <sup>4</sup>	221 x10 <sup>4</sup>	227 x10 <sup>4</sup>	231 x10 <sup>4</sup>	235 x10 <sup>4</sup>	237 x10 <sup>4</sup>

Table 9: The percentage yeast viability before and after fermentation.

Sample	Before fermentation(%)	After Fermentation (%)
Barley malt	96	98.5
CSR-01 malt	96	97.5
CSR-02 malt	96	97.5
Samsorgh-17 malt	96	97.5
50% Barley malt & 50% CSR-01 malt	96	97.5
50% Barley malt & 50% CSR-02 malt	96	98
50% Barley malt & 50% Samsorgh-17 malt	96	96.5
25% Barley malt & 75% CSR-01 malt	96	97.5
25% Barley malt & 75% CSR-02 malt	96	97.5
25% Barley malt & 75% Samsorgh-17 malt	96	96.0



Table 10: Apparent fermentability of worts from barley malt, malted sorghum varieties and their combinations.

Sample	Apparent Fermentability (%)
Barley malt	72.00
CSR-01 malt	37.50
CSR-02 malt	44.00
Samsorgh-17 malt	36.00
50% Barley malt & 50% CSR-01 malt	50.00
50% Barley malt & 50% CSR-02 malt	62.00
50% Barley malt & 50% Samsorgh-17 malt	48.00
25% Barley malt & 75% CSR-01 malt	42.00
25% Barley malt & 75% CSR-02 malt	53.00
25% Barley malt & 75% Samsorgh-17 malt	42.00

## DISCUSSION

The malt of sorghum varieties and their combinations were mashed using single infusion method with the addition of external enzymes which helped in the saccharification of the produced starch granules in the mash and consequently, the breaking down of protein into individual wort amino acids. During this process, different saccharification temperature/time and filtration time were noted for the Barley, Sorghum varieties and their combinations. In table 1, saccharification temperature of the samples ranged from 68 to 76°C, where Barley had the lowest saccharification temperature of 68°C, followed by CSR-02 of 72°C among the Sorghum varieties analyzed and 50% Barley & 50% CSR-02 combination had a saccharification temperature of 70°C closer to that of Barley while Samsorgh-17 and its 50% Barley & 50% Samsorgh-17 combination have the highest saccharification temperature of 76 and 74 °C respectively and this was attributed to its low level of  $\beta$ -amylase enzyme (diastatic power). In table 2, the filtration time was reported and it ranged from 65 to 82 (min), Barley had the shortest filtration time of 65 min followed by CSR-02 with 71 min filtration time as compared with the other sorghum varieties understudied and 50 % barley & 50 % CSR-02 combination had the closest filtration time of 69 min in relation to that of barley. At the end of mashing process (saccharification) and filtration, the worts obtained were further subjected to wort analysis which include; wort colour evaluation, hot water extract, apparent extract value, free alpha amino nitrogen determination. In table 3, the wort colour was analysed and the result of the absorbance recorded which ranged from 0.196 to 0.282. Table 4 showed the hot water extract value of the barley, sorghum varieties cum their combinations and it ranges from 324.16 to 506.50 °L/kg, Samsorgh-17 had the highest hot water extract value of 445.72 °L/kg when compared with other sorghum samples analyzed and 50 % Barley & 50 % Samsorgh-17 combination had the highest hot wort extract value of 465.98(°L/kg) generally, while CSR-01 had the lowest hot water extract value of 324.16 °L/kg in relation to that of barley wort. In table 5, the apparent extract value of the samples were represented appropriately and it ranged from 8.0 to 12.5 °P as samsorgh-17 (11.0 °P) and its 50% Barley & 50% Samsorgh-17 (11.5 °P) had the highest apparent extract values when relating to sorghum samples and their combinations analyzed respectively, whereas CSR-01 (8.0 °P) and its 25% barley & 75% CSR-01 (9.5 °P) had the lowest apparent extract value when relating to sorghum samples and their combinations analyzed respectively. The free alpha amino nitrogen (FAN) of the samples are reported in table 6, barley wort contained FAN of 150 mg/l, followed by CSR-02 with 70 mg/l and its 50% barley & 50% CSR-02 wort combinations contained FAN value of 99 mg/l closer to that of barley wort, while Samsorgh-17 contained lowest FAN of 48 mg/l when compared with other sorghum varieties studied. There was a slight drop in the pH of the sample worts as reported in table 7.

The worts obtained from barley, sorghum varieties and their combinations were further pitched/inoculated with the same quantity of fresh wet yeast (*Saccharomyces cerevisiae*) for fermentation process. During this

fermentation, the Apparent extract value, pH and FAN were still checked, while the yeast concentration, performance and viability were monitored and reported in tables 8 and 9 respectively. There was a slight drop in the pH, while the apparent extract values and FAN continued to decrease as the yeast concentration got increased. These were evidences that fermentation occurred, though with differences in the rate of occurrences. Table 10 showed the apparent fermentability (AF) of the samples, Barley had the highest apparent fermentability value of 72 % followed by CSR-02 with apparent fermentability of 44 % whereas its 50 % barley & 50 % CSR-02 had apparent fermentability of 62 % which was closer to that Barley, while Samsorgh-17 that contained the highest extract value tends to be the lowest in terms of fermentability, this suggested that the high extract value of Samsorgh-17 did not influenced its fermentability directly, hence it contained less fermentable sugars, in accordance with [14], that suggested that a low level of fermentable sugars is produced in sorghum malt mashing, which has been attributed to the high starch gelatinization temperature and low  $\beta$ -amylase activity in sorghum compared with Barley.

## CONCLUSION

The research work focused on fermentation analysis of three sorghum varieties and their combinations with barley in different proportions. The study revealed the 50 % barley & 50 % CSR-02 malts had a better brewing potentials as compared to other samples studied, this was evidenced in the high quality characteristic shown after several analysis such as its high content of free alpha amino nitrogen, apparent extract level and apparent fermentability percentage. The infusion mashing method used during the mashing process had shown that 50% barley & 50 % CSR-02 malt produced more fermentable sugars in the wort. This resultant wort when subjected to fermentation after inoculation of *Saccharomyces uvarum*, provided a nutrient balanced medium for the fermentation process as the apparent fermentability is 62%. In this study, 50% barley & 50 % CSR-02 malts had better and higher results in malting qualities, wort analysis and fermentation analysis and hence, can be used in the brewing industries to curtail the high cost of barley importation in Nigeria.

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