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Influence of Germination Time, During Malting, on Selected Anti-Nutritional Factors of Some Sorghum [*Sorghum bicolor* (L.) Moench] Types

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ABSTRACT

The influence of germination time, during malting, on selected anti-nutritional factors (phytate and tannin) of some sorghum types was investigated. The local, improved and hybrid sorghum types were used for the study and respectively subjected to malting while phytate and tannin were periodically evaluated, during the germination period, using standard procedures. The results showed that the phytate concentration of all the sorghum types, before germination, ranged from 459 to 1097.9 mg/100g sample while that of barley (control) was 620.8 mg//100g sample. The phytate content of all the sorghum types got reduced by 32.7-56.3% level after 48-hour germination. Most of the reduction levels of phytate for the sorghum types were higher than that obtained for barley (42.2%) which served as the control. At 96-hour germination period, the reduction levels of phytate increased by 52.1 to 79.2% while that of barley also increased by 66%. The tannin level of all the sorghum types, before germination, ranged from 0.12-4.44 mg/g sample while that of barley (control) was 0.04 mg/g sample. The tannin level during the germination period showed a reduction level of 13.2 to 50% at 48-hour germination while the reduction level for barley was 77.5%. At 96-hour germination time, the reduction level increased to 26.3 - 43.5% while no tannin detection was observed for hybrid D of the sorghum grain and barley respectively. The conclusion from this study revealed that it might be impossible to have a total elimination of phytate and tannin from most sorghum types at 96-hour germination period during malting.

Keywords: *Phytate, tannin, sorghum types, germination time, malting.*

INTRODUCTION

Sorghum grain is comparable to some other cereal grains in terms of nutrient content. However, it contains some anti-nutrient factors that affect the nutrient absorption by human body system (Stephen,2008).These anti-nutrients serve as limiting factors in the utilization of sorghum for both human and animal as they make bioavailability of certain nutrients impossible (Khattab and Arntfield 2009). Reduction of these anti-nutrient factors such as Phytic acid and tannin is therefore important during sorghum grain utilization. Phytic acid is stored primarily in the aleurone layer in phytin bundles and to a lesser extent in the germ. Phytic acid complexes with essential dietary minerals like Ca, Zn, Fe and Mg to form phytates (Abd-El-Rahaman *et al*., 2007) thereby causing the minerals to be unavailable for absorption. The binding can result in very insoluble

salts with poor bioavailability of the minerals. Another unique chemical property of sorghum is its unusually high content of tannins and polyphenols. Tannins are plants secondary substances (not in metabolic pathways, providing energy for growth and reproduction) that are characteristically rich in phenolic hydroxyl groups (Afify, 2012). Stephen (2008) and Sokrab*et al*.(2012)all reported that the condensed tannins and other polyphenols of sorghum grain naturally provide agronomic advantages such as bird resistance, but can be harmful in the diet, causing reduced weight gain of rats and chicks. The high content of tannins and other polyphenols creates serious brewing problems of chill haze in beer and the nutritional problem of unavailability of sorghum protein when consumed directly as food (Bressani *et al*., 1982). Attempts to reduce tannin and phytate have been tried by different means. Phytate content can be reduced by abrasive decortication, milling into flour, soaking of the grain and activation of indigenous enzyme phytase by fermentation or malting (Ghavidel and Prakash, 2007). Tannins may be alleviated by treating the grain with dilute aqueous ammonia, use of strong alkali and formaldehyde or by dehulling (Mohammed-Nour *et al*., 2010). However, Stephen (2008) reported that whether any of these measures are suitable and simple enough for wide spread adoption is not clear but enzymic methods of phytate and tannin removal by fermentation or malting have been found to be more effective than the physical extraction methods. The objective of this study, therefore, was to evaluate the effect of germination time, during malting, on selected anti-nutritional factors (phytate and tannin) of some sorghum types including local, improved and hybrid sorghum grains.

MATERIALS AND METHODS

Sources of Materials

Three categories of sorghum were used for this study. Local sorghum grains (Pelipeli, Kwaya, Kilburi and Telleri) were obtained from Adamawa Agricultural Development Agency, Yola, Adamawa State, Nigeria. The improved sorghum grains (Samsong 17, Samsong 41, FF Katsina and White Kaura) were sourced from the Institute of Agricultural Resarch (IAR), Samaru, Nigeria. The hybrids samples coded A, B, C and D; were sourced from Lake Gerio Research Farm of River Basin Development Authority (RBDA), Yola, Adamawa State, Nigeria. The barley sample which served as the control was obtained from Lake Chad Research Institute, Maiduguri, Borno State, Nigeria. All samples were stored in a dry environment in different polyethylene bags in the laboratory at room temperature of 32±2^{o}C and 65% relative humidity (RH) until required.

Malting of sorghum grains

Experimental samples (300 g) of each sorghum grain type and barley were respectively taken and the malting process follows the procedure of Palmer (1989). The cleaned grains were steepedin thrice quantity of water for 12h with 1h air rest after 6h 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 5min 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^{o}C). During the germination stage, samples were periodically taken for phytic and tannin contents determination at 0, 48 and 96 h; representing the initial, optimum and maximum germination time as described by Obizoba and Atii (1994).

Determination of Phytate Content

Phytic acid content of samples was determined by the method described by Davies and Reid (1979). Samples (I g each) was finely grounded and was extracted in 40 ml of 0.5M nitric acid for 1h, filtered and 5.0ml of standard ferric chloride solution (2 mg/l) was added to each filtrate and incubated at 100^{o}C for 20min. 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 600nm. The results were converted to milligrams of phytate using the 4:6 atomic ratios for Fe: P in ferric phytate (Garcia-Estepa *et al.,* 1999) and the results expressed as mg/100g sample.

Determination of tannin content

Quantitative estimation of tannins was carried out using the modified vanillin–HCl procedure (Gomez *et al.,* 1997). 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 5ml 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. Five 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 30^{o}C 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.

Statistical Analysis

In this study, the general linear model (GLM) of SPSS statistical package (version 16.0) was used for the statistical analysis of results. All the results obtained for the statistical analysis were carried out in triplicate and were subjected to analysis of variance (ANOVA) to determine differences within the samples (Snedecor and Cochran, 1987)and separation of mean values was by Duncan Multiple Range Test (Duncan, 1955) to determine the differences within the variation at 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Phytate Contents of Some Sorghum Types, during Malting, as Influenced by Germination Time

The results of phytate content of some sorghum types, during malting, as influenced by germination time are presented in Table 1. The phytate concentration of all the sorghum types, before germination, ranged from 459 to 1097.9 mg/100g sample while that of barley (control) was 620.8 mg//100g sample. There were significant variations ($p<0.05$) observed in the phytate contents of the sorghum samples. After 48h germination time, the phytate content varied from 457 mg/100 g to 626 mg/100 g; indicating that the phytate content of all the sorghum types got reduced by 32.7-56.3% level. Most of the reduction levels of phytate for the sorghum types were higher than that obtained for barley (42.2%) which served as the control. At 96-h germination time, the phytate content varied from 215 mg/100 g to 298 mg/100 g. At different levels of germination time, the phytate contents of the hybrids were found to be lower than the other sorghum types. This implies that the levels of this anti-nutrient in hybrid sorghum samples were lower than that in the other sorghum types.Idris *et al.* (2006) also observed higher phytate contents in the sorghum grains in their study. The value obtained for barley in this study was observed to be slightly lower than that reported for barley (632 mg/100 g) by Abd-El-Rahaman *et al*. (2007). The slight difference could be attributed to varietal differences. Other workers have reported similar variations in levels of phytate content of some cereals (wheat, rice, barley, millet and rye) (Idris *et al.,* 2006). It was also reported that germination time had a substantial effect on the reduction of phytate content due to the action of endogenous phytases released during germination that degraded the phytate into inorganic phosphorus and inositol and its intermediate forms (Stephen, 2008; Sokrab*et al.,* 2012).

Tannin Contents of Some Sorghum Types, during Malting, as Influenced by Germination Time

The tannin contents of the sorghum samples are presented in Table 2. The tannin level of all the sorghum types,

before germination, ranged from 0.12-4.44 mg/g sample while that of barley (control) was 0.04 mg/g sample. The result indicated that there were significant differences ($p<0.05$) in the tannin content between the sorghum types at different levels of germination time. After 48h of germination time, the tannin content varied from 0.06 mg/g sample to 3.35 mg/g sample. The tannin level during the germination period showed a reduction level of 13.2 to 50% at 48-hour germination while the reduction level for barley was 77.5%. At 96-hour germination time, the tannin concentration reduced to a range of 0.13 to 3.07 mg/g sample in all the sorghum types and this indicated that at 96-hour germination time, the reduction level increased to 26.3 - 43.5% while no tannin detection was observed for hybrid D of the sorghum grain and barley respectively. However, the tannin content of the hybrid sorghum was found to be consistently lower than those recorded for the other sorghum types throughout the germination period. Generally, the tannin content recorded for sorghum samples in this study agrees with the values reported by Stephen (2008) in terms of low concentration. Tannin contents as low as 0.20 mg/g and as high as 8 mg/g were reported from different sorghum varieties in grain quality evaluation while some other varieties contain no tannin at all(Afify, 2012). Agronomic advantages such as resistance to bird depredation are associated with high–tannin sorghums which have relatively low nutritional value for non-ruminant (Afify, 2012; Sokrab*et al*., 2012).

CONCLUSION

Elimination or inactivation of anti-nutritional compounds such as phytate and tannin is absolutely necessary to improve the nutritional quality of sorghum and effectively utilize its full potential as human food. From this study, phytate and tannin content of the hybrids were found to be consistently lower than those recorded for the other sorghum types throughout the period of malting. Hybrid sorghum type will be better suited for brewing in the brewing industry because of its low tannin content. The conclusion from this study revealed that it might be impossible to have a total elimination of phytate and tannin from most sorghum types at 96-hour germination period during malting.

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Table 1: Effect of germination time, during malting, on the phytate content of some sorghumtypes.

Sorghum type	0-hour germination	48-hour germination		96-hour germination	
	Phytate concentration (mg/100g sample)	Phytate concentration (mg/100g sample)	Reduction capacity[1] (%)	Phytate concentration (mg/100g sample)	Reduction capacity (%)
Local sorghum grain:					
Pelipeli	1044.9±2.1^{f}	617±2.3^{b}	40.9	298±3.2^{a}	71.5
Kwaya	1094.8±1.3^{a}	626±1.5^{a}	42.8	266±3.2^{d}	75.7
Kilburi	1084.9±4.1^{b}	558±5.1^{f}	48.6	277±3.1^{c}	74.5
Telleri	1074.8±1.3^{c}	542±1.5^{g}	49.6	285±2.1^{b}	73.5
Improved sorghum grain:					
Samsorg 17 (SK5912)	1052.8±1.3^{e}	543±2.3^{g}	48.4	269±3.5^{d}	74.4
Samsorg-41 (1CSV400)	1068.8±4.3^{d}	599±5.3^{c}	44.0	271±2.6^{d}	74.6
White Kaura (SV20043)	1097.9±3.1^{a}	589±4.2^{d}	46.4	285±3.1^{b}	74.0
F.F. Katsina(SSV200503)	1036.1±2.1^{g}	572±2.1^{e}	44.8	278±3.8^{c}	73.2
Hybrid sorghum grain:					
Hybrid A	1004.9±1.3^{h}	483±1.2^{i}	51.9	215±2.2^{fg}	78.6
Hybrid B	459.1±3.2^{j}	309±4.2^{l}	32.7	220±1.7^{ef}	52.1
Hybrid C	1044.8±3.2^{f}	457±4.2^{j}	56.3	217±3.6^{f}	79.2
Hybrid D	1031.9±3.3^{g}	495±2.3^{h}	52.0	225±3.8^{e}	78.2
Barley (control)	620.8±5.6^{i}	359±6.3^{k}	42.2	211±4.2^{g}	66.0

Values are mean ± standard deviation of three determinations. Values with different superscripts in a column are significantly different at $p<0.05$.

[1]Reductioncapacity (%) was calculated with respect to the initial total phytate content in the raw grain.

Table 2: Effect of germination time, during malting, on the tannin content of some sorghumtypes.

Sorghum type	0-hour germination	48-hour germination		96-hour germination	
	Tannin concentration (mg/g sample)	Tannin concentration (mg/g sample)	Reduction capacity[1] (%)	Tannin concentration (mg/g sample)	Reduction capacity (%)
Local sorghum grain:					
Pelipeli	0.29±0.01^{f}	0.23±0.05^{cd}	20.7	0.20±0.02^{bcd}	31.0
Kwaya	0.41±0.15^{cd}	0.32±0.03^{bc}	22.0	0.25±0.03^{bcd}	39.0
Kilburi	0.44±0.12^{c}	0.35±0.02^{bc}	20.5	0.30±0.03^{b}	31.8
Telleri	0.34±0.0^{e}	0.27±0.11^{bcd}	20.6	0.24±0.03^{bcd}	29.4
Improved sorghum grain:					
Samsorg 17 (SK5912)	0.38±0.03^{de}	0.33±0.05^{bc}	13.2	0.28±0.09^{bc}	26.3
Samsorg-41 (1CSV400)	0.25± 0.01^{fg}	0.20±0.05^{d}	20.0	0.18±0.06^{a}	28.0
White Kaura (SV20043)	4.44±0.05^{a}	3.35±0.19^{a}	24.5	3.07±0.24^{b}	30.9
F.F. Katsina (SSV200503)	0.49± 0.01^{b}	0.38±0.05^{b}	22.4	0.30±0.03^{b}	38.8
Hybrid sorghum grain:					
Hybrid A	0.23±0.03^{g}	0.15±0.06^{de}	34.8	0.13±0.03^{d}	43.5
Hybrid B	0.23±0.01^{g}	0.18±0.03^{d}	21.7	0.14±0.02^{d}	39.1
Hybrid C	0.24± 0.01^{g}	0.17±0.05^{de}	22.2	0.15±0.02^{cd}	37.5
Hybrid D	0.12±0.02^{h}	0.06±0.01^{ef}	50.0	ND[2]	100.0
Barley (control)	0.04±0.01^{i}	0.009±0.001^{f}	77.5	ND	100.0

Values are mean ± standard deviation of three determinations. Values with different superscripts in a column are significantly different at $p<0.05$.

[1]Reductioncapacity (%) was calculated with respect to the initial total tannin content in the raw grain.

[2]ND= Not detected.