Studies on the Malting Parameters of Two Acha Species *Digitaria exilis* and *Digitaria iburua*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article	

ABSTRACT

The malting parameters of two acha species (*Digitaria exilis*-white and *Digitaria iburua*-black), a small annual herbaceous indigenous African cereal grain were studied. This study was aimed at assessing the brewing potential of the two acha species. The research was carried out in Microbiology Laboratory of Nnamdi Azikiwe University, Awka. The brewing properties assessed were; moisture content, cold water extract, hot water extract, diastatic power, total nitrogen, cold water soluble protein, total soluble protein and free amino nitrogen. Extraction, digestion, titration, colorimetric and spectrophotometric methods were used to assess the above brewing properties. The moisture content of the white species malt (11.0 - 12.4%) was higher than that of the black (10.3 – 11.2%) and increased with germination time but decreased after the peak (4th) day. The lower moisture content of the black acha malts gave a resultant effect of more extract production from cold water extract (CWE 27.5, 30.10%), hot water extract (HWE 278, 304°l/kg) and diastatic power (DP 19 ,23°L) of the two acha malts on the fourth (peak) day of germination. The peak values

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for total nitrogen (1.6, 1.75%), cold water soluble protein (120, 128%), total soluble protein (6.2, 7.5%) and free amino nitrogen (FAN 105, 114.5%) were also higher for the black acha on the peak day of germination. The correlation studies between the HWE (R^2=0.9903, 0.9551), Cold water soluble protein (CWS-P- R^2= 0.9809, 0.9285) and Diastatic power for the black and white species, respectively, were positively significant depicting a strong relationship between the correlated parameters. The black acha malt recorded more extract yield than the white acha and the result of the two acha species assessed showed that they possess brewing properties.

1. INTRODUCTION

Acha grains are mostly consumed whole, perhaps because of their small size [1]. Whole grain cereals have been found to be good sources of nutritionally valuable substances such as dietary fiber, antioxidants and minerals [2-4]. A wide range of these compounds is affected by germination, while some compounds, such as beta-glucans are degraded, others like starch proteins and vitamins can be increased by means of malting [5-7]. Therefore, germination and malting of cereals is a way not only to produce fermentable extract for the brewing and distilling industries, but can also be a way to produce ingredients enriched with health promoting compounds [8,9]. Acha grains have considersble potential in foods and beverages [10]. The low starch gelatinization temperature and high beta-amylase activity of acha shows the brewing potential of acha grains in partial substitution of barley malt [11,12] .Thus, this study is a comparative analysis aimed at determining the malting parameters of two species of acha.

2. MATERIALS AND METHODS

2.1 Acha Species Used

The acha species used in this study are white (*Digitaria exilis*) and black (*Digitaria iburua*). They were bought from a local market in Onitsha, Anambra State, Nigeria and identified by the Botany Department, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria.

2.2 Grain Sorting and Cleaning

The grains were thoroughly cleaned and surface sterilized with 1% (v/v) sodium hypochlorite to check for microbial contamination. Afterwards, grains were subsequently washed and drained.

2.3 Steeping and Germination of Grains

One kilogram of each acha species was steeped in water at room temperature for 12 hours followed by 4 hours air rest and further 12 hours wet-steep. Grains were germinated for 6days and were daily turned and sprayed with water to avoid matting and also ensure equal germination. Thereafter, grains were kilned at 45°C for 24 hours and samples were stored for analysis.

2.4 Moisture Content Determination

The European Brewery Convention method was adopted [13].The percentage moisture content of the sample was calculated using the formula

$$M = \frac{W1 - W2}{W1} \times 100$$

Where

W1 = weight of sample before drying
W2 = weight of sample after drying

2.5 Determination of Germinative Capacity

As described by EBC method [13], 100 g grains from each variety were steeped in 100ml of 0.375% (v/v) hydrogen peroxide for two days. After steeping, the liquor was drained off and replaced with fresh 100ml of water for 24h after which the water was drained off and the number of germinated grains counted. The germinative capacity was determined using the formula

$$G.C. = 100 - n + \frac{d}{2}$$

Where

n = grains that did not germinate
d = damaged grains [13].

2.6 Determination of Germinative Energy

The Institute of Brewing method was used. Germinative energy was determined as the

percentage of the grains that germinated at the end of 72 hours of germination [14].

3. MALT ANALYSIS

3.1 Determination of Malting Loss

Ten grams (10g) of Acha from each species was weighed before and after malting. The loss in weight was determined as the difference between the unmalted grain and the malted ones. Malting losses were then calculated using the formula:

$$\text{Malting loss (\%)} = \frac{W1 - W2}{W1} \times 100$$

W1 = Weight of unmalted grains
W2 = weight of malted grains [15].

3.2 Determination of Cold Water Extracts (CWE)

The cold water extract of malted acha varieties was determined using (10g) ten grams of ground malts suspended in 200ml of distilled water containing 12ml of 0.1M ammonia solution for 3hrs with stirring every 30mins at room temperature [14]. Cold water extract was determined using the formula:

$$\text{CWE (\%)} = \frac{G}{3}.86 \times 20$$
G = 1000 (S.G -1)
G= Gravity; S.G = Specific gravity

3.3 Determination of Hot Water Extracts (HWE)

The hot water extract which is a measure of dissolved materials in wort was prepared by infusion mashing method [13]. The extract yield was obtained from the relation:

$$HWE = G \times 10.13$$

Where

G =1000 (S.G – 1)
G= Gravity; S.G = Specific gravity

3.4 Determination of Diastatic Power

Infusion extract of the malts were prepared and 3ml aliquots of the extracts were separately pipetted into 100 ml of 2% 0.1M citrate phosphate buffer starch solution in 200ml flasks.

The mixtures were shaken and maintained at room temperature for 1hr from the time the aliquots were added. At the end 30ml of 0.1M NaOH was added to stop the reaction and total volume raised to 200ml with distilled water. The diastatic power of the malts was then determined by titrating the starch digests against 5ml of Fehling's solution (i.e. equal volumes of solution A and B mixed together) contained in a 150ml conical boiling flask. The flask contents were boiled as titration continued until the blue colour of Fehlings solution discharged. Three drops of 1% (w/v) aqueous solution of methylene blue were added and the titration and boiling continued to the end point when the methylene blue was decolourized and the reaction mixture became bright red. The diastatic power (D.P) in degrees linter (^{0}L) was calculated from the relation [15].

$$D.P = \frac{2000 - 200}{Xy - Xs}$$

Where

x = no of ml of malt extract used in digesting starch
Y = no of ml of starch digest used in titration
S = titre for starch blank

3.5 Total Nitrogen Determination of Acha

The total nitrogen of the malt was determined using the Kjhedahl method. The percentage nitrogen in dry acha (N) is determined using the formular

3.6 Determination of Total Soluble Nitrogen

The total soluble nitrogen (i.e. the amount of the total nitrogen solubilized during mashing) was also determined by Kjhedahl method described in total nitrogen determination. The total soluble nitrogen of the sample was calculated as follows:

$$TSN = \frac{titre\ (ml) \times 14.008}{DM}$$

Where

TSN = total soluble nitrogen content of malt in % dry matter
1.0 ml of titre = 1.4008 mg nitrogen.
DM = Dry matter

3.7 Determination of Cold Water Soluble Protein (CWS-P)

The soluble protein in the cold water extract was determined using Biuret reagent and expressed as: mg CWS-P% dry matter [1]. The biuret reagent was prepared by dissolving 0.3 g sodium potassium tartrate in 300 ml of 0.5 M potassium hydroxide and 200ml of 0.024M copper sulphate pentahydrate. The mixture was diluted with 500 ml of iso-propanol and kept in amber bottle. About 0.5 ml of the reagent was added to 25 ml of the sample, mixed and Incubated in a water bath at 40^0C for 30 mins and cooled at room temperature for 5 mins. The absorbance was read within 30 mins at 550 nm against a blank without copper sulphate in the solution The CWS-P was calculated using the formula

CWS-P (%) = 0.855 x Abs. at 550 nm
CWS-P = Dry matter

3.8 Determination of Free Alpha Amino Nitrogen (FAN) (Ninhydrin Method)

Two milliliters of clear sample solution was mixed with 1ml of ninhydrin solution (coloring agent) stoppered and heated in a boiling water bath for 16mins. After cooling for 20mins, 5ml of diluting reagent (potassium iodate solution) was measured at 570nm within 30mins. A standard glycine solution was treated at exactly as test solution. Alpha amino nitrogen (mg/litre) was calculated from the relation:

$$\frac{\text{OD of test solution} \times 2 \times \text{dilution factor}}{\text{OD of standard solution}}$$

4. RESULTS AND DISCUSSION

4.1 Analysis of Unmalted Grain

The moisture content (8%, 5%) of the two grains are within the ranges reported for acha grains and for sorghum varieties [1]. The germinative energies (96%, 92%) and germinative capacities (95%, 90%) fell within the range reported for barley [16], showing that the acha grains has high malting potential.

4.2 Malting Properties of Acha Varieties

The moisture content of the two malts is shown in Fig. 1. The black acha malt had less moisture content than the white acha malt as germination days progressed. This observation is in line with the report by Rourke [17] that darker malts tend to have lower moisture contents and this results in more or higher extract yield. Thus, the higher the moisture content of grains, the less their extracts yield per kilogram. The cold water extract of the two malts (27.5%-white,30.10%-black) on the fourth day of germination were similar to that recorded for sorghum varieties [18] and less than that recorded for maize [19] but higher than the value (17-20%) recommended for well modified barley [14].

Hot water extract value is one of the most important measurements in judging malt quality. It measures the soluble materials from the malt when some hydrolytic enzymes have acted optimally. The hot water extract of the black acha malt on the 4th day of germination (278%-white, 305%-black) is in accordance with the specified range recorded for well modified barley malts [14] and the two malts recorded a similar result obtained with that of two millet varieties [20].The diastatic power of the two acha malts increased with germination time (days) and decreased after the peak day (4th day) thus, showing the amylolytic activity of the malts.

Figs. 5 & 6 show the correlation between hot water extract and diastatic power. The results recorded correlation values of $R^2 = 0.991$, and $R^2 = 0.948$ for white and black acha malts respectively. The high values of correlation factor R^2 show that the two species have good potential of producing high quality malt. Thus, there is a strong relationship between hot water extract and diastatic power.

Malting loss occurs as a result of material loss in weight due to respiration by the embryo. In Fig. 7, the malting loss of the two varieties increased as germination days progressed.

The total nitrogen and protein represented in Figs.6 and 7 show that on the 3rd and 4th day of germination, the acha malts produced recommended values. Usually, the recommended percentage of nitrogen required in brewing is between 1.4 to 1.8% [14]. The result is also in accordance with that reported for sorghum varieties [21].

The total soluble protein is the fraction of protein that is water soluble. It is also expressed as a percentage. The soluble nitrogen (protein) is part of the measure of the level or degree of modification. The greater the value of soluble nitrogen, the higher the modification. The values recorded (6.5%-white, 7.5%-black) were higher than that reported for white acha [22].
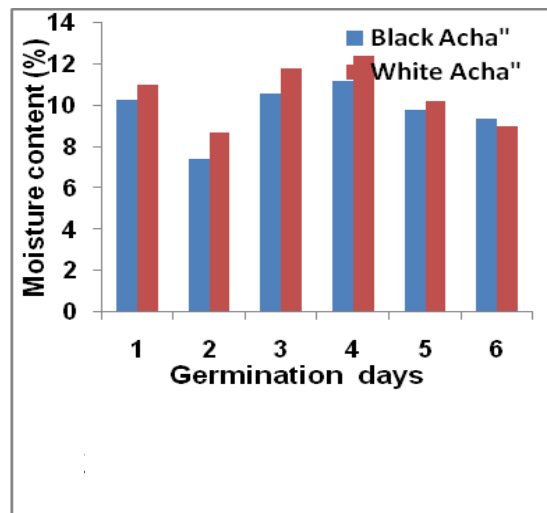
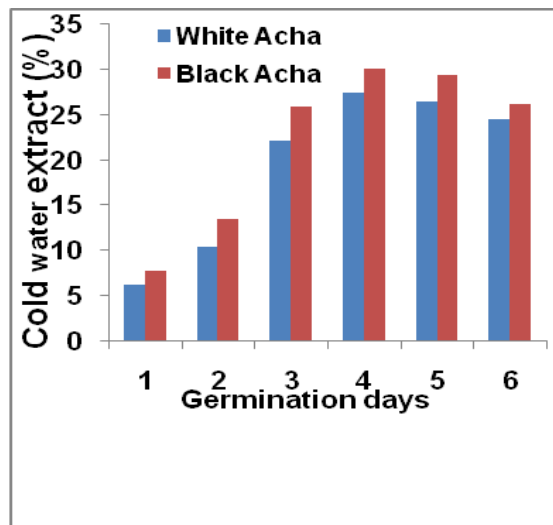**Fig. 1. Mosture content of Acha malts with germination time**

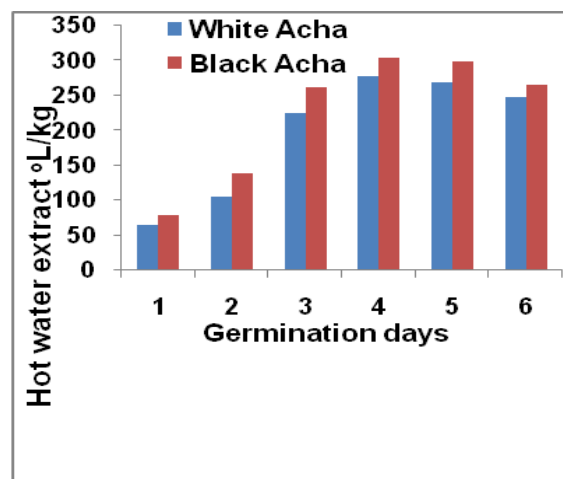**Fig. 2. Cold water extract of Acha malts with germination time**

Fig. 3. Hot water extract of Acha malts with germination time
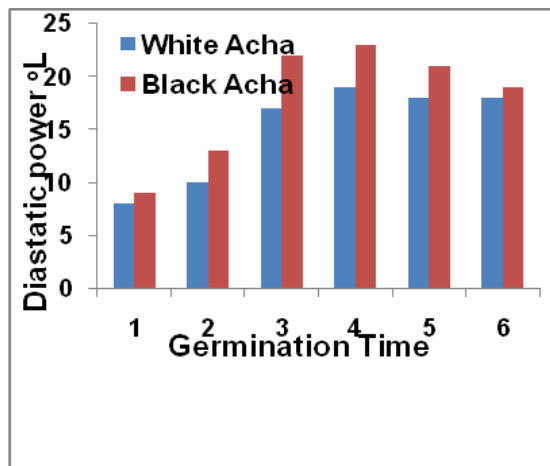
Fig. 4. Diastatic power of Acha malts with germination time
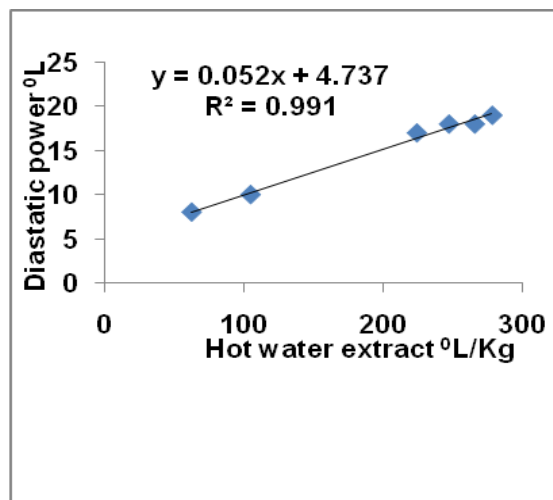

Fig. 5. Correlation analysis of hot water extract and diastatic power of white acha specis
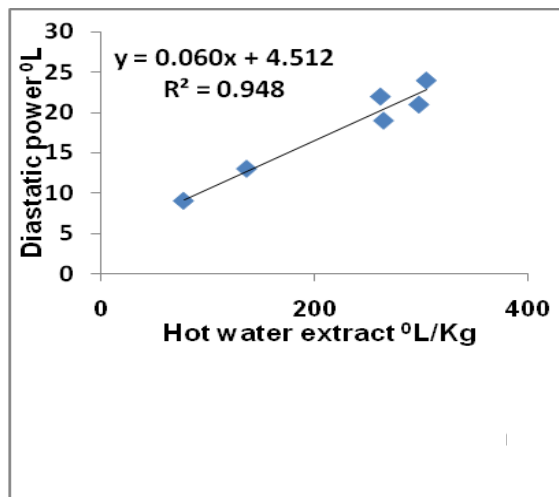

Fig. 6. Correlation analysis of hot water extract and diastatic power of black acha specis

Cold water soluble protein is important as a measure of the level of proteolytic activity during malting [23]. The cold water soluble nitrogen comprises high molecular weight products of the proteinase activity during grain germination [19]. The cold water soluble protein production by the two species increased along the germination days and reduced after the 4th day with the black species giving a higher value. The higher value of CWS-P of the black species is probably the reason for the corresponding increase or release of more soluble nitrogen (protein). This also indicates or supports a higher proteolytic activity of the black acha species.
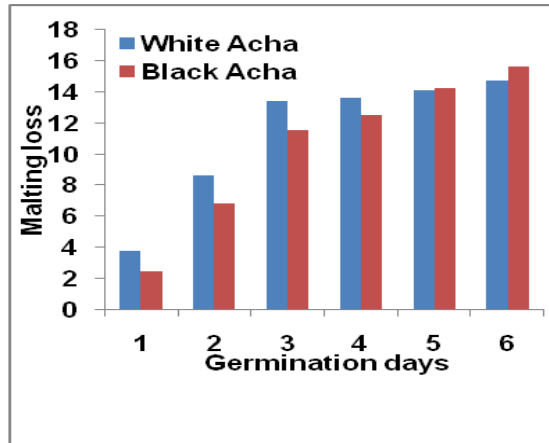
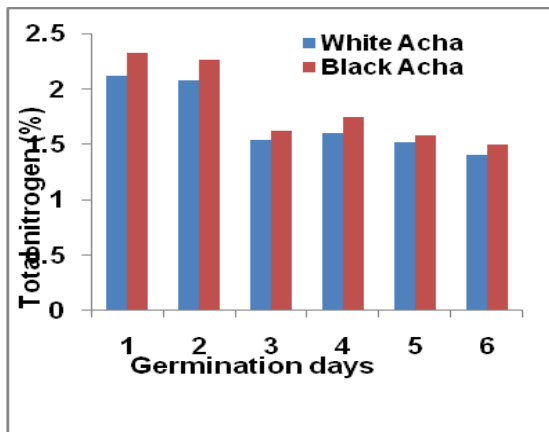**Fig. 7. Malting loss of Acha malts with germination time**

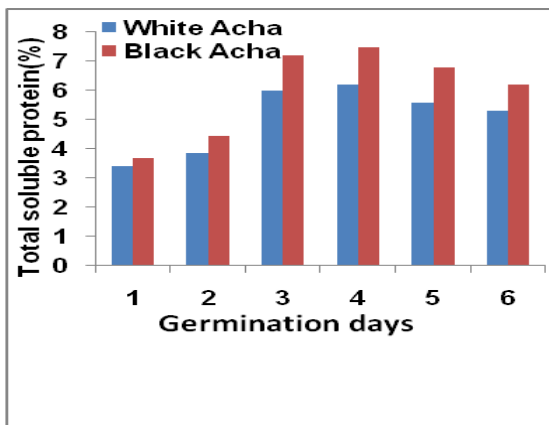**Fig. 8. Total nitrogen of Acha malts with germination time**

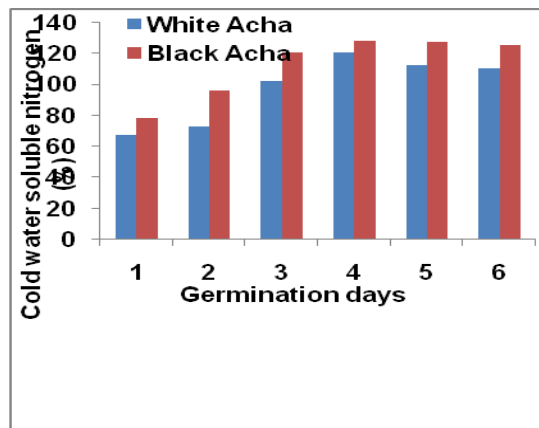**Fig. 9. Total soluble protein of Acha malts with germination time**

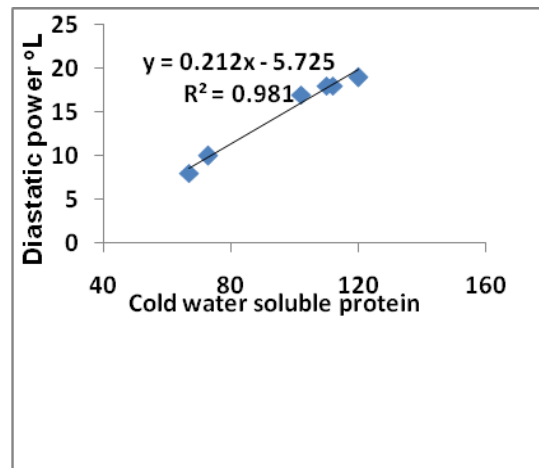**Fig. 10. Cold water soluble nitrogen of Acha malts with germination time**

Fig. 11a. Correlation analysis of hot water soluble protein and diastatic power of white acha specis
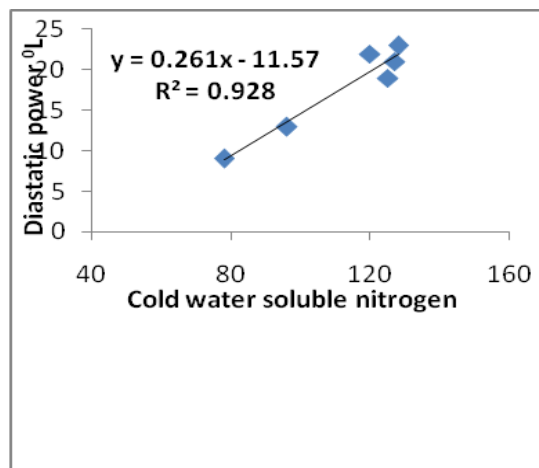
Fig. 11b. Correlation analysis of cold water soluble protein and diastatic power of black acha specis
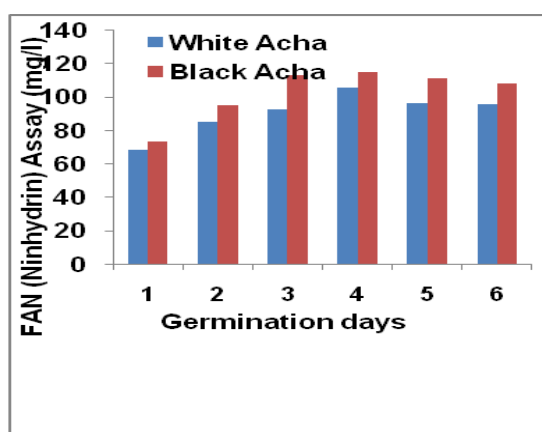
Fig. 12. FAN (Ninhydrin) assay of Acha malts with germination time

The free alpha amino nitrogen is a measure of the concentration of individual amino acids and small peptides which can be utilized by yeast during fermentation. The two acha malts produced FAN values (105-115mg/l) which fall within the range recommended for typical malt. The result also gave similar ranges with that recorded for rice germinated at 25°C [24].

5. CONCLUSION

This study has shown that Acha has potential brewing properties that are yet to be exploited. Despite its small size which poses a threat to its usage in commercial brewing (for example, being a substitute grain in brewing), its indicated malt qualities can be enhanced and used as a minority malt or adjuncts in brewing industries, thus reducing the cost of the bulk grains (sorghum, maize).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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