Selected Anti-nutritional Factors and *In-vitro* Protein Digestibility of Some Sorghum Types as Influenced by Germination Time During Malting

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Abstract: The effect of germination time, during malting, on phytate and tannin concentration as well as on the *in-vitro* protein digestibility of some sorghum types was investigated. The increase in the germination time led to a decrease in the phytate content from the initial range of 1004.6-1097.4 mg/100g, at zero-hour germination, to a range of 457.2-626.3 mg/100g at 48-hour germination time. A further decrease in the phytate content was obtained at 96-hour germination time with a range of 215.4 -298.3 mg/100g. A maximum of 71.5-79.2% phytate reduction capacity at 96-hour germination time was attainable with the sorghum types. The increase in germination time also caused the tannin concentration to decrease. The initial tannin concentration (0.23-4.47 mg/g), at zero-hour germination time, decreased to 0.15-3.35 mg/g at 48-hour germination time while a range of 0.13-3.07 mg/g was obtained at 96-hour germination time. A maximum of 25.9-45.8% tannin reduction capacity at 96-hour germination time was attainable with the sorghum types. The *in-vitro* protein digestibility of the sorghum types increased from the initial level (32.1-45.1%), at zero-hour germination time, to 42.8-61.3% at 48-hour germination time while that of 96-hour germination time was 46.7-64.8%. It may be concluded that the decrease in the anti-nutritional factors and improvement in the protein digestibility of sorghum types during the malting process was essentially a function of germination time and sorghum grain variability.

Keywords: Sorghum, Protein Digestibility, Germination, Malting, Anti-nutrient

1. Introduction

Sorghum (*Sorghum bicolor* (L) Moench) is an important cereal grain in the tropical arid and semi-arid zones of the world, and has been noted to be the fifth ranked cereal after wheat, maize, rice and barley in importance [1]. The consumption of sorghum serves as a major source of proteins and calories in the diets of people particularly in Africa [2], thereby meeting their food security requirements. The utilization of sorghum grains includes human food uses, serving as one of the most important weaning food cereals in low-income and high-income countries [3]. Other areas of sorghum usage, particularly in Africa, include the production of many traditional food products such as *ogi, eko, kunnu* and *tuwo* [4], fermented beverages such as *mahewu* [5], *couscous* and *dolo* [6], *injera, kisra* and *ugali* [7], among others. The nutritional quality of sorghum is dictated mainly by its chemical composition while the constraints that limit its utilization as food or feed is the occurrence of anti-nutrients such as phytate and tannin [8]. The phytate has been implicated to cause the essential minerals (Ca, Mg, Mn, Zn, Fe and Cu) not to be bioavailable and can also form a phytate-protein complex thereby interfering in protein utilization [9]. The negative consequence of tannin in sorghum-based diet has to do with its interference with protein digestion by binding dietary protein into an indigestible form [10].

Digestibility of sorghum protein is of immense interest particularly in Nigeria and to many African families who

depend on sorghum as staple food. In such situations, sorghum is often the main source of dietary protein. Another nutritional constraint to the use of sorghum in human and animal diets is the poor digestibility of sorghum proteins on cooking [11]. Certain factors that have been implicated to contribute to poor protein digestibility of sorghum include grain organizational structure [12], possible interaction of sorghum proteins with non-protein components [13], and possible changes within the sorghum proteins themselves and these are disulphide crosslinking of protein molecules [14], racemization of amino acids and isopeptide formation [15]. The scientific efforts that have been made to improve sorghum protein digestibility essentially include the use of reducing agents in sorghum cooking [16], subjecting of sorghum grains to fermentation [17], the use of extrusion cooking in sorghum processing [18], and malting of sorghum grains [10].

In Africa and Asia, there have been series of genetic improvement on sorghum varieties and the areas of focus include that of disease resistance, high yielding capability, yield stability and nutrient enhancement, among others [19, 20]. The use of newly developed sorghum types is therefore very important so as to know the appropriateness of their food and feed usage. The present study was aimed at evaluating the role of germination time, during malting, on selected anti-nutritional factors and in-vitro protein digestibility of different sorghum types.

2. Materials and Methods

2.1. Sources of Materials

Three classes of sorghum grains were used for this study and these include (1) Local sorghum type (*Pelipeli and Kwaya*) obtained from Adamawa Agricultural Development Agency, Yola, Nigeria; (2) Improved sorghum type (SAMSORG-14 and SAMSORG-17) obtained from the Institute of Agricultural Research (IAR), Samaru, Nigeria; and (3) Hybrid sorghum type (Hybrid A and B) sourced from Lake Gerio Research Farm of the River Basin Development Authority (RBDA), Yola, Nigeria. All samples were respectively oven-dried to 8.9-10.2% moisture level at 50°C and then stored in different polyethylene bags at ambient temperature (32±2°C) and 65% relative humidity until required.

2.2. Malting of Sorghum Grains

Experimental samples (300 g) of each sorghum grain type and barley were taken and the malting process follows the procedure of Palmer [21]. The grains were steeped in thrice quantity of water for 12 h with 1h air rest after 6 h of steeping. For each air rest, the steeping water was changed. After steeping, the grains were sterilized by soaking in a solution of 1% sodium hypochlorite for 5 min before it was drained prior to germination. The steeped grains were spread on wet jute bags and covered with moist cotton cloth and left to sprout at room temperature (32±2°C). The phytate, tannin and protein digestibility of the samples were determined immediately at 0 h at the onset of germination and later at 48 h and 96 h germination times respectively as described by Obizoba and Atii [22].

After germination, the grains were dried in Gallenkamp oven (BS model OV-160, England) at 50°C for 24 h. Rootlets and shoots of the grains were separated from the kernels by rubbing the grain in a sieve (Endecotts Ltd, London, England) of 0.6 mm mesh size. The sieve allowed the rootlets and shoots to pass through but retained the kernels [23]. The unmalted and malted sorghum and barley samples were milled into flour with a hammer mill (Gibbons Electric, Essex, UK) to pass through 1-mm mesh size screen into fine flour. The samples were then stored at room temperature (32±2°C) for subsequent analysis.

2.3. Determination of Phytate Content

Phytic acid content of samples was determined by the method described by Davies and Reid [24]. Samples (1 g each) was finely grounded and was extracted in 40 ml of 0.5M nitric acid for 1hr, filtered and 5.0 ml of standard ferric chloride solution (2 mg/l) was added to each filtrate and incubated at 100°C for 20 min. This was again filtered and 3 ml 0.004M ammonium thiocyanate added to the filtrate. The absorbance of the standard ferric chloride solution and the free Fe^{3+} remaining in solution was read on a spectrophotometer (SP6-400 UV spectrophotometer, PYE UNICAM) at 600 nm. The results were converted to milligrams of phytate using the 4:6 atomic ratios for Fe: P in ferric phytate [25] and the results expressed as mg/100g sample.

2.4. Determination of Tannin Content

Quantitative estimation of tannins was carried out using the modified vanillin –HCl procedure [26]. Sample (0.25 g) was extracted using 10 ml of 4% (v/v) concentrated HCl in methanol for 20min in flask capped with parafilm. Individual test sample and sample blank was prepared by adding 5 ml of 4% HCl in methanol to 1ml aliquots of the sample extract. A set of catechin standard solution were prepared from the catechin stock solution. 5 millilitres of vanillin-HCl reagent (freshly prepared) was added to the extract (1 ml) and to each (1 ml) of the standard solution The colour was developed after 20min at 300C and the absorbance of standard solution, sample extract and sample blank was read in a spectrophotometer at 500 nm. A standard curve was prepared expressing catechin concentration, i.e amount of catechin (μg/mL) which gives a colour intensity equivalent to that given by tannins from the catechin standard solution readings after correcting for sample blank absorbance. The results were expressed as mg/g sample.

2.5. Determination of In-vitro Protein Digestibility

In-vitro protein digestibility was determined by the method described by Mertz *et al.* [27]. Initial protein content of the sample was determined by micro-Kjeldahl nitrogen

determination method. Sample of 200 mg was mixed thoroughly with 20 ml of buffered pepsin and later with 15 ml of buffered pepsin solution. A blank sample was similarly prepared the same way. The samples were then digested with pepsin and the sample residue collected after digestion were rolled up and inserted into Kjeldhal flask to determine the protein content of the sample using the micro-Kjeldahl nitrogen determination procedure. The percent protein before and after pepsin digestion was calculated using the formula given in the micro-Kjeldahl nitrogen determination method. The result of the percentage protein before and after pepsin digestion was inserted into the following equation:

$$Protein\ digestibility(\%) = \frac{A-B}{A} x100 \qquad (1)$$

where; A = protein in the sample
    B = protein after pepsin digestion

2.6. Statistical Analysis

The SPSS statistical package (version 16.0) was used for the statistical analysis of results. All the results obtained for the statistical analysis were subjected to analysis of variance (ANOVA) to determine differences within the samples [28] and Duncan Multiple Range Test was used to determine the differences within the variation at 95% confidence level (p ≤ 0.05).

3. Results and Discussion

3.1. Influence of Germination Time, During Malting, on the Phytate Content of Some Sorghum Types

Table 1 shows the effect of germination time, during malting, on the phytate content of some sorghum types. The phytate content of unmalted sorghum types ranged between 1004.6 and 1097.4 mg/100g, with hybrid sorghum grain (Hybrid A) giving the lowest value while White Kaura (SSV2005503) gave the highest value. The barley grain (control) exhibited lowest phytate level (620.7 mg/100g) than the sorghum types. Phytate is usually formed during plant seed maturation and so the differences in the phytate content may be a function of degree of maturation and other agricultural practices such as fertilizer application [29].

Table 1. Effect of Germination Time, during Malting, on the Phytate Content of Some Sorghum Types[1].

Sorghum type	Unmalted (Zero-hour germination) Phytate content (mg/100g)	48-hour germination Phytate content (mg/100g)	Contributory reduction capacity[2] (%)	96-hour germination Phytate content (mg/100g)	Contributory reduction capacity (%)
Local sorghum grain					
Pelipeli	1044.7±1.4[d]	617.1±2.3[a]	41.0	298.3±3.2[a]	71.5
Kwaya	1094.5±5.3[a]	626.3±5.5[a]	42.8	266.1±3.2[d]	75.7
Kilburi	1084.9±1.6[ab]	558.2±5.1[d]	48.6	277.2±3.1[bc]	74.5
Telleri	1074.7±2.8[bc]	542.1±1.5[e]	49.6	285.3±2.1[b]	73.5
Improved sorghum grain					
Samsorg 17 (SK5912)	1052.5±2.7[d]	543.2±2.3[e]	48.4	269.1±3.1[b]	74.5
Samsorg-41 (ICSV400)	1068.4±4.4[c]	599.3±5.3[b]	44.0	271.2±2.6[cd]	74.6
White Kaura (SSV2005503)	1097.4±3.4[c]	589.2±4.2[b]	46.4	285.3±3.1[b]	74.0
FF Katsina (SSV200503)	1047.6±2.5[d]	572.1±2.1[c]	45.4	278.2±3.8[bc]	73.5
Hybrid sorghum grain					
HybridA	1004.6±3.6[f]	483.3±1.2[g]	51.9	215.4±2.2[f]	78.6
Hybrid B	1010.5±6.5[f]	459.1±3.2[h]	54.6	220.2±1.7[ef]	78.2
Hybrid C	1044.7±3.5[de]	457.2±4.4[h]	56.3	217.1±3.6[ef]	79.2
Hybrid D	1031.5±3.6[e]	495.4±2.3[f]	52.0	225.3±3.8[e]	78.2
Barley (Control)	620.7±2.1[g]	359.1±6.6[i]	42.2	211.1±4.2[f]	66.0

[1]Values are means ± standard deviation of three determinations. Mean values within the same column having the same superscript are not significantly different at p≤ 0.05.
[2]Contributory reduction capacity (%) was calculated with respect to the initial total phytate content at zero-hour germination.

The phytate content of sorghum types, at 48-hour germination time, was significantly lower (p≤ 0.05) than those of the unmalted counterparts. The phytate reduction capacity at 48-hour germination time was between 41.0 and 56.3% with *Pelipeli* giving the lowest reduction capacity while hybrid sorghum grain (Hybrid C) gave the highest reduction capacity. The phytate reduction capacity in barley at 48-hour germination time was observed to be 42.2%. The general decrease in the phytate content of sorghum types at 48-hour germination time could be attributed to an increase in the phytase (enzyme) activity during the germination stage and this might have led to phytate degradation [30, 31].

There was further decrease in phytate content of the sorghum types at 96-hour germination time leading to an overall phytate reduction capacity of 71.5 to 79.2%. The phytate reduction capacity in barley at 96-hour germination time was 66%. The elongated germination time might have given rise to an increased phytate-phytase interaction thereby leading to a further degradation of phytate in the grains [31].

3.2. Tannin Content of the Sorghum Types as Influenced by Germination Time

Table 2 shows the effect of germination time, during malting, on the tannin content of some sorghum types. The

tannin content of the unmalted sorghum grains ranged between 0.23 and 4.47 mg/g with the hybrid sorghum grain (Hybrid B) giving the lowest value while White Kaura (SSV2005503) gave the highest value. Tannin was not detected in hybrid sorghum grain (Hybrid D) and barley respectively. Tannins are essentially one of the phytochemicals present in plant seeds such as cereal grains and leguminous seeds [32]. They are usually deposited rapidly during the milk stage of development underneath the seed coat [33] and are principal polymerized products of flavan-3-ols and/or flavan-3,4-diols [34].

Table 2. *Effect of Germination Time, during Malting, on the Tannin Content of Some Sorghum Types[1].*

Sorghum type	Unmalted (Zero-hour germination) Tannin content (mg/g)	48-hour germination Tannin content (mg/g)	Contributory reduction capacity (%)	96-hour germination Tannin content (mg/g)	Contributory reduction capacity (%)
Local sorghum grain					
Pelipeli	0.27±0.01[bc]	0.23±0.05[cd]	14.8	0.20±0.02[cd]	25.9
Kwaya	0.38±0.02[bc]	0.32±0.03[bc]	15.8	0.25±0.03[bc]	34.2
Kilburi	0.43±0.02[bc]	0.35±0.02[bc]	18.6	0.30±0.03[b]	30.2
Telleri	0.35±0.01[bc]	0.27±0.11[c]	22.9	0.24±0.03[bc]	31.4
Improved sorghum grain					
Samsorg 17 (SK5912)	0.45±0.02[bc]	0.33±0.05[bc]	26.7	0.28±0.09[b]	37.8
Samsorg-41 (ICSV400)	0.25±0.05[bc]	0.20±0.05[d]	20.0	0.18±0.06[cd]	28.0
White Kaura (SSV2005503)	4.47±0.21[a]	3.35±0.19[a]	25.1	3.07±0.24[a]	31.3
FF Katsina (SSV200503)	0.47±0.02[b]	0.38±0.05[b]	19.1	0.30±0.03[b]	36.2
Hybrid sorghum grain					
HybridA	0.24±0.01[bc]	0.15±0.06[e]	37.5	0.13±0.03[de]	45.8
Hybrid B	0.23±0.01[bc]	0.18±0.03[e]	21.7	0.14±0.02[de]	39.1
Hybrid C	0.24±0.01[bc]	0.17±0.05[e]	29.2	0.15±0.02[de]	37.5
Hybrid D	ND[3]	ND	-	ND	-
Barley (Control)	ND	ND	-	ND	-

[1]Values are means ± standard deviation of three determinations. Mean values within the same column having the same superscript are not significantly different at p≤ 0.05.
[2]Contributory reduction capacity (%) was calculated with respect to the initial total tannin content at zero-hour germination.
[3]ND= Not detected.

At 48-hour germination time, the tannin content was observed to decrease in all the sorghum types. Tannin was not detected in Hybrid D and barley respectively at 48-hour germination time. The decrease in the tannin content may be attributed to its partial break down during germination [35]. The tannin reduction capacity in the sorghum types at 48-hour germination time ranged between 14.8 and 37.5% with *Pelipeli* (local sorghum grain) giving the lowest reduction capacity and Hybrid A giving the highest value. At 96-hour germination time, all the sorghum types experienced further break down of tannin giving a range of 0.13 -3.07 mg/g. The tannin reduction capacity was observed to range between 25.9 and 45.8%, with *Pelipeli* (local sorghum grain) giving the lowest tannin reduction capacity while Hybrid A gave the highest reduction capacity.

3.3. Effect of Germination Time on In-vitro Protein Digestibility of Sorghum Types

The effect of germination time, during malting, on *in-vitro* protein digestibility of some sorghum types is presented in Table 3. The *in-vitro* protein digestibility of the unmalted sorghum grains ranged between 32.1 and 45.1%, with *Kilburi* (local sorghum grain) exhibiting the lowest value while Hybrid B had the highest value. The protein digestibility of barley was observed to be 60.8%. Protein digestibility is essentially a measure of the susceptibility of a protein to proteolysis [11]. A protein with high digestibility is regarded as being potentially better nutritionally than one of low digestibility due to its ability of providing more amino acids for absorption on proteolysis [11]. At 48-hour germination time, the *in-vitro* protein digestibility of each sorghum type was observed to increase significantly (p≤ 0.05) ranging between 42.8 and 61.3% with *Kilburi* and Hybrid A giving the lowest and highest values respectively. The improved protein digestibility of sorghum types at 48-hour germination time may be attributed to increased enzymatic activities such as proteases which are capable of breaking down proteins during germination [36]. The incremental level in protein digestibility, at 48-hour germination time, ranged between 7.8 and 18% while the incremental level for barley was 6.7%. The general increase in protein digestibility could also be attributed to the reduction in inhibitory activity of protein-complexing reactions specifically through phytate and tannin respectively. The reduction in the level of phytate, which is capable of forming phytate-protein complex [9] as well as reduction in the concentration of tannin, which is capable of binding dietary protein into an indigestible form [10], all contributed to an improved protein digestibility.

Table 3. *Effect of Germination Time, during Malting, on the In-vitro Protein Digestibility of Some Sorghum Types[1].*

Sorghum type	Unmalted (Zero-hour germination)	48-hour germination		96-hour germination	
	In-vitro protein digestibility (%)	*In-vitro* protein digestibility (%)	Incremental level in protein digestibility[2] (%)	*In-vitro* protein digestibility (%)	Incremental level in protein digestibility (%)
Local sorghum grain					
Pelipeli	40.8±0.4e	55.6±0.4d	14.8	58.3±0.2g	17.6
Kwaya	35.6±0.2g	51.2±0.4f	15.6	52.1±0.1h	16.5
Kilburi	32.1±0.1h	42.8±0.1h	10.7	46.2±0.2i	14.1
Telleri	35.6±0.2g	43.8±0.3h	8.2	48.3±0.1h	12.7
Improved sorghum grain					
Samsorg 17(SK5912)	43.2±0.1c	56.5±0.1d	13.3	59.8±0.1f	16.7
Samsorg-41(ICSV400)	37.3±0.1f	46.3±0.3g	9.0	48.5±0.1h	11.2
White Kaura(SSV2005503)	35.7±0.1g	43.5±0.2h	7.8	46.7±0.2i	11.0
FF Katsina(SSV200503)	42.2±0.1d	56.2±0.1d	14.0	58.1±0.1g	15.9
Hybrid sorghum grain					
HybridA	43.1±0.1c	61.7±0.1b	18.0	63.1±0.1d	20.0
Hybrid B	45.1±0.2b	61.2±0.5b	16.0	64.8±0.2b	19.7
Hybrid C	40.7±0.1e	54.4±0.1e	13.7	60.5±0.3e	19.8
Hybrid D	42.6±0.1cd	58.2±0.1c	15.6	63.7±0.1c	21.1
Barley(Control)	60.8±0.1a	67.5±0.3a	6.7	79.5±0.1a	18.7

[1]Values are means ± standard deviation of three determinations. Mean values within the same column having the same superscript are not significantly different at $p \leq 0.05$.
[2]Incremental level in protein digestibility (%) was calculated on the basis of initial digestibility at zero-hour germination.

At 96-hour germination time, there were further increases in the protein digestibility of all the sorghum types ranging between 46.7 and 64.8%. The incremental level in protein digestibility, at 96-hour germination time, was between 11 and 21.1% with Kaura (SSV2005503) giving the lowest incremental level while Hybrid D gave the highest level. The elongated germination time might have increased the activities of the proteases thereby making the protein highly susceptible to digestibility. The degree of protein digestibility may be used as an indicator of protein availability in a food material [11].

4. Conclusion

The germination time during the malting process of sorghum types had revealed a positive influence on phytate and tannin content reduction as well as an improvement in protein digestibility of the sorghum types. At 96-hour germination time, the phytate reduction capacity could range between 71.5 and 79.2% while that of tannin could range between 25.9 and 45.8%. In the case of *in-vitro* protein digestibility, the incremental level at 96-hour germination time could range between 11 and 21.1% for all the sorghum types evaluated under this study.

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