

PRODUCTION OF MALT-BASED SUGAR SYRUP FROM ENZYMATIC HYDROLYSIS OF MALTED SORGHUM AND MILLET GRAINS.

Abstract

The suitability of sorghum and millet grains in the production of malt-based syrups was determined. The grains were steeped for 50 h, germinated for 5 days at room temperature and were kilned for 48h to produce the malts. Mashing was carried out by three-stage decoction method and the resulting wort further hydrolysed with glucoamylase enzyme to yield malt syrups. Proximate analysis results showed that the crude protein contents in sorghum (11.3%) and millet (10.8%) malts were significantly ($p < 0.05$) higher than in sorghum (10.36) and millet (8.58%) grains. Cereal grains (sorghum, millet) were higher in fat (6.83, 7.30%), ash (2.41 and 3.16%), fibre (3.31 and 2.63%), moisture (9.93 and 9.95%) and total carbohydrate (71.63 and 53.35%) contents when compared with the malts. Results for malting characteristics of the grains showed that sorghum had significantly ($p < 0.05$) higher germinative energy (82.53%), germinative capacity (90.50%) diastatic power (32°L) and lower malting loss (13.50%) than millet grains: 76.6%, 85.67%, 27°L and 18.47% respectively. Mashing temperature and pH optima results for amylase activity were $60-70^{\circ}\text{C}$ in sorghum, $40-45^{\circ}\text{C}$ in millet and pH 6-7 in sorghum and millet respectively. Results obtained on the analysis of the malt syrup samples (sorghum and millet) were (%): moisture (12.35, 13.46), ash (0.02, 0.04), pH (4.5, 5.0), total solids (82.20, 80.1), Dextrose equivalent (85, 81) and reducing sugar (70.30, 65.45) respectively. Viscosity, colour and taste of the end products were physically checked. Sorghum grain exhibited better potential for syrup production.

Keywords: Malt, sugar syrup, glucoamylase, sorghum, millet.

Introduction

The malt-based syrup is a concentrated aqueous solution of mixed nutritive saccharides of edible starches of malted cereal grains. Malting is essentially a biological process in which the germination of cereal grain is carried out in a controlled environment [1]. The technically important features of germination are the synthesis of hydrolytic enzymes which breaks macromolecules into compounds of low molecular weight of **desired** characteristics [2]. When the process reached the **desired** stage, the germination is interrupted by drying or kilning.

The development of malt-based syrups involves three fundamental stages production of malt, preparation of wort from the malt by infusion or decoction mashing process and further saccharification of the wort to malt syrup using external glucoamylase [3]. The resulting syrup which is a mixture of saccharides is concentrated to about eighty per cent (80%) solids. Enzymatic hydrolysis is now largely replacing the acid hydrolysis because it is easy to control, effective even in mild ambient conditions and do not give rise to any by-product [4].

Malt-based syrups are increasingly being used as natural food colourants, thereby replacing caramels. In the baking industry, diastatic malt syrups may be used in bread as a yeast food releasing sugars naturally and contributing to loaf volume and texture. Malt based syrup is also used in brown bread and dark cake manufacture, breakfast cereals and biscuit production. In the pharmaceutical industry, malt syrups could be incorporated into infant liquid mixture as sweetening, colouring and flavour carrier [5].

Glucose syrup is traditionally produced from corn starch. Currently, it is not sufficiently manufactured in Nigeria, but most industries make use of it. Import information reveals that in the year 2008 and 2010, \$40,015,825 and \$24,049,485 were used in the importation of glucose and glucose syrup containing less than 20% fructose respectively [6].

Cereal grains such as maize, millet, and sorghum are important stable foods found in the diet of the people of northern Nigeria. In 2015-2017, Nigeria rose to 2nd place in the world with regards to sorghum production behind the United States of America. USA's output was projected at 8.4m metric tonnes and Nigeria with 6.4 m metric tonnes in 2017 [7]. Pearl millet is the most commonly grown millet type in Nigeria followed by finger millet [8]. In 2016, global production of millet was 28.4million tonnes, led by India with 36% of the world total. Nigeria is the fifth producer of millet in the world as of 2016 with an annual tonnage of 1.5million tonnes [9].

In Nigeria, the relative abundance of sorghum and millet crops has prompted the current research efforts towards foreign exchange conservation. Wheat and corn are today in Nigeria 'Golden cereals' and hence the quest for substitute the blends.

Malting of the local cereals generate endogenous malt amylases which augment the imported microbial amylases used in syrup production, thereby saving cost. The study was carried out to prepare malt-based syrups from locally available cereal grains. The specific objectives were to determine the malting conditions necessary for optimising the cereals' malt diastatic power, to determine which of the two cereal, sorghum and millet has higher malting potential and finally to determine the level of microbial amylase and conditions suitable for the production of relatively cheap malt-based syrup.

2.0 MATERIALS AND METHODS

2.1 Materials.

Millet grains (*Pennisetum typhoides*) and sorghum grains (*Sorghum bicolor*) were purchased from Orba market, Nsukka, Nigeria

2.2 Methods

2.2.1 Determination of germinative characteristics of sorghum and millet grains.

Thousand corn weight, germinative capacity and germinative energy of the cereal grains were determined following the method of Institute of Brewery [10]. Percentage malting loss was determined by the method described by Novellie *et al.* [11].

2.2.2 Determination of Diastatic power

Diastatic power was determined using the ferricyanide method [10]. Malt extract was obtained by extracting with water for 2 hours in a temperature-controlled water bath (Model NoDK 600 Gulflex England). About 3 ml of the unfiltered malt extract supernatant was transferred into a 250 ml Erlenmeyer flask containing 100 ml buffered starch solution maintained at 30 °C in a water bath. After 1 hour thorough mixing, 5 ml portion of the digested starch solution was mixed with 10 ml of alkaline ferricyanide and left to stand in boiling water for 20 minutes. On cooling to 30 °C, 25 ml acetic acid salt and 1 ml potassium iodide solutions were added and the solution titrated with 0.05 mol/l sodium thiosulphate solution to the complete disappearance of the blue colour thus formed. A blank was prepared for the unfiltered malt infusion and 2% buffered starch solution.

The diastatic power (Dp) was calculated as follows: $Dp(IOB) = B - A(23 + 200/250 \times 1/C)$. Where, A = Volume of sodium thiosulphate used for direct titration, B = Volume of sodium thiosulphate used for blank determination, and C = Volume of unfiltered malt extract used for digestion. The diastatic power (Dp^{IOB}) was converted to $Dp^{(L)}$ as follows: $Dp^{(L)} = (Dp^{IOB}) \times 1.1$

2.2.3 Determination of reducing sugar content

The reducing sugar/dextrose equivalent (DE) values of the syrup samples were determined according to the Institute of Brewery method of analysis [10] as follows: 10 % (w/v) solution was prepared by weighing 25.00 g of the syrup in a glass dish and dissolving same by gradual stirring in warm water. This was transferred quantitatively to a 250 ml graduated flask after adjusting the temperature to 20 °C, it was made up to the mark at that flask and diluted to mark at 20 °C. This was mixed well and filtered. It was used as the "diluted solution".

25 ml of mixed Fehling's solution was pipette into a 15ml boiling flask, and an almost sufficient volume of the diluted solution was added from the burette to the cold Fehling's solution to effect reduction, so that, if possible, not more than 1ml was required later to complete the titration. The contents of the flask were mixed and heated over a wire-gauze, kept in moderate ebullition for 2 minutes and three drops

of methylene blue indicator added without removal of the flame; then the titration was completed in one minute with continuous ebullition.

The endpoint (decolourisation of the methylene blue) was taken as the volume at which the reaction mixture turned red. The titre was recorded.

The results were calculated as the most appropriate sugar (glucose or maltose) using the appropriate factor from the Lane & Eynon Table. For the dilution given, the percentage reducing sugar in the sample 'as is'

$$\% \text{ Reducing sugar} = \frac{\text{Lane \& Eynon factor} \times 100}{\text{Titre}}$$

$$\text{Dextrose Equivalent (DE)} = \frac{\% \text{ reducing sugar (as glucose)} \times 100}{\% \text{ total solids}}$$

2.2.4 Proximate composition analysis

Analysis of the moisture, crude protein, fat, ash, fibre, and total carbohydrate contents of sorghum/millet grains, malts and syrups were performed according to standard methods [12].

2.2.5 Determination of optimum malting conditions for the cereal grains

2.2.5.1 Moisture Content as a function of steep time

Eight Petri-dishes lined with filter papers at their bottom were provided and filled with equal volumes of water. Twenty grams (20 g) of the grains were cleaned and steeped at room temperature in each of the Petri-dishes for various times, 10-80 h, with eight hourly change of steep liquor. At the end of each steep period, the grains were drained, surface water blotted with filter paper, then the moisture content determined.

2.2.5.2 Determination of optimum Steep time

The grains (20 g) were steeped at various times, 10-80 hrs as described above. Each of the eight sets was allowed to germinate for 4 days in a dark cupboard and then kilned for 48 h at 55 °C, after which the malt's diastatic power was determined.

2.2.5.3 Determination of optimum germination period

The gains (20 g) were steeped for 50 h and germinated for various periods, 1-7days in a dark cupboard, later kilned for 48 h at 55 °C and the malt's diastatic power determined.

2.2.5.4 Determination of effects of kilning at 45°C and varying drying time on the moisture content of the malts:

Samples of malted grains at optimum malting conditions (50 h steeping and 5 days germination) were kilned at various periods (12 h, 24 h, 36 h, 48 h, 60 h) at 45 °C and moisture content determined

2.2.5.5 Determination of malting loss as a function of the germination period

The grains (20 g) were steeped for 50 h and germinated for various periods, 1-7 days, then the resulting malting losses per nth day of germination determined.

2.3 Production of sorghum and millet Malts

1 kg of each of the grains was cleaned and steeped in ordinary tap water for 50 hours at room temperature as follows: 8 hr steeping: 2 hours air rest: 8 hours steeping: 2 hours air rest. Air rests were done by draining off the steep water. After the last 2 hours air rest, the grains were placed on a cotton cloth sterilized with sodium hypochlorite (3.5 % in 175 ml distilled water), covered with jute bag and germinated at room temperature with water sprinkled at intervals and turning the grain to avoid matting. The green malt was harvested after 5 days of continuous germination and dried in a hot air oven at 45 °C for 48 hours. The polished malts were milled into flour to pass through 1mm mesh screen and packaged into plastic containers and stored in a cool place.

2.4 Extraction of malt amylase

Malt amylase was extracted according to Shambe *et al.* method [13]. The malted grains (5.0 g) were ground separately in a mortar and quantitatively transferred into 100 ml standard volumetric flask by washing with distilled water, then made up to 100 ml. It was incubated at 37 °C for 3 h in 250 ml conical flask and 2.0ml samples withdrawn, centrifuged at 8000 g, in 0.5 h and the supernatant stored in a refrigerator.

2.5 Preparation of 1% buffered starch substrate

5 g starch (dry mass) was turned into a paste with a little cold water and then poured into 400 ml of boiling water. The mixture was boiled for 2 minutes and then cooled. Adequate quantity of each of the buffer solutions prepared earlier was added and each of the mixtures was made up to 500 ml resulting to 1% buffered starch substrate solution of pH 4, 5, 6, 7 and 8 respectively.

2.6 Preparation of maltose calibration curve

A series of maltose solutions were prepared so that 2 ml contain 0.4 – 2.0 mg anhydrous maltose as follows: Into each of ten test tubes were added 0.4 – 2.0ml of stock standard maltose solution containing 2 mg/ml respectively. The solutions were respectively made up to 2 ml each by addition of the appropriate amount of distilled water. 1ml of the 1% starch solution and 2 ml of DNS reagent were added.

The test-tubes were transferred to a rack in a boiling water bath and heated for five minutes and then cooled to room temperature after which the content of each tube was diluted by making-up the volume to 20 ml with distilled water. A suitable amount of each sample was poured into a colourimeter cuvette for optical density determination at 505nm against a reference blank which contains only 2 ml water, 1ml starch and 2 ml DNS reagent.

2.7 Determination of optimum pH for amylase activity

Two millilitres (2 ml) of the diluted amylase extract (2 ml extract in 200 ml distilled water) was added to test tubes in a rack-containing 1ml each of the 1% buffered starch substrate solution at the pH 4, 5, 6, 7, 8 previously prepared. The tubes were shaken for 5 minutes to mix it properly and incubated in a thermostatically controlled water bath at 37 °C for 10 minutes.

The diastatic reaction was stopped by the addition of 2ml DNS colour reagent. All the tubes were heated in a boiling water bath for 5 minutes and then cooled to room temperature after which the contents of the tubes were diluted to 20ml with distilled water. The absorbance was read at 505 nm against a reference blank. The blank was prepared by boiling the amylase extract for 5 minutes before adding to the 1% buffered starch substrate solutions. The concentration of the reducing sugars as maltose in the starch hydrolysate was calculated by extrapolating its absorbance value from the maltose calibration curve.

2.8 Determination of optimum temperature for amylase activity

Two millilitres (2ml) of the dilute amylase extract was added to 1ml of 1% starch (substrate) solution buffered at optimum pH range 6-7 and incubated for 10 minutes at various temperatures, 30, 40, 50, 60, 70 and 80 °C. Two millilitres (2 ml) of (DNS) colour reagent was added (and other procedures repeated) and the concentration of reducing sugar as maltose was calculated

2.9 Determination of mashing conditions for malt syrup production

2.9.1 Evaluation of effects of varying mash concentrations and saccharification time on the reducing sugar content of worts.

Mash concentrations, 25%, 35%, and 45 % and total saccharification time of 1 h., 2 h., and 3 h. were employed in the production of worts. Saccharification by the malt amylase was at a mash temperature range of 70-80 °C and pH 6-7. The resulting in reducing sugar contents (as glucose) were determined.

2.9.2 Evaluation of the effects of varying concentrations of glucoamylase and saccharification time on the reducing sugar content of syrups.

Worts were prepared from a 25 % mash. Its pH was adjusted to 4.3 with a 2 M HCL solution, and temperature maintained at 55 °C. Varying concentrations of glucoamylase, 0.05%, 0.01%, and 0.15 % (dry weight basis of the mash) were added respectively, then saccharification carried out for 12 h., 24 h., and 36 h. The resulting hydrolysates were neutralised, filtered and analysed for reducing sugar content as glucose.

2.10 Production of Malt based Syrups

A three-stage decoction method as described by [14] was used in this work. Fifty grams (50 gm) of Sorghum malt milled to 1-2 mm particle size (in a Thomas Willy Mill Model ED 5) was mixed with 200 ml of tap water, to give 25 % mash. This was incubated at 40°C for 30 minutes. The one-third portion of mash was withdrawn, boiled for 5 minutes, and returned to the main mash. The temperature of the mash rose to 50 °C and was maintained at this temperature for 15 minutes at a pH 6.5. (This was adjusted with 2 M (Ca(OH)₂ solution). The one-third portion of the mash was again removed, boiled for 5 minutes and returned to the main mash. The

temperature of the mash was raised to 60 °C, and this was maintained at a temperature range of 60-65 °C for 30 minutes.

A further one-third portion of the mash was removed, boiled for 5 minutes and returned to the mash. The temperature rose and was maintained at 70 – 75 °C (the mashing off temperature for 30 minutes). The pH of the resulting wort was adjusted to 4.3 using 2 M HCL. Its temperature was maintained at 55 °C using a thermostatically controlled water bath (Model No DK 6 Gulfex medical England,). Glucoamylase solution, 0.15 % (DWB of the mash) was added and incubated for 36 h (with constant shaking) until the desired DE value was attained. The malt hydrolysate solution produced was neutralised with 2 M Na₂CO₃ solution. A ten-fold dilution of it was made, filtered using (Whatman filter paper) and finally concentrated to a syrupy consistency by evaporation on a boiling water bath

2.11 Analysis of malt syrups.

2.11.1 Determination of percentage of total solids of the syrups.

A sample of the syrup was transferred into a previously dried and weighted stainless steel dish. The dish and content were later placed on a boiling water bath and evaporated to dryness. This was weighed and then placed in an oven and dried for 3 hr at 105 °C. The dish was returned to the oven and weight checked at thirty-minute intervals until no further loss in weight could be detected. The dish was cooled in a desiccator for 20 minutes and the weight taken.

The percentage of total solids was calculated thus:-

$$\% \text{ Total solids} = \frac{\text{Wt of the sample after dry}}{\text{Wt.of the sample before drying}} \times 100$$

2.11.2 Determination of pH

Ten millilitres (10 ml) of the sample was measured into a 100 ml beaker and the pH was determined with the aid of a previously standardised pH meter (Model 5 Horiba Kyoto Japan). The pH meter was calibrated using pH 4.0 and 7.0 buffer.

2.11.3 Determination of Specific gravity/Degree Baume of the Syrups

The specific gravity of the syrups was determined according to the procedure described in [12] method of analysis. A 50ml specific gravity bottle was filled with distilled water stoppered and immersed in a water bath at 20 °C. After 30 minutes, the specific gravity bottle was removed, dried with filter paper and weighed. The same procedure was repeated with the samples. The specific gravity of the samples was calculated as follows:

$$SG = \frac{\text{weight of liquid held in SG bottle}}{\text{weight of water held in SG bottle}}$$

Degree Baume (°Be): This is related to Specific gravity by, the following formula (Corn Refiners Association, 1965):

$$^{\circ}\text{Be} = 145 - \frac{145}{SG}$$

3.0 RESULTS AND DISCUSSION

3.1 Germination properties of sorghum and millet malts

Table 1 shows the germination properties of cereal grains. The values of 33.3 g and 6.8 g 1000-kernel weight obtained respectively for sorghum and millet grains were smaller compared to 37-47 g for barley reported by [15]. However, since these grains are smaller in size, it is expected that their weight will be smaller. There were significant variations in the germinative properties of the cereal grains ($P \leq 0.05$). Germinative energies of 82 % and 76 %, germinative capacities of 90% and 85% and malting losses of 13.5 % and 18.7 % were obtained for sorghum and millet malts respectively. Hough *et al.* [14] reported that grains intended for malting should have satisfactory germination properties of over 90%. Sorghum showed good potential for use as malting grain. High germination capacity, germination energy and malting yield and/or low malting loss are indicators of good malting barley [16]. Physiological and structural differences of the grains may be responsible for the differences in their malting characteristics [17].

Table 1 Germination properties of sorghum and millet malts

Property	Sorghum malt	Millet malt
Thousand kernel weight(g)	33.43 ^a ± 0.61	6.95 ^b ± 0.15
Germinative energy (%)	82.53 ^a ± 0.30	76.76 ^b ± 0.35
Germinative capacity (%)	90.50 ^a ± 0.40	85.67 ^b ± 0.49
Malting loss (%)	13.50 ^a ± 0.30	18.47 ^b ± 0.37
Malt yield (%)	87.70 ^a ± 0.36	72.76 ^b ± 0.35

Results are the means of three replications values carrying different superscriptions in the same row are significantly different ($P < 0.05$)

3.2 Optimum malting conditions of the cereal grains

Figures 1 to 5 present the optimum malting conditions of the cereal grains. The variation of moisture content (%) against steeping time (hr) is shown in figure 1. The result indicates a sharp rise in water uptake during the first 10 hours. Further steeping above 20 hours showed marginal increases. It is evident from the results that sorghum grains absorbed moisture faster than millet grains; this may be due to its relative large corn size. Dahlstron *et al.* [18] observed that larger corns absorb water more rapidly than smaller ones initially, and the difference in water absorption after 24 hr is marginalised. Also, Hartong and Kretschmer [19] found that samples of grains that absorbed faster gave better malts than grains that absorb water more slowly.

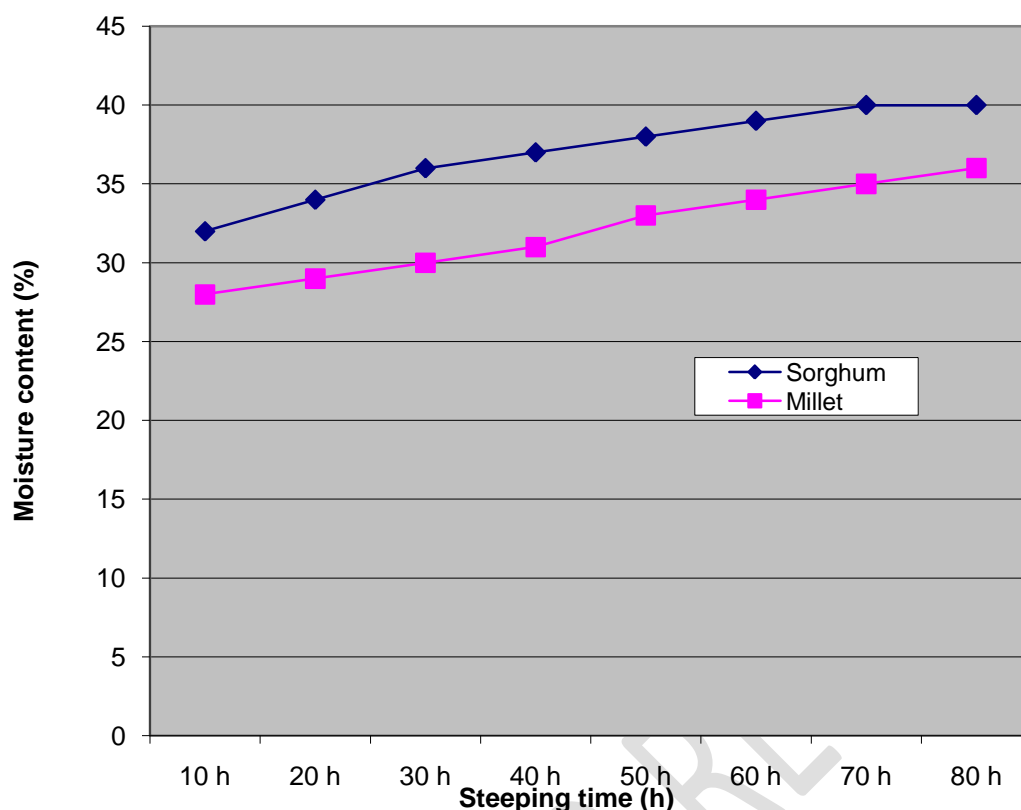


Fig. 1 Moisture content (%) against steeping time (hr)

The result obtained from the plot of diastatic power ($^{\circ}$ L) against steep time (hr) (fig. 2) showed that the optimum steep time was 50 hours. At this time, the two cereal showed maximum values of diastatic powers after the grains were allowed to germinate for four days. Furthermore, extrapolating the 50-hour optimum steep time to results of the plot of moisture content (%) against time (hr) as shown in Fig. 1 gave variously 38% and 33% optimum moisture contents for sorghum and millet grains respectively. Nout Davis, [20] obtained 45% moisture content after 35 h and 20 h of steeping sorghum 'Andivo' and sorghum 'igumba' respectively. Taylor and Robbins [21] showed that high germination moisture gave the highest malt *beta*-amylase activity. Aisien and Ghosh [22] reported that the optimum moisture content for rapid germination of Guinea corn (*sorghum vulgare*) was between 35 and 40%, at the optimum temperature of 22 $^{\circ}$ C. Dewar *et al.* [23] found that sorghum malt diastatic power (combined *alpha*- and *beta*-amylase activity) increased with time of steeping and was directly related to steep-out moisture. Ezeogu and Okolo [24] found that steeping regime, and in particular, the use of air-rests enhanced sorghum malt quality, including *beta*-amylase activity. It is probable that the use of air-rests simply provides more oxygen and hence more rapidly increases seedling metabolic activity. Steeping involves immersing the grains in water until they have imbibed a suitable amount of water at a temperature of about 30-40 $^{\circ}$ C to support growth and biochemical changes during germination [14].

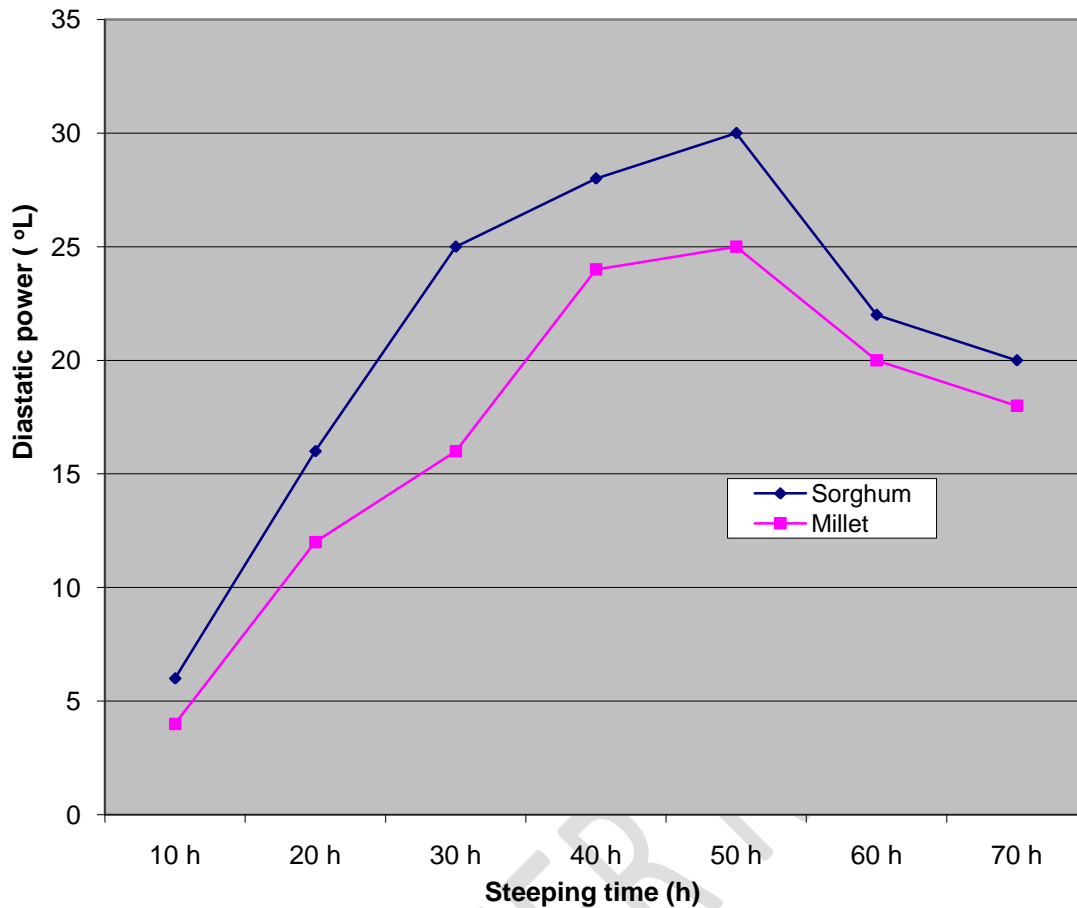


Fig. 2 Diastatic power (°L) against steeping time (h) after 5 days of germination

Figure 3 shows the plot of diastatic power (°L) against germination period (days). The optimum germination period of 5 days was obtained after steeping the grains for 50 hours with intermittent use of air-rests. Diastatic power of 32°L and 27°L were obtained respectively for sorghum and millet malts under the same stipulated malting conditions. The result shows that there was no diastatic power measurable in ungerminated grain but rises sharply from 1-3 days reaching its peak after 5 days of germination. This suggests that diastatic enzymes absent in ungerminated grain develop with germination [21]. In general, the DP of the malts increased with increasing germination time to about 5 days (Fig. 3). This is in agreement with what has been reported by others [23, 25]. Germination process promotes the production of several enzymes, notably α -amylase and β -amylase, which convert the starch in the grain into sugar [26].

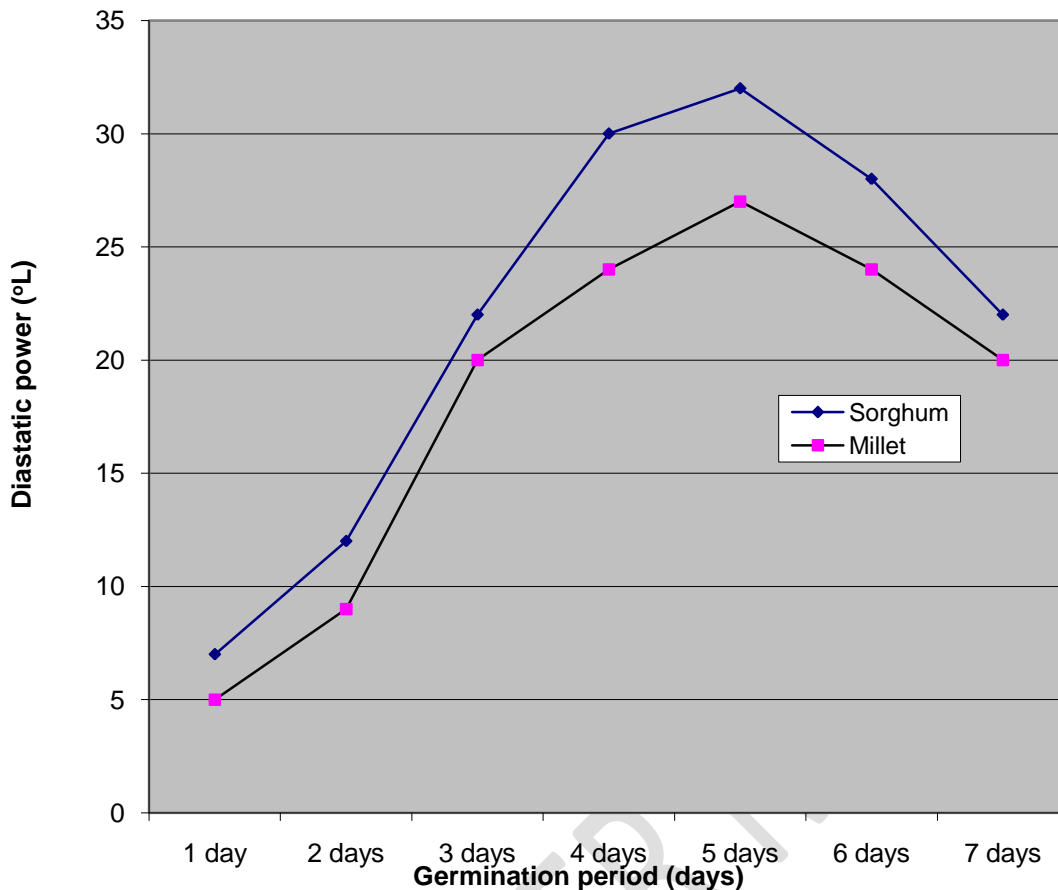


Fig. 3 Diastatic power (° L) against germination period (days) after 50 hr of steeping

Figure 4 shows the result of malting loss (%) measurements during the various periods of germination (days). The result showed that the malting loss of **the** grains increased with increase in the **duration** of germination. Significant increases in malting loss were recorded **ed** between 2-4 days of germination, which correspond to the periods of the significant drop in kernel weights during germination. The ranges of 12 – 16% and 16 – 20% malting losses were obtained respectively for sorghum and millet malts within 4 to 7 days of germination. The malting losses for barley have been given by **Hough et al.** [14] as 6 – 12% and **Makeri et al.** [15] as 11-18% respectively. The high malting loss of the millet grains could be due to excessive serratation during steeping leading to grains growing uncontrollably during germination [14]. The malting losses observed in the test were adequate because an average of 10 -15 % respiration / metabolic loss is expected in well-malted sorghum with good diastatic power [27].

Malting loss could also result from the long steeping period as materials tend to be leached into the steep water. Malting loss which is comprised of physiological, moisture and vegetative loss is inversely related to the malt yield. Sorghum with less malting loss recorded higher malt yield. Malt yield is a critical factor in malting as it reflects the amount of extract obtainable from the cereal grain concerned.

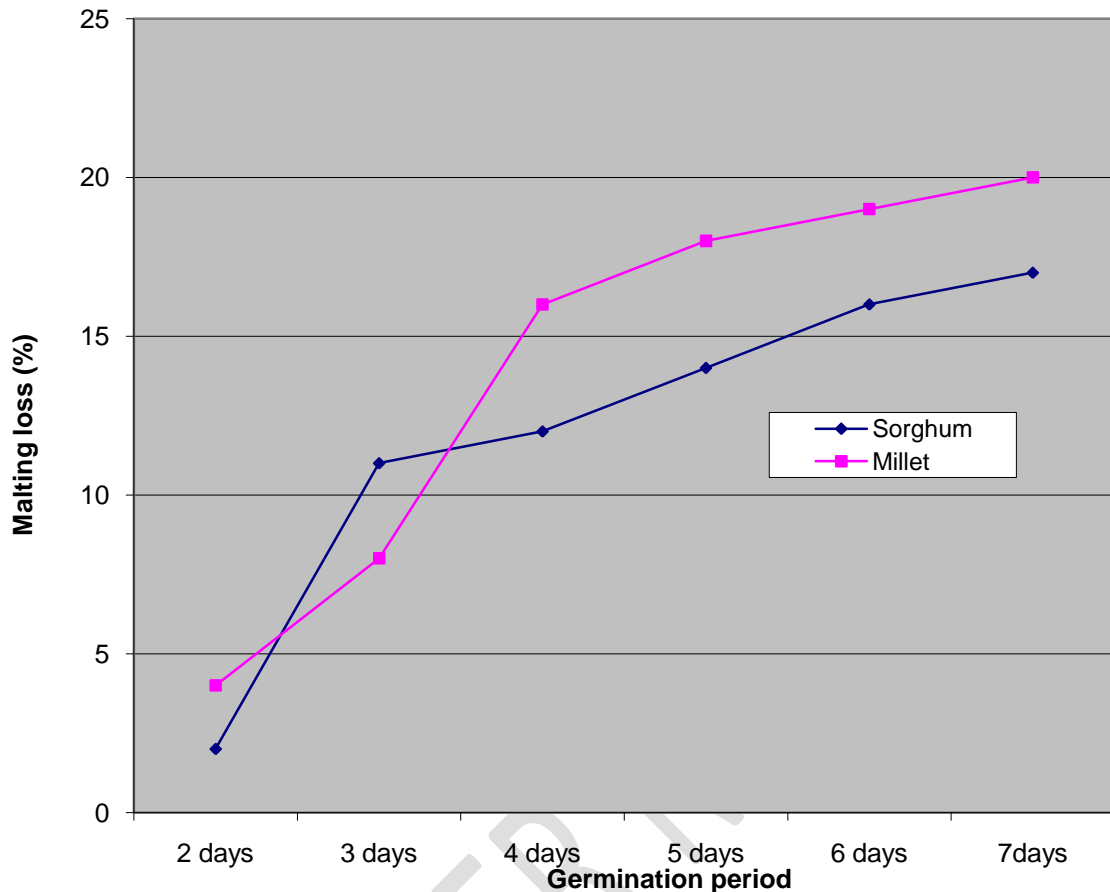


Fig. 4 Malting loss (%) against germination periods (days)

The result of kilning studies (Fig. 5) shows that moisture release is reciprocally related to moisture absorption. A significant loss in moisture was recorded after 12 hours of kilning at 45 °C. With regards to the moisture level about storage qualities of malts, optimum kilning can be carried out at 45°C for 24 – 48 hours depending on the moisture level of the green malt and the moisture content of the malt required. The effect of temperature on the malt characteristics was not investigated, however, other workers [20, 11] showed that only kilning at 70°C resulted in a significant loss in diastatic activity. Kilning in the range of 40 to 60 °C caused only negligible destruction.

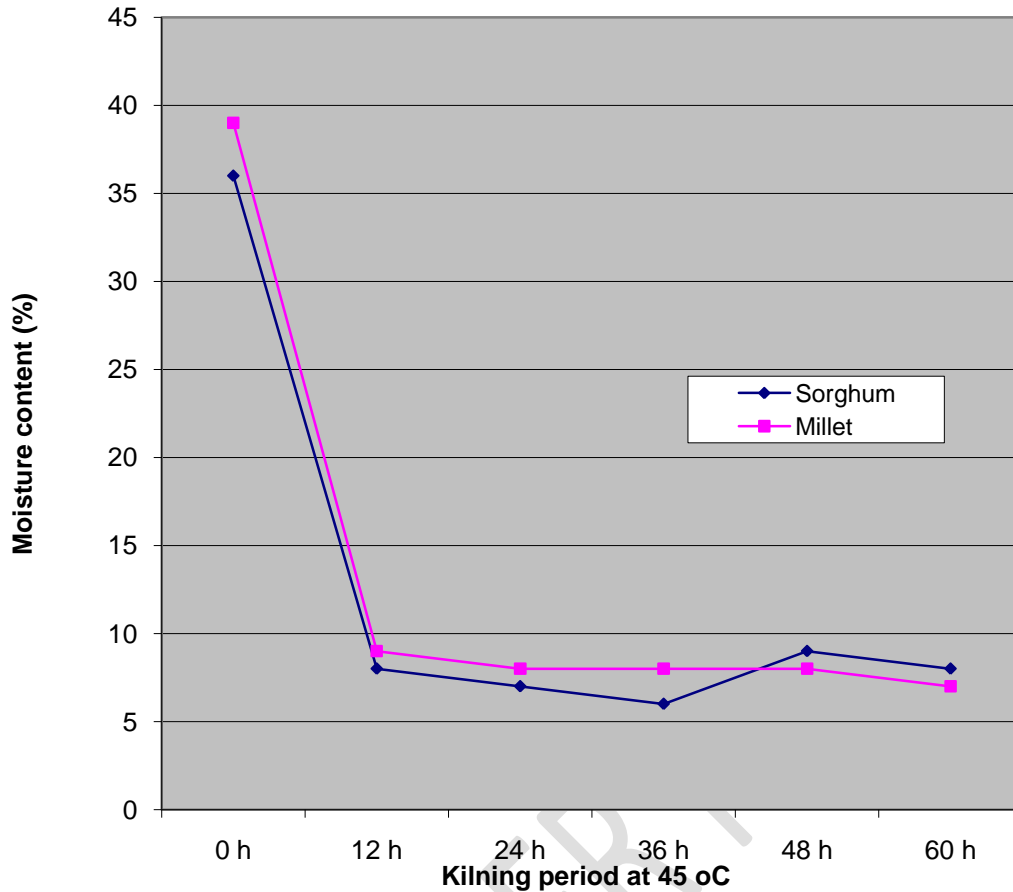


Fig. 5 Moisture content (%) against kilning periods (h) at 45^oC

3.3 Determination of optimum pH and temperature conditions for malt's amylase activity

The results of the optimum pH for the amylase activity of the malts were presented in Fig. 6. The optimum pH of 6-7 obtained in this study agrees with the values reported by [13]. Sills and Stewart [28] reported an optimum pH range of 5.5-6.5 for purified barley *alpha*-amylase. Hough *et al.* [14] observed that the pH value of 6.0 was quite suitable for amylase activity at 65^oC. The result is also in agreement with the pH optimum of 7.0 for *alpha*-amylase enzymes in barley [29]

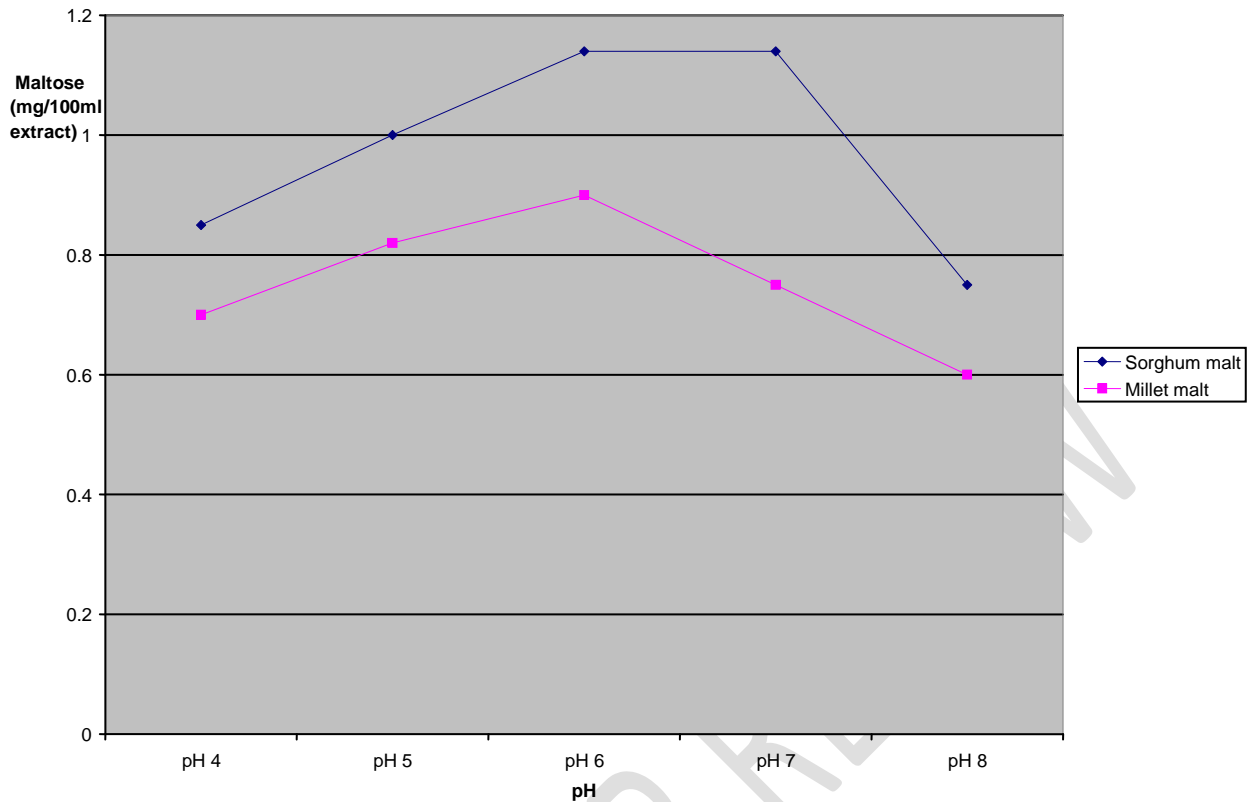


Fig. 6 Optimum pH determination for amylase activity

The results for the optimum temperature for the amylase activity of the malts were presented in Fig. 7. The amylase activity of the sorghum malt peaked at 60-70 °C while that of millet reached a maximum value at 40-50 °C. Two major starch-digesting enzymes, α - and β -amylases are released from malt. Temperature range of 60-65 °C maximise the activity of β -amylase whilst 65-70 °C is necessary to allow α -amylase to operate optimally [30]. Shambe *et al.* [13] reported the optimum temperature range of 35-45 °C for millet and 50 – 75 °C for sorghum malt amylase activities. The temperature optima in both cases are below gelatinisation temperature ranges of millet and sorghum starches obtained by other workers. Sorghum starch gelatinization temperature of 67-73°C have been reported for sorghums grown in Southern Africa [31] and 71-81°C for sorghums grown in India [32]. The millets have high starch gelatinization temperatures; pearl millet (*Pennisetum glycon(L)*), 61-68 °C and finger millet (*Eleusine coracana (L)Gaerthn*) 65-69°C [33]. These are far higher than the range quoted for barley starch of 51-60°C [34], a necessary prerequisite for effective degradation of starch by amylases. The general poor malting quality of sorghum and millet grains as reported by other authors is thus partly due to this inherent high gelatinization temperature of the grains' starches.

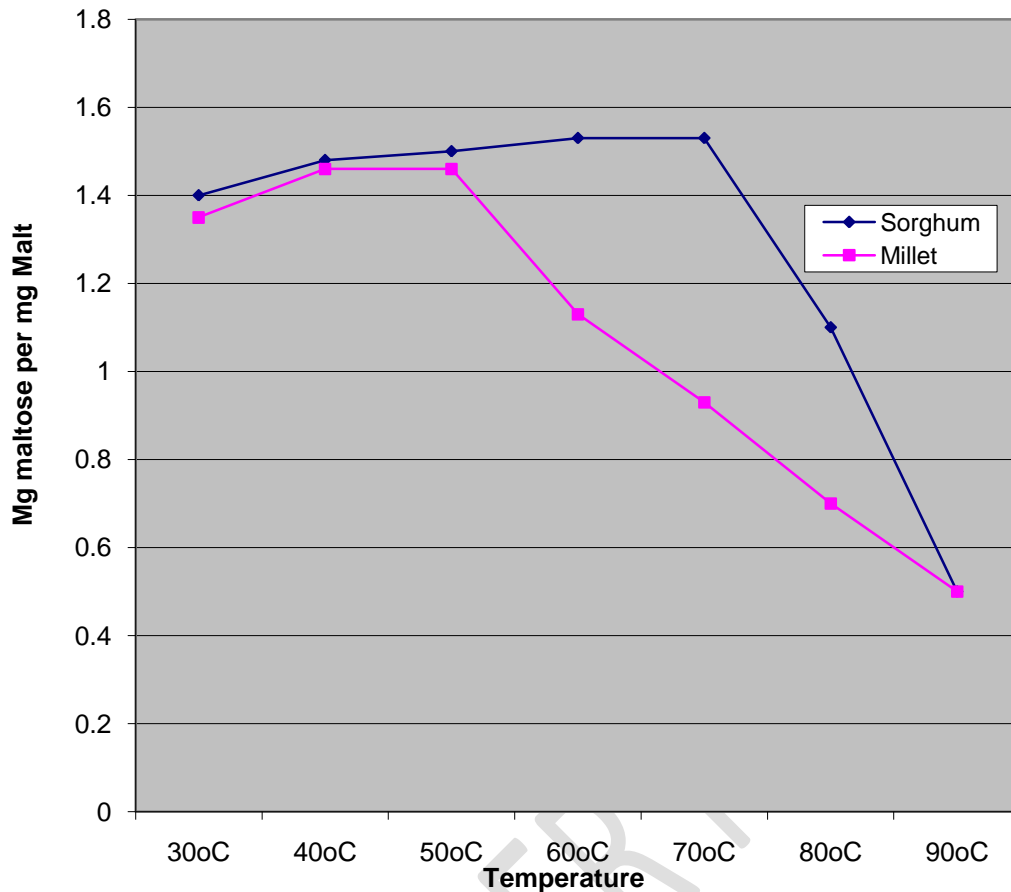


Fig 7. Optimum temperature determination for amylase activity in the malts

3.4 Effects of varying mash, glucoamylase concentrations and saccharification time on reducing sugar contents of the malt syrups:

Figures 8 and 9 show respectively the results of the effects of varying mash concentrations and saccharification time, glucoamylase concentrations, and saccharification time on the reducing sugar content of malt syrups. Results show that maximum conversion was attained at the lowest mash concentration (25 %), highest enzyme-substrate ratio (0.15 % glucoamylase) and at a longer saccharification time (36 hr). Jamie Ranshaw [35] showed that thicker mashes increase the stability of mash enzymes, while thinner mashes, dilute enzymes and substrates, lead to quick conversion but rapid destruction of enzymes. A three-stage decoction mashing method was used for the wort extraction. Highest extract and high fermentable sugars were obtained with a triple-decoction mashing process. Decoction-mashing type also has been found by other workers to be an effective process for sorghum malt production [36, 37]. The removal and boiling of portions of the mash enable complete gelatinisation of the sorghum malt starch in those portions removed. This facilitates rapid saccharification of the starch by the malt α -amylase when the portions are returned to the main mash. The low temperature (60° C) mashing period facilitates sugar formation by the β -amylase. Palmer *et al.* [37] observed that the average level of extract (88 %) obtained from sorghum malt by the decoction procedure is much higher than that which can be obtained from barley because sorghum is a huskless grain. Hence, the starch-containing endosperm tissue forms a higher proportion of the kernel than in barley.

The main factors that regulate the activities of mash enzymes are temperature, pH, time, and concentration of the wort [38]. The thermostability of starch-hydrolysing enzymes is critical for the fermentable sugar yield during mashing. The mashing temperature profile is a balance between the temperature required for starch gelatinisation needed to enable efficient hydrolysis and the rate of thermal inactivation of these enzymes [39]. The mashing temperature of 65 °C is generally used in mashing barley malt, but when sorghum malt was mashed at the same temperature the result was inadequate gelatinisation of the starch and sub-optimal release of sugars even when commercial enzymes were added. However, at a mashing temperature of 85°C and above, sorghum starch was gelatinised effectively and sugars released into the wort was higher than at 65°C, and even higher when commercial enzymes were included at a very low rate. Meanwhile, at 65°C mashing temperature, triple decoction mashing method gave good results. High conversion syrups are prepared almost exclusively by enzyme-enzyme conversion process [40]. Aderibigbe *et al.* [41] observed that acid catalysed hydrolysis of starch is not capable of giving practical hydrolysates with more than about 90% D-glucose owing to acid catalysed reversion and dehydration reactions resulting in a sizable loss of glucose.

The objective of the liquefaction process (mashing) is to convert a concentrated suspension of starch granules into a solution of soluble dextrans of low viscosity for convenient handling and easy conversion to glucose by glucoamylase. However, there are feasible ranges of interacting variables during the saccharification process of syrup production. The 25-35% mash concentration range struck a balance between the costs of removing excess water from hydrolysates against higher conversion to glucose attainable at low mash concentration. The Saccharification range of 12-36 hours chosen for the experiment was based on the enzyme-substrate ratio used and the extent of conversion desired. The optimum temperature range of glucoamylase activity is 50-63°C, where the upper limit is dependent on glucoamylase stability, and a lower limit on the need to inhibit microbial contamination during long saccharification periods. Furthermore, the pH range, 4-5 is dependent on the source of glucoamylase, and also sugars are most stable at this pH range.

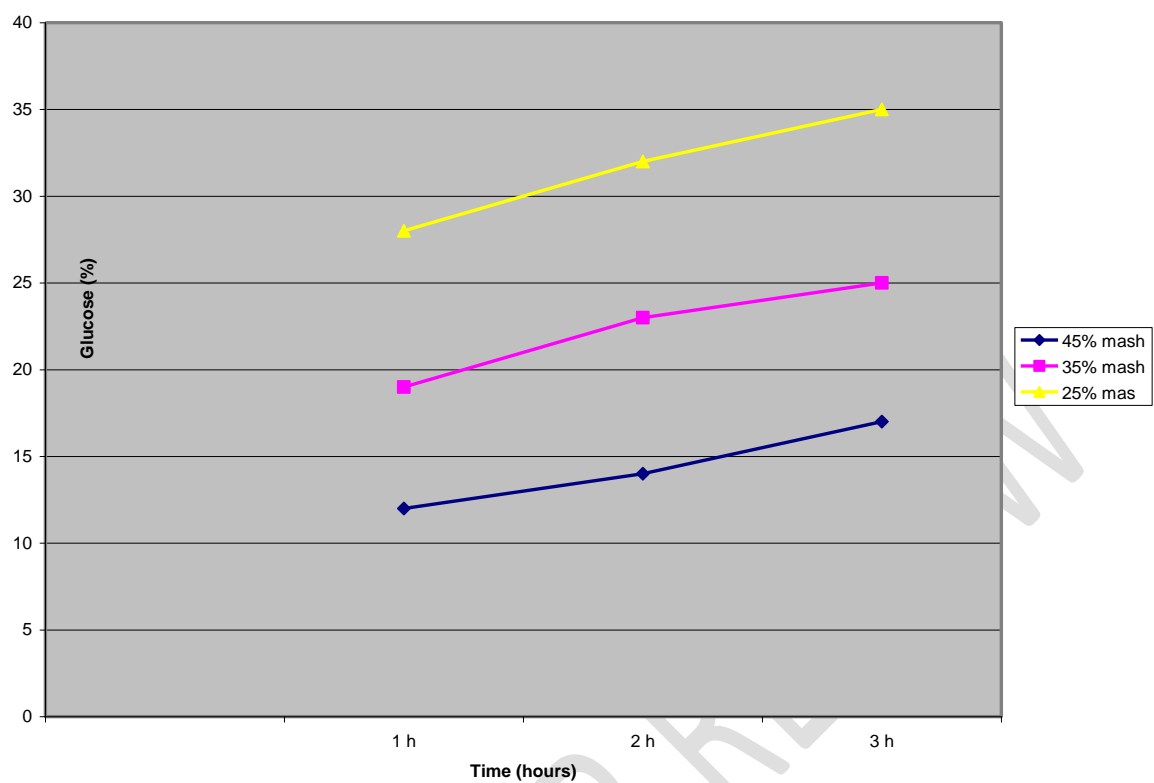


Fig. 8 Effect of varying mash concentrations and saccharification time on reducing the sugar content of the wort.

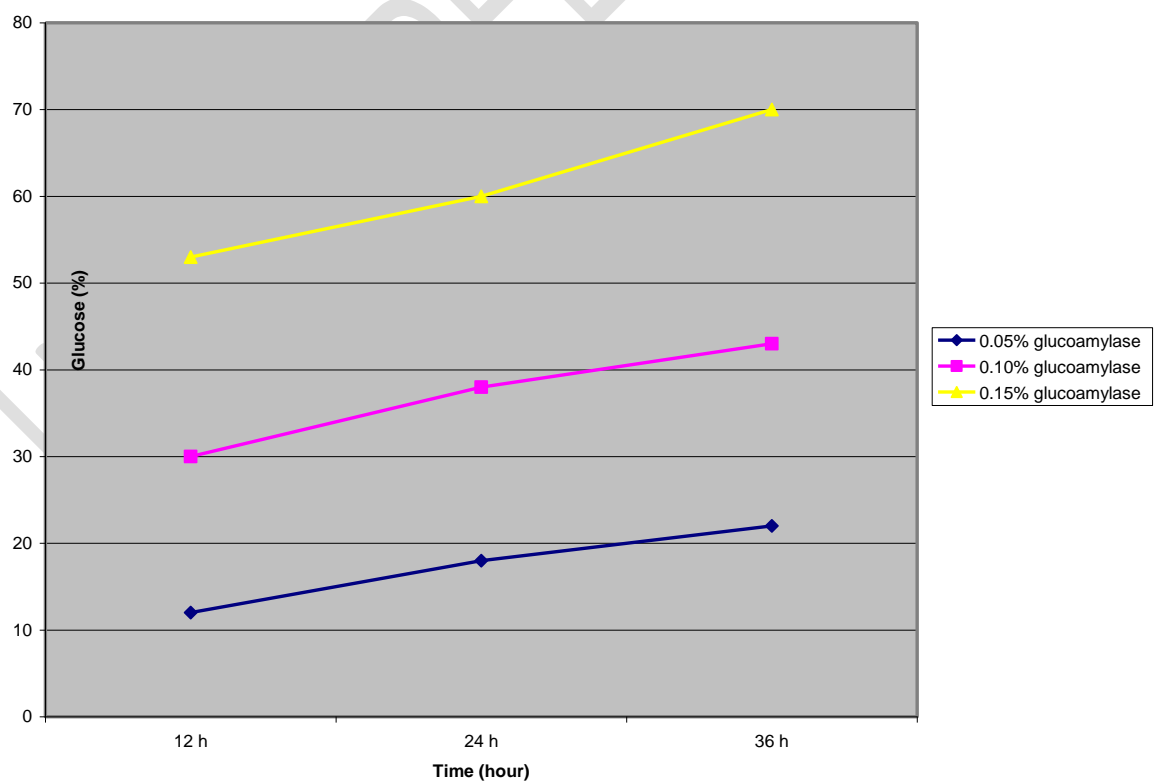


Fig 9. Effect of varying concentrations of glucoamylase and saccharification time on the reducing sugar content of the syrup.

3.5 Proximate composition of sorghum/millet grains and malts

Table 2 shows the results of the proximate composition of Sorghum, millet grains and malts. The moisture content of sorghum and millet grains (9.93 and 9.95 %) are significantly ($p < 0.05$) higher than the malts (7.50 and 6.53 %). This could be attributed to the loss of water during kilning which involves drying of the green malt in a kiln at 45 °C for 24 h. The crude protein contents of millet grains (8.58 %) and sorghum grains (10.36 %) were significantly ($p > 0.05$) lower in the millet malt (10.8 %) and sorghum malt (11.30 %). This could be attributed to improvement in Free Amino Nitrogen, FAN. The FAN content of the malt is a product of both the catabolic processes which degrade the storage proteins into peptides and amino acids and anabolic processes which synthesise them into new proteins during germination [42]. FAN is produced during malting by the action of endogenous proteinase and peptidase enzymes on the protein reserves of the grain [43] and the breakdown products are collectively referred to as FAN. Tatsadjien *et al.* [44] reported an increase in protein content during prolonged germination of sorghum.

The fat content of millet and sorghum grains (7.30 and 6.83 % respectively) were significantly ($p < 0.05$) higher than the malts (2.75 and 2.40% respectively). This is due to the increased activity of the lipolytic enzymes. They hydrolyse fats to simpler products which can be used as a source of energy for the developing embryo. Similar observations were made for malted millet [45] and sorghum malt [46]. This decrease in fat content implies an increased shelf-life for the malts compared to the cereal grains. Malting of millet and sorghum grains decreased the fibre content from 2.63 and 3.31% to 1.48 and 3.11% respectively as shown in Table 2. This could be attributed to the loss of the pericarp layer of the grains which are rich in fibre during germination [47].

There were significant ($p < 0.05$) decrease in the ash content of millet and sorghum grains (3.16 and 2.41 % respectively) when compared to the malts (1.20 and 1.30 % respectively). This is expected because, during soaking and germination processes, the pericarp or aleurone layer was lost thus resulting in the much decrease. Most mineral elements reside either in the pericarp or aleurone layer of the grains.

The total carbohydrate content of the millet and sorghum grains (53.35 and 71.63 % respectively) were significantly ($p < 0.05$) lower than the resulting malts (47.93 and 54.40 % respectively). The observed decrease in carbohydrate content could be as a result of metabolism due to the high level of amylase activities. The amylases break down complex carbohydrates to simpler and more absorbable sugars which are utilised by growing seedlings during the early stages of germination [48]. The decrease in the total carbohydrate content was corroborated by the same observation made by Inyang [45] and Yagoub *et al.*, [49].

Table 2 Proximate composition of sorghum/millet grains and malts

Parameter	Grainsmalts			
	Millet	Sorghum	Millet	Sorghum
Moisture content (%)	9.95 ^a ±0.39	9.93 ^b ±0.56	6.53 ^c ±0.15	7.50 ^c ±0.10
Protein(%)	8.58 ^a ±0.45	10.36 ^b ±0.15	10.8 ^b ±0.30	11.30 ^c ±0.10
Fat(%)	7.30 ^b ±0.20	6.83 ^c ±0.25	2.75 ^a ±0.05	2.40 ^a ±0.09
Fibre(%)	2.63 ^b ±0.20	3.31 ^b ±0.16	1.48 ^a ±0.18	3.11 ^b ±0.17
Ash (%)	3.16 ^c ±0.35	2.41 ^b ±0.16	1.20 ^a ±0.10	1.30 ^a ±0.10
Total carbohydrate(%)	53.35 ^a ±0.53	71.63 ^c ±0.25	47.93 ^b ±0.15	54.40 ^d ±0.26

Results are the means of three replications. Values carrying different superscriptions in the same row are significantly different ($P < 0.05$)

3.6 Physicochemical properties of malt-based syrups

Table 3 shows the physicochemical properties of malt-based syrup samples produced from sorghum and millet malts. Sorghum sample has significantly $p < (0.05)$ higher total solids, dextrose equivalent (DE), and total reducing sugar contents when compared with millet sample. While millet sample has significantly $p < (0.05)$ higher ash and moisture contents than sorghum sample. Ash (0.02 and 0.04% for sorghum and millet respectively) and moisture (12.35 and 13.46% for sorghum and millet respectively) contents of the syrups are within the Standard Organization of Nigeria [50] specification (0.03 and 18% max for sorghum and millet respectively). The value of 43°Be density was obtained for malt syrups. The higher value may be due to its relatively higher content of cellulosic materials.

The pH of the samples varies between 4.5 and 5.0. Foods at a pH range, 4.4-5.0 are medium acid foods and they resist to a very large extent microbial activity. Percentage of the total solids content of the syrup samples are millet (80.21) and sorghum (82.20%). The maximum total solids for this kind of product is >70 as reported by [51]. The DE of the syrups varies from 81.60 to 85.52%. The minimum recommended DE for glucose syrup is >20 . The high DE value was as a result of the three-stage decoction mashing, further hydrolysis of wort by amyloglucosidase and duration of saccharification process.

High reducing sugar values for millet, 65.45 % and sorghum, 70.30 % were obtained (Table 3). This could be attributed to the conversion of amylose and amylopectin portions of starch by α -amylase to a collection of linear and branched dextrans during mashing processes. The linear dextrans are rapidly and almost totally converted to D-glucose by glucoamylase enzyme. The branched dextrans are much less susceptible to hydrolysis [52]. The samples were concentrated at the same time and temperature. Physical check on the syrup samples shows that sorghum sample was more viscous than millet sample. The samples were sweet, brown, viscous liquid with caramel odour.

Table 3 Physicochemical properties of malt-based syrups

sample Property	Syrup sample	
	Sorghum	Millet
Ash content (%)	0.02 ^a ±0.003	0.04 ^b ±0.003
Moisture (%)	12.35 ^a ±0.13	13.46 ^b ±0.25

Total solids(%)	82.20 ^a ±0.10	80.21 ^b ±0.12
Degree Baume(^o Be)	43.00 ^a ± 0.13	43.00 ^a ±0.10
Dextrose equivalent	85.52 ^a ±0.26	81.60 ^b ±0.30
Total reducing sugar (glucose)(%)	70.30 ^a ±0.26	65.45 ^b ±0.37
the pH of	4.5 ^a ±1.20	5.0 ^a ±1.40
Physical check		
Viscosity	viscous	more viscous
Colour	brown	brown
Taste	sweet	sweet

Results are the means of three replications. Values carrying different superscriptions in the same row are significantly different (P<0.05)

4. CONCLUSION

The broad objective of the present study was to produce malt-based syrups principally from locally available cereal grains for industrial applications. Malting and malt's quality characteristics of the grains studied indicated that sorghum generated better malt than millet. Fifty hour (50 hr) of steeping and 5 days of germination at room temperature gave the optimum diastatic power development in sorghum (32 °L) and millet (27 °L). Thicker mash, further hydrolysis with amyloglucosidase and longer saccharification period yielded high DE values for sorghum (85) and millet (81) syrup samples. The method of malt-based syrup production presented in this study, when adopted by small and medium entrepreneurs will go a long way in creating wealth and conserve foreign exchange spent on glucose syrup importation. Malt syrup, as well as glucose syrup, **has** existing markets in food and pharmaceutical industries based in Nigeria.

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