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Raw grain characteristics and endoglucanase activity of sorghum (*S. bi-color*) and millet (*Pennisetum typhoides* and *Digitaria exilis*) malts

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Abstract: *Endoglucanase activity has been a major concern in malting process due to the need to de-polymerize the high molecular weight cellulosic material. Sorghum and millet varieties were screened for their physicochemical properties and endoglucanase activity. Effect of plant hormone (gibberellic acid) on the production of endoglucanase during germination was also investigated. All the varieties screened apart from fonio-zegla, showed negligible water sensitivity, high germination energy and desirable germination capacity. Endoglucanase activity increased with the malting time and this was associated with malt modification during germination. The endoglucanase activity was optimal on the 3rd day for sorghum and on the 4th day for millet. Application of gibberelic acid enhanced endoglucanase activity. Composite malt from sorghum and millet is recommended for brewing, weaning foods, animal feeds and other industrial purposes.*

Keywords: *endoglucanase, germination energy, gibberellic acid, malting, millet, sorghum*

I. Introduction

The need to reduce the high import cost of barley as well as improve the extract yield and nutritive value of weaning foods, animal feeds and bakery products have necessitated research into local cereals like sorghum and millet [1], [2]. Malting process has been used for centuries to impact distinctive flavours and colours and most importantly to promote solubilization of starch and proteins, thus improving the acceptability, digestibility and nutritive value of the grains [3]. These effects arise from the activation of hydrolytic enzymes such as α-amylase, cellulase, lipase and protease, which are stimulated in the aleurone cells and scutellum during germination [4], [3] by the action of gibberellic acid [5], [6], [7]. Cellulase is a β-glucan degrading complex enzyme system consisting of three major components: endo-β-glucanase (EC.3.2.1.4), exo-β-glucanase (EC.3.2.1.91) and β-glucosidase (EC.3.2.1.21) [8], [9]. The three enzymes act together to decompose the cell wall cellulosic biomass thus opening doors for other hydrolytic enzymes to penetrate into the endosperm and have access to the nutrient reserves. Endo-β-glucanase which is also called 1,4-β-D-glucan glucanohydrolase or CMCase randomly cleave the internal β-glycosidic linkages of cellulosic chains, yielding glucose and cello-oligosaccharides, hence a rapid decrease in polymer length and gradual increase in the reducing sugar concentration [10], [11]. Endoglucanase play key role in increasing the yield of fruit juices, oil extraction, improving the nutritive value of weaning foods, animal feeds and bakery products [1].

Gibberellic acid also known as gibberellin is a tetracyclic di-terpenoid plant hormone which stimulates seed germination, promotes growth and development of cells [12]. Application of gibberellic acid at very low concentrations has been noted to increase the stimulation of hydrolytic enzymes like α-amylase in finger millet [13], cause several fold increase in protease activity of malted barley [14] and diastatic enzymes in millets and sorghum [15], [16]. Brigs and MacDonald discovered in their study of the pattern of modification in malting barley, that 6ml of 0.2 mg GA_3/kg grain sprayed one hour after steep out, elevated the amount of cell wall degrading enzymes from aleurone layer and contributed to the rapid modification rates of the grain [17].

Sorghum and millet malts are used in Africa in the production of weaning foods, animal feeds, bakery products, local beer and alcohols like kaffir beer, burukutu and opaque beer which contain undegraded starch thereby reducing the product yield and nutritive quality [18], [13]. Sorghum and millet are the most drought-tolerant grain crops of the drylands, with early maturity and serves as staple food and feed during the hunger season. *Sorghum bicolor* is valued for its relatively high protein content among the cereal grains [19]. Sorghum though the fifth most important cereal in the world after wheat, rice, maize and barley [20] is the most under-utilized grain of the semi-arid Africa [21]. *Digitaria exilis* commonly called 'acha' or 'hungry rice' in Northern Nigeria grows well on poor, sandy or ironstone soil in areas of low rainfall [22] and has been reported to have high brewing and malting potentials [15].

This study was aimed at improving grain modifications and development for Industrial purposes with focus on the key enzyme endoglucanase. Some varieties of sorghum and millet were investigated for malting characteristics and endoglucanase activity and the effect of gibberellic acid on the endoglucanase activity determined.

II. Materials And Methods

2.1 Materials

Three varieties of sorghum (*sorghum bicolor*; 'white farafara', yellow sorghum – 'kaura' and red sorghum – 'jardawa') and two varieties of pearl millet *(Pennisetum typhoides*; 'dauro' and 'gero') were purchased from Samaru market Zaria. Two varieties of fonio millet (*Digitaria exilis*; 'fonio bhull' and 'fonio zegla') were obtained from Chori, Kaduna State. The name bhull and zegla are based on their sources while the others are their Nigerian local names. Materials were identified using breeder's characteristics.

2.2 Preparation of the raw grains

About 1.50 kg of each of the samples were weighed and cleaned to remove dirt, stones and broken kernels. 50 g of each sample was milled into powdered form using a Thomas Wiley Laboratory mill and then stored safely prior to analysis.

2.3 Determination of raw grains characteristics

2.3.1 Proximate compositions of the grains were determined according to the methods of Association of Official Analytical Chemists [23].

2.3.2 Germination energy and germination capacity

The method of American Society of Brewing Chemists was adopted [24]. One hundred grains of each of the samples were distributed in a compact single layer in the centre of one-half of moistened (with distilled water for 5 seconds and drained) Whatman filter paper placed on a glass sheet. The other half of the filter paper was used to cover the grains before placing in desiccators containing water at the bottom to maintain humidity near saturation. After 72h, the percentage of the sprouted kernels was reported as germination energy. Kernels that showed no evidence of growth were replaced between damp filter paper and returned to the desiccators for additional 48h. At the end, kernel showing evidence of germination were counted and added to the number of kernels germinated within 72h after steeping. The sum was recorded as percentage germination capacity.

2.3.3 One thousand kernel weight and malting loss.

The analysis of the Institute of Brewing [25] was adopted for the determination of one thousand kernel weight and malting loss. One thousand grains of the different samples were counted and weighed in triplicates before malting and after kilning. The difference between the weight of the resulting malt and the original grains was calculated as a percentage of the grain weight to give malting loss.

2.4 Malting procedure

The malting procedure of [26] was employed with few modifications. Steeping was at room temperature ($28\text{-}30^0$C). Some samples were treated with 8 ml of 2.5 µg/l gibberellic acid (GA_3) after steep out. Samples were withdrawn from each variety at 0, 2, 3, 4 and 6 day and kilned at 50°C until the rootlets could be removed by hand (between 16-24h).

2.5 Preparation of extracts

5 g of the pulverized samples were dissolved in 10 ml of 0.05 M citrate buffer at pH 4.8 and thoroughly shaken (150 oscillations per minutes) at 4°C for 1h. The slurry were centrifuged twice at 3000 rpm for 10 min and then filtered through whatman No.1 filter paper. The crude extract was dialyzed against 3 volumes of the same buffer for 48h with three changes.

2.6 Enzyme assay procedure

Enzyme assay was by the DNS method as described by [27]. The reducing sugar was determined as glucose by reading at 540 nm, with a blank in a spectrophotometer. Blanks of buffer enzyme without substrate and substrate without enzyme were used. All assays were in triplicates.

III. Results And Discussion

The summary of the raw grain characteristics and proximate compositions of the various varieties of sorghum (white farara, kaura and jardawa), pearl millet (gero and dauro) and fonio millet (bhull and zegla) are presented in table 1.0. One thousand kernel weights of the grains ranged from 0.45g in fonio-zegla to 38.21g in

kaura. The result showed significant ($p<0.05$) differences in 1000 kernel weight of samples among the groups and within the representative groups of sorghum, pearl millet and fonio millet. The 1000 kernel weight of sorghum varieties are within the range (22–50 g) observed in barley but that of pearl millet and fonio millet fall far below the range [15]. Therefore the 1000 corn weight in the range 0.45-8.23g observed in pearl millet and fonio varieties is very small and suggests that the cereals have tiny grains compared to sorghum and barley.

Table 1.0: Raw Grain Characteristics

S/No	Sample	1000 Kernel Wt. (g)	Moisture Content (%)	Protein Content (%)	Lipid Content (%)	Ash Content (%)	Carbohydrate content (%)	Germination Energy (%)	Germination Capacity (%)
1	**'White farafara'**	36.30 ±0.05[a]	12.10 ±0.02[a]	8.90 ±0.00[a]	3.21 ±0.05[a]	1.77 ±0.02[a]	73.55 ±0.04[a]	95 ±0.47[a]	98 ±0.47[a]
2	**'Kaura'**	38.21 ±0.25[b]	12.60 ±0.11[b]	9.09 ±0.05[a]	2.63 ±0.02[b]	1.56 ±0.01[b]	74.05 ±0.08[b]	95 ±0.47[a]	97 ±0.47[b]
3	**'Jardawa'**	30.08 ±0.20[c]	10.80 ±0.08[c]	7.84 ±0.12[b]	3.79 ±0.02[c]	1.96 ±0.02[c]	75.72 ±0.09[c]	91 ±0.94[b]	94 ±0.00[c]
4	**'Gero'**	6.57 ±0.16[d]	12.00 ±0.05[a]	10.77 ±0.07[c]	4.03 ±0.01[d]	1.83 ±0.05[a]	71.46 ±0.08[d]	91 ±0.94[b]	96 ±0.00[d]
5	**'Dauro'**	8.23 ±0.19[e]	11.50 ±0.02[d]	11.30 ±0.05[d]	3.50 ±0.08[e]	1.57 ±0.00[b]	70.86 ±0.07[e]	93 ±0.47[c]	98 ±0.00[a]
6	**Fonio (Bhull)**	0.46 ±0.01[f]	9.76 ±0.05[e]	9.88 ±0.02[e]	4.08 ±0.05[d]	1.97 ±0.01[c]	74.29 ±0.03[f]	95 ±0.47[a]	99 ±0.00[e]
7	**Fonio (zegla)**	0.45 ±0.00[f]	10.10 ±0.10[f]	7.82 ±0.18[b]	4.37 ±0.05[f]	2.16 ±0.05[d]	75.80 ±0.07[c]	69 ±0.05[d]	73 ±0.00[f]

Values are mean ± standard deviation of triplicate determinations. Values with different superscripts per column are statistically significant ($p<0.05$).

The percentage moisture contents of the samples ranged from 10.00% to 13.00%. The values varied significantly among the groups except farafara and gero. Moisture percentages of less than 13% are within the range in which the grains can safely be stored before malting [28]. Varieties of pearl millet had the highest protein contents and lowest carbohydrate contents. The lipid composition of kaura was significantly the lowest. The ash contents of all the samples were approximately 2.0%. Proximate analysis (%protein, %lipid, %ash) is consistent with the values obtained in barley and those required for malting; (<13% protein, <5% lipid and 2.5% ash). Low protein content preferably between 8.0-10.5% dry matter is preferred for malting barley [29]. Generally, low protein content results in high starch content and consequently high sugar content in the final malt. During malting and mashing, proteins are partly degraded to amino acids and soluble peptides, which are needed as nutrients for yeasts and to produce good foam in beer. High percentage protein may retard water up-take during steeping and result in high soluble protein content in wort, which may lead to problem of haze formation in beer. Though, higher protein content is desired for animal feeds [29]. High lipid content affects the foam quality and beer staling [30].

Apart from fonio-zegla, other samples showed negligible water sensitivity and had high germination energy indicating that more than 90% of the grains germinated under the conditions of the test (Table 1.0). Germination energy is the percentage of the grains that will germinate under the conditions of a specified test while germination capacity is the percentage of living grains in the sample [28]. Germination capacity of the samples except fonio-zegla is within the range of 95-100% desirable for malting [13]. Low germination energy in fonio-zegla might be related to drowning of the grains within the 18 hours of steeping process or poor preservation or the nature of the variety. The difference between germination capacity and germination energy is a measure of the percentage dormancy of sample grains [28]. The study results showed a close range between germination energy and germination capacity.

Malting loss of the samples significantly ($p<0.05$) increased with the germination time (Table 2.0) and was highest in *Digitaria exilis* ('bhull'), on the sixth day. The loss in dry weight is mainly due to increase in metabolic activity and partial degradation and utilization of carbohydrate material [31]. The gradual increase in malting loss observed in sorghum and pearl millet varieties is an indication of a gradual loss of the dry matter. 'Kaura' and 'gero' had the best dry matter conservation, a quality that is desirable in malting. The low malting loss in fonio-zegla compared to fonio-bhull may be due to poor germination. The percentage malting losses shown by all the varieties (except fonio-bhull (39.3%) on the fourth day of germination are within the range reported for barley (23%), wheat (22.25%) and corn malts (24%) [13], but lower than 32.5% reported for ragi (finger millet) by Nirmala *et al*. [31]. The high loss in fonio-bhull on the fourth day and other varieties on the sixth day of germination are consistent with values (32.1-41.7%) reported for some varieties of fonio millet [16]. The high loss in fonio millet is probably due to the considerable length of the radicles compared to the grain dimension as suggested by Nout and Davies [32].

Table 2.0: Percentage malting loss of samples dried at 50°c after steeping

After steeping	'White farafara'	'Kaura'	'Jardawa'	'Gero'	'Dauro'	Fonio-Bhull	Fonio-Zegla
Day 0	3.1 ±0.11[a]	2.9 ±0.30[ab]	4.6 ±0.15[c]	2.6 ±0.12[b]	5.9 ±0.14[d]	4.5 ±0.00[c]	2.0 ±0.00[e]
Day 2	10.8 ±0.12[a]	9.9 ±0.10[b]	11.9 ±0.29[c]	7.7 ±0.09[d]	15.4 ±0.08[e]	13.2 ±0.01[f]	6.9 ±0.01[g]
Day 4	23.2 ±0.20[a]	16.4 .±0.16[b]	17.9 ±0.00[c]	14.3 ±0.19[d]	29.9 ±0.08[e]	39.3 ±0.01[f]	20.2 ±0.05[g]
Day 6	39.7 ±0.30[a]	34.8 ±0.05[b]	26.0 ±0.05[c]	27.4 ±0.12[d]	42.0 ±0.05[e]	51.3 ±0.00[f]	33.6 ±0.00[g]

Values are mean ± standard deviation of triplicate determinations. Values with different superscripts per row are statistically significant ($p<0.05$).

Endoglucanase activity of the different malts had negligible activity (<0.08 absorbance) with DNS assay method and CMC as substrate despite the day of germination and quantity of enzyme flour (0.05 – 5.0g/ml) till the extracts were dialysed. The low activity was suspected to be due to product inhibition by glucose or cellobiose. After dialysis, there was profound activity in the various malts with 'kaura' having the highest activity followed by 'dauro' (Fig 1.0). Based on the enzyme activities, 'kaura', 'dauro' and fonio-bhull were selected from the representative group and malted for six days to determine the effect of germination time endoglucanase activity. Fig 2.0 showed that enzyme activity increased with malting time and was maximum on the 3rd day in 'kaura' and 4th day in 'dauro' and 'bhull'. The sharp decline on the 3rd day in 'kaura' is an indication of early and rapid modification which is desirable for malting [26]. Enzyme activity in 'dauro' slowed down on the 3rd possibly because of change in room temperature. Further study on the purified endoglucanase from 'dauro', 'kaura' and 'fonio-bull', showed a sharp decrease in enzyme activity after the optimum temperature in 'dauro', unlike the gradual decrease noted in 'kaura' and 'fonio-bhull' [2].

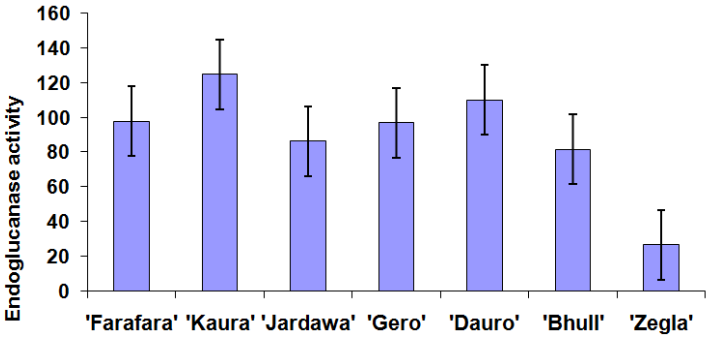

Fig. 1.0: **Screening of samples based on Endoglucanase activity after Dialysis**

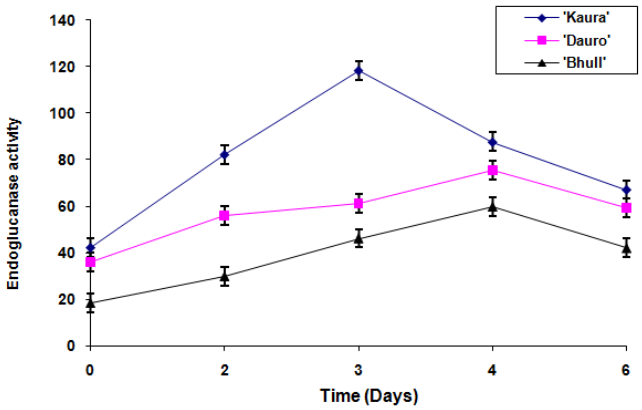

Fig. 2.0: **Effect of germination time on endoglucanase activity**

The results of the samples that were malted with gibberelic acid are presented in fig 3.0. The application of gibberellic acid caused an increase in the reducing sugar of the selected samples, indicating accelerated development of endoglucanase activity by gibberellic acid treatment. Modification was significantly

correlated ($r \geq +0.99$, $p=0.01$) between the sample groups. The effect on 'dauro' was more significant ($p=0.27$) compared to those of 'kaura' and fonio-bhull. Thus hormones such as gibberellic acid can be used to enhance modification and product yield. The hormone did not push forward the optimum time of germination in these cereals as reported for α and β-amylases of finger millets [32].

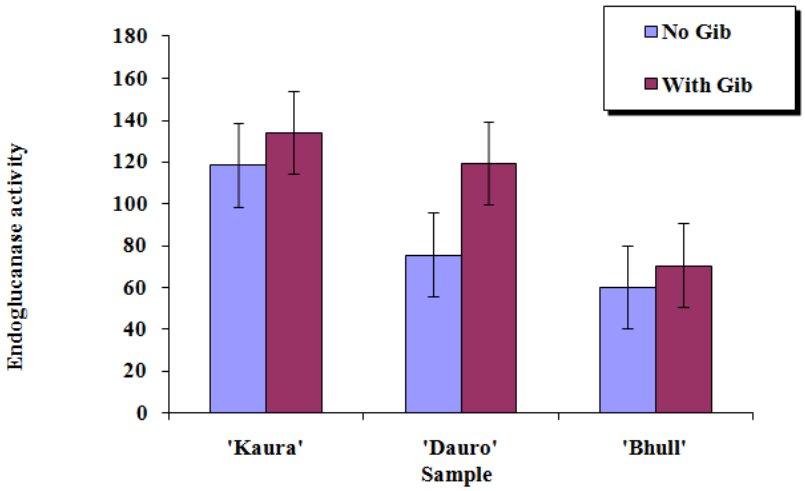

Fig.3.0: Effect of Gibberellic treatment on the highest day ('kaura'-3rd day; 'dauro' and fonio-bhull - 4th day) of Endoglucanase activity.

IV. Conclusion

The results of the raw grain characteristics as well as the endogucanase activity of sorghum and millet varieties indicated that 'kaura', 'dauro' and fonio-bull had better characteristics within their various groups, but 'kaura' and 'dauro' were found to be more suitable for malting process than fonio-bhull. Application of gibberellic acid enhanced endoglucanase activity of the cereals. A blend of sorghum and pearl millet malt is recommended for high quality malt.

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