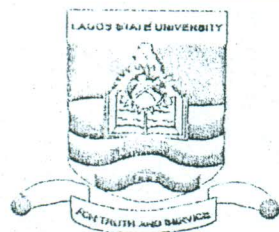


Handwritten signature



A JOURNAL OF THE
FACULTY OF SCIENCE
LAGOS STATE UNIVERSITY

QUANTITATIVE ASSESSMENT OF CHANGES IN TANNIN, SOLUBLE PROTEIN CONTENTS AND DIASTATIC ACTIVITY DURING GERMINATION OF SOME IMPROVED SORGHUM CULTIVARS

B.O. OYEDOYIN, C.O. ORISHAGBEMI AND A.K. LAWAL

FEDERAL INSTITUTE OF INDUSTRIAL RESEARCH, C ODI, P.M.B. 21023, IKEJA, LAGOS.

ABSTRACT

Three improved sorghum cultivars, SK5912, L1499 and L533, were each subjected to conditioned germination for 1,2,3,4 and 5 days and investigations on changes in their respective tannin, soluble protein contents and diastatic activity were carried out to determine likely effect on the nutritive value of germinated sorghum. The initial level of each component in ungerminated sorghum samples was determined. Both tannin and soluble protein levels of ungerminated seeds varied among the 3 sorghum cultivars, and none of them was found to show any diastatic activity. For all the cultivars considered, the tannin, soluble proteins and diastatic activity increased with duration of germination up to 5 days considered. SK5912 had the highest percent increase in tannin (110%, equivalent to 0.063mg/ml), soluble protein (169.6%, as 48.42mg/g) and lowest percent increase in diastatic activity, but with highest value (800.7%, as 38.641 I.O.B. units), while L1499 and L533 cultivars had lower diastatic activity values of 25.76 and 32.50 I.O.B. units respectively. Germination of sorghum seeds irrespective of cultivar enhanced increase in the tannin, soluble protein contents and diastatic activity (SK5912 cultivar showing the most remarkable increases) thereby contributing to improvement of their nutritive value especially when converted to sorghum malt. Key words: Sorghum, germination, tannin, proteins, diastatic activity.

INTRODUCTION

Sorghum (*Sorghum bicolor*) has been a vital staple food for millions of people in Semi-Arid Tropics (Ahmed et al, 1996). It is also one of the main staple crops in the food systems of Western Africa and represents about 37% of the total food grain production (Debrah, 1993).

In different parts of the world vigorous efforts are directed towards coupling the beneficial effects of harmful constituents such as tannin and cyanide in sorghum seeds by direct removal of seed testa, fermentation or by extraction (Ahmed et al, 1996). The tannin content of different cultivars of sorghum have been shown to increase slightly when seeds were germinated for varying periods of time (Glennie 1983).

Cereal grains are known to have a low protein content and the protein quality is limited by deficiencies in some essential amino acids, mainly lysine. According to Hammed et al (1996), it has been reported that the protein quality of sorghum is similar to that of other cereals except rice. Wang, 1977 has also shown that one of the feasible methods of improving the protein quality of sorghum is by germinating the seeds.

The malting process of sorghum seeds that generates the fermentable mono and disaccharides is dependent upon the activity of α and β - amylases that develop in sorghum seeds during germination (Hulbe et al, 1980). Bureng and Wergan, 1982, reported that activity of amylases increased appreciably during malting.

The study therefore investigated the effect of germination on the tannin, soluble protein contents and diastatic activity of sorghum seeds.

MATERIALS AND METHODS:

Three different improved varieties of sorghum namely S.K. 5912, L533 and L1499 were obtained from Institute for Agricultural Research (IAR), Zaria and used for this work.

GERMINATION:

One hundred gram (100g) sample of each variety was cleaned steeped, germinated and kilned. The steeping procedure involved wet steeping in water at the ambient conditions for 10 hours followed by air resting for 18 hours. The steeped seeds were germinated for 5 days at a temperature ranging between 26°C and 28°C. During germination, humidification by wetting with water as well as regular turning of the grains were done. Samples were taken for analysis each day.

The green malt was kilned for 10 hours at 65°C, 7 hours at 75°C and 6 hours at 85°C.

Analytical Procedures:

The ungerminated sorghum samples were analysed for moisture, protein and tannin contents. Also diastatic activity was investigated. On daily basis, samples of the germinating seeds were analysed for moisture, diastatic activity and protein contents. Also, the tannin contents of germinated seed samples were determined for days 1, 3 and 5.

MOISTURE AND TANNIN DETERMINATION:

To express results on a 105°C dry matter basis, moisture was determined according to the AOAC (1965) method. Tannins were estimated by the modified procedure of Maxon and Rooney, as described by Price et al (1978). A 200mg sample was extracted with 5ml 4% (v/v) concentrated HCL in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5ml) was added to the extracted (1ml) and the absorbance of the colour developed after 20 min at 30°C was read at 520nm. A standard curve was prepared expressing the results as catechin equivalents, that is the amount of catechin (mg/ml) which gives a colour intensity equivalent to that given by tannins after correcting for the blank.

DIASTATIC ACTIVITY MEASUREMENT:

Diastatic activity, i.e. the combined α and β -amylase activity was determined by the alkaline ferricyanide method according to recommended methods of Analysis of Barley, malt and Adjunct as described by Anon (1971). Results are expressed as Institute of Brewing Units (10B units). This is the amount of enzyme that catalyses the conversion of 10 μ mol of starch per min under defined conditions.

PROTEIN CONTENT DETERMINATION:

The total soluble protein of the ungerminated and germinating samples were determined according to the method of Lowry (1941).

Each experiment was carried out in triplicate and average values determined.

RESULTS AND DISCUSSION

The results of analysis of ungerminated and germinated sorghum seeds of 3 different varieties are presented in table 1 and 2.

Table I: Results of analysis of Ungerminated Sorghum samples

Cultivars	Moisture Content (%)	Protein (mgPrg ⁻¹)	Tannin (mg/ml)	Diastatic Activity (10B units)
S.K. 5912	7.0	15.76	0.030	0
L533	6.83	21.09	0.085	0
L1499	7.0	20.40	0.102	0

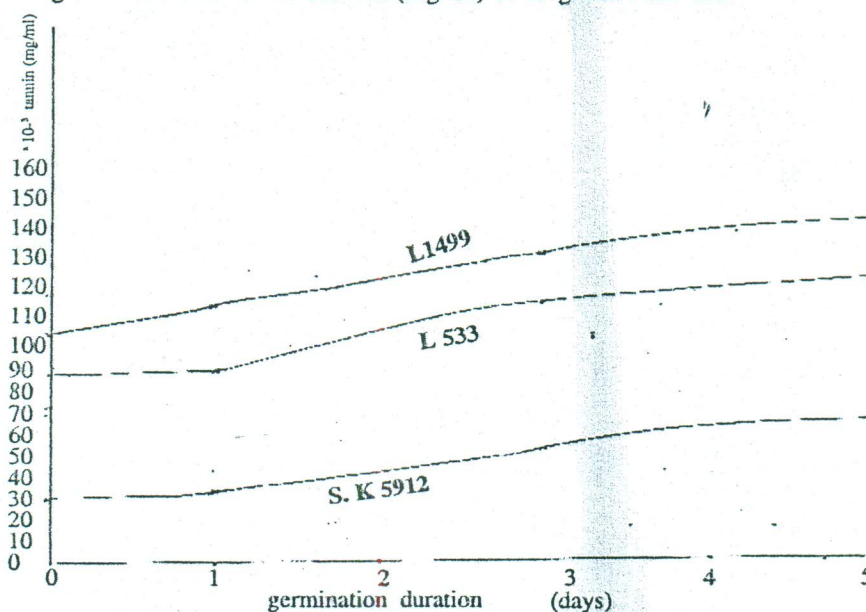
Values presented are mean of three experiments.

Table 2: **Results of Analysis of Germinated Sorghum Samples:**

Cultivar	Germination Duration (days)	Moisture content (%)	Tannin (mg/ml)	Protein (mg/ml)	Diastatic (mgPrg ⁻¹)	Activity (10B Units)
S.K. 5912	1	8.0	0.033		17.96	4.29
	2	9.4	-		23.41	27.60
	3	8.0	0.051		36.60	28.21
	4	9.0	-		43.82	34.55
	5	8.0	0.063		48.42	38.64
L1499	1	7.0	0.116		20.40	1.84
	2	7.6	-		25.87	11.04
	3	6.8	0.139		27.30	20.24
	4	7.8	-		34.63	21.78
	5	7.8	0.153		34.81	25.76
L533	1	7.8	0.087		21.66	2.45
	2	8.6	-		27.50	7.97
	3	8.0	0.116		37.44	19.63
	4	9.0	-		41.84	21.47
	5	7.8	0.126		45.72	32.50

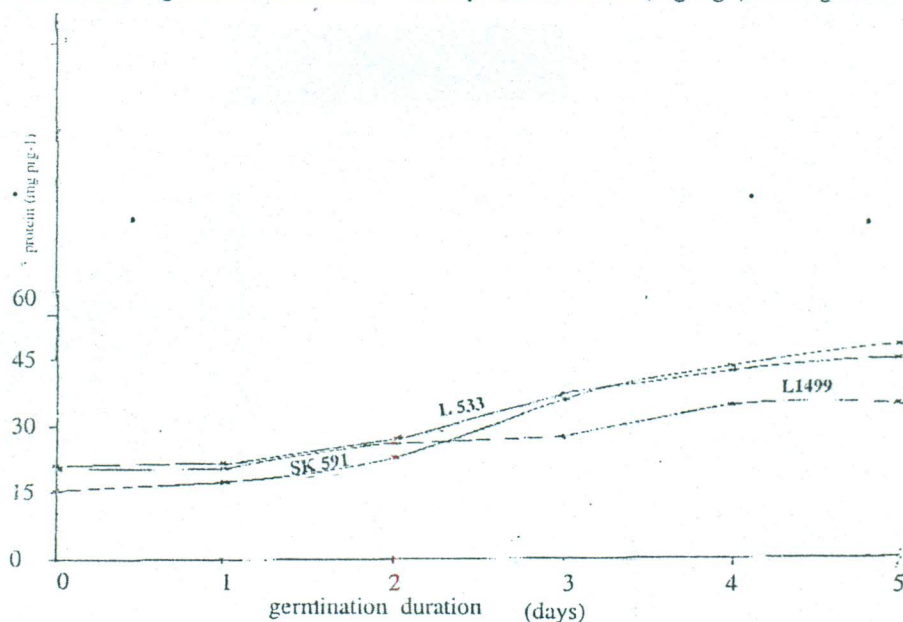
Values presented are mean of three experimtns.

Figure 1 shows the effect of germination on tannin content (mg/ml) of sorghum cultivars



Varieties. Tannin content of ungerminated seeds were 0.030, 0.085 and 0.102 mg/ml for cultivars SK 5912, L533 and L1499 respectively. Germination period up to 5 days markedly increased the tannin content to 0.063, 0.126 and 0.153 for cultivars SK 5912, L533 and L1499 respectively. SK 5912 had the highest percentage increase (110%) followed by L1499 (80%) and L533 (23.52%). This could be as a result of solubilization of tannin when seeds were soaked in water and migration of tannin to the outer layer as a result of germination as indicated by the browning of the germinated seeds. This is in line with results obtained by Ahm. et al. 1996.

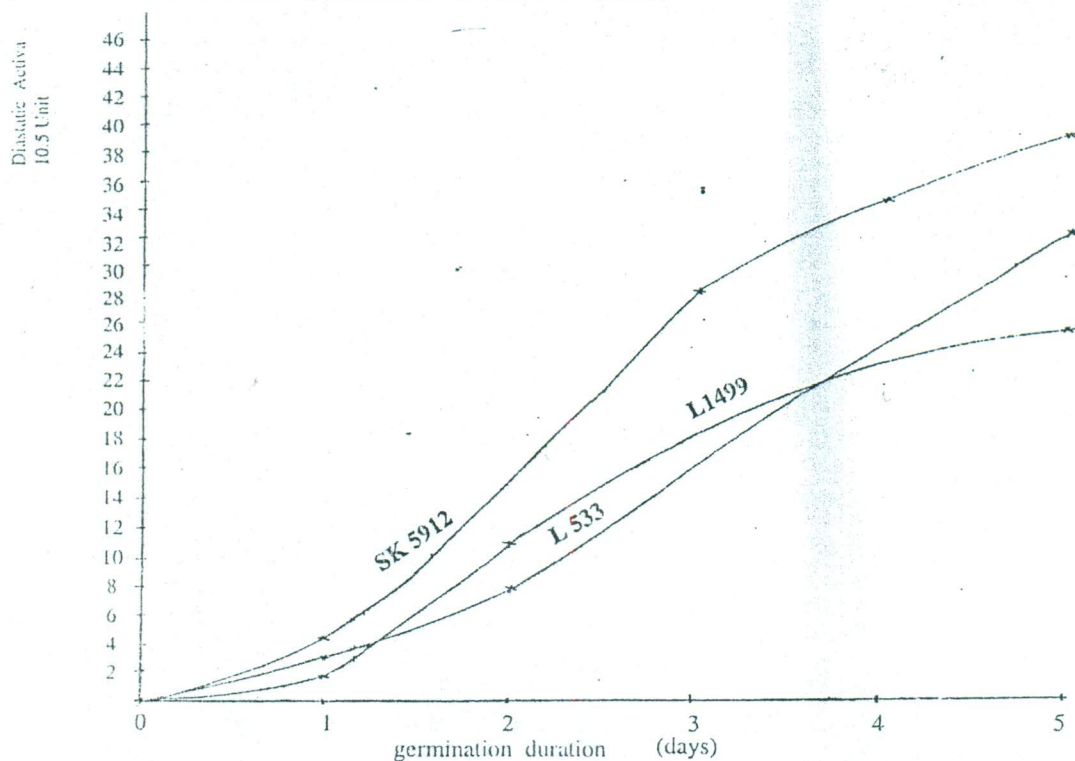
Figure 2 shows the effect of germination on the soluble protein content ($\text{mgPr}g^{-1}$) of sorghum.



The soluble protein content of unfermented seeds were 15.76 , 21.09 and $20.40 \text{ mgPr}g^{-1}$ for cultivars S.K. 5912, L533 and L1499 respectively. Germination of seeds for 1 day slightly increased soluble protein content to 17.96 , 20.51 and $21.66 \text{ mgPr}g^{-1}$ for the cultivars SK 5912, L533 and L1499 respectively. Increase in the germination period up to 5 days markedly increased the soluble protein content to 48.42 , 34.81 and $45.92 \text{ mgPr}g^{-1}$ for the cultivars S.K.5912, L533 and L1499 respectively.

S.K. 5912 had the highest percentage increase (169.6%) followed by L533 (111.1%) and L1499 (70.72%). For all the cultivars, result revealed that soluble protein content was increased slightly as seeds were germinated for different periods. Ziad et al (1996), reported slight increases in the soluble protein content of corn varieties when germinated for period up to 72 hours. Also the values of soluble protein analysis of different sorghum varieties using Lowry's method obtained by Serna-Saloivar et al (1988) seem to agree with the stated result. The high protein content indicate a high enzymatic activity of the germinated seeds.

Figure 3 shows the effect of germination on diastatic activity,



i.e. the combined α and β amylase activities. For all cultivars, diastatic activity as Institute of Brewing (10B) units of ungerminated seeds were nil. Diastatic activity significantly increased up to 38.64, 25.76 and 32.50 for S.K. 5912, L1499 and L533 cultivars respectively, when seeds were germinated for up to 5 days. This confirms high enzymatic activity of the germinated sorghum seeds.

CONCLUSION

This study had shown that germination of sorghum seeds irrespective of cultivar enhanced increase in the tannin, soluble protein contents and diastatic activity (S.K. 5912 cultivars showing the most remarkable increases) thereby contributing to improvement of their nutritive value especially when converted to sorghum malt.

ACKNOWLEDGEMENT

The authors remain grateful to the management of Federal Institute of Industrial Research, Oshodi (FIIRO) for the provision of necessary facilities required for this work.

REFERENCES

- Ahmed, S.B., Mahgoub, S.A. & Babiker, B.E. (1996). Changes in tannin and cyanide contents and diastatic activity during germination and the effect of traditional processing on cyanide content of sorghum cultivars. *Food Chemistry* 56 (2), 159 – 162.
- Anon, K.B. (1971) Measurement of diastatic activity. In *Recommended methods of Analysis of Barley, Malt and Adjuncts Adopted by the Council of the Institute of Brewing* pp. 163 –5.
- AOAC (1965) *Official Methods of analysis*, 70th edn. Association of Official Agricultural Chemists, Washington, DC.
- Bureng, P.N. and Wogan, J.T. (1982). Properties of amylases and α -glucosidase in Feteria (sorghum) malt. *Sudan J. Food Sci. Technol.* 14, 34 – 40.
- Debrah, S.K. (1993) Sorghum in Western Africa. In *sorghum and millets community and research environments* (Byth D.E. ed). Patancheru, A.P. 502324, India. International Crops Research, Institute for Semi-Arid Tropics.
- Glennie, C.W. (1983) Oxyphenolase changes in sorghum grain during malting. *J. Agric. Food Chem.* 31 1295 – 9.
- Hulse, J.H., Laing, E.M. & Pearson, O.E. (1980). *Malting and brewing in sorghum and the millets. Their composition and nutritive value.* Academic Press, New York, pp. 453 – 67.
- Price, M.L., Soroyoc, S.V. & Bultler, L.G. (1978). A Critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric- Food. Chem.*, 26, 1214-18.
- Serna – Saloivar, S.O. (1988). Effect of soyabeans and sesame addition on the nutritive value of maize and decorticated sorghum Tortiles. *Cereal chem.* (65) (1) 44 – 48.
- Wang, Y.D. (1977). *Enrichment of Ingredients for fabricated foods by fermentation and germination of corn and sorghum* Ph.D. Thesis, University of Missouri, Columbia, M.O.
- Ziad, S. Abdel Moueium, Abdullahi H. EL Tiny & Abdel H. Abdalla (1996). Effect of germination on protein fractions of corn cultivars. *Food chem.* 56 (2), 381 – 384.