

The Effects of Malting Conditions on the Diastatic Power of Three Malted Nigerian Sorghum Cultivars

¹Moneke, A. N., ¹Okolo, B. N., ¹Orji, N. O. and ²Ire, F. S.

¹Brewing Science Laboratory, Department of Microbiology, University of Nigeria, Nsukka, Nigeria

²Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria

Corresponding author: Moneke, A. N. Brewing Science Laboratory, Department of Microbiology, University of Nigeria, Nsukka, Nigeria. Email: annymoneke@yahoo.com Phone: +234 803 3357 734

Abstract

The effects of malting conditions on the diastatic power of three malted sorghum cultivars (SK 5912, ICSV 400 and KSV 8) were investigated. The three Nigerian sorghum cultivars were steeped in 0.01M NaOH, 0.01M Ca(OH)₂ and distilled water, respectively, under air-rest and continuous steeping regimes. SK 5912 steeped in Ca(OH)₂ gave higher hot water and cold water extract values than when steeped in NaOH and distilled water. KSV 8 when steeped in distilled water gave peak values for cold water extract during air-rest and continuous steeping regimes. For hot water extract, KSV 8 had peak values when steeped in NaOH whereas SK 5912 gave the least values when steeped in distilled water. Cold water soluble-carbohydrate gave peak values for ICSV 400 when steeped in Ca(OH)₂ and the least values were recorded in NaOH steeped grains. The diastatic power of the three sorghum cultivars improved with increase in germination time except for ICSV 400-steeped in Ca(OH)₂ and KSV 8 steeped in distilled water which showed decrease in activity on the 4th day in continuous steeping regime. The results show that steeping in dilute alkali solutions gave higher values than distilled water while air-rested steeping regime gave better values than continuous steeping regime. The results from this study suggest that increase in germination time enhanced the diastatic power of the malted grain.

Keywords: Sorghum varieties, Malting, Diastatic power, Cold water extract, Hot water extract

Introduction

Sorghum (*Sorghum bicolor* (L) Moench) is a major food crop and is ranked fifth in terms of world cereal production after wheat, rice, maize and barley (Taylor and Belton, 2002, Doggeth, 1988). Much sorghum is malted to brew opaque beer in most parts of Africa including Nigeria and European type lager beer and non-alcoholic malt beverages in several African countries (Taylor and Dewar, 2001; Beta *et al.*, 1995). The art of malting has been with Africans for long and the malting procedure is basically the same in all parts of the continent (Abiodun, 2002). Malting is the limited germination of cereals in moist air, under controlled conditions, with the objective of mobilizing the endogenous hydrolytic enzymes especially amylase of the grain which attacks the α -1-4 glucosidic bonds in starch molecules (Kanauchi and Bamforth, 2008; Taylor and Belton, 2002). Malting involves steeping, germination and kilning of the grain until the food store (endosperm) which is available to support the development of the germ of the grain, has suffered some degradation from enzymes. The primary aim of malting is to develop and activate enzymes for the conversion of the grains storage protein reserves into assimilable nitrogenous forms for yeast growth and fermentation (Baxter, 1981; Pierce, 1982). The activities of these enzymes are terminated by kilning the young plant (green malt) so that the endosperms are not completely depleted through respiration of the embryo and its growth (Ogu *et al.*, 2004). Sorghum has replaced barley in Nigeria as the primary source of extract for brewing. The primary malting characteristics of sorghum

(*Sorghum bicolor*) have been compared, and diastatic power, α -amylase, amyloglucosidase and protease activities increased with malting time and malt modification (Okungbowa *et al.*, 2002; Muoria *et al.*, 1998). Low temperature kilning schedules produce sorghum malts with greater diastatic power and α -amylase. However, improvement in sorghum cultivar selection, manipulation of steeping regimes and germination conditions improve sorghum malt quality for lager brewing. It has been noted that higher germination temperature resulted in high malting losses (Badau *et al.*, 2006). An important feature of malt quality for brewing is the level of amylase activities in hot water and cold water extracts, which develop during germination of sorghum grains. Several researchers have observed that steeping sorghum grain in dilute NaOH gave malts with improved diastatic power and free amino nitrogen (FAN), reduced malting loss, enhanced carbohydrate and protein mobilization without any adverse effect on the grains (Beta *et al.*, 1995; Agu and Palmer, 1996). The combined and coordinated action of starch degrading enzymes is termed diastatic power, with α -amylase and β -amylase activities most highly correlated (Lefyedi and Taylor, 2006). High level of diastatic power is required in brewing processes and is an important characteristic for estimating the quality of malt for beer production (Chen *et al.*, 2006; Evans *et al.*, 1995). Georg-Kraemer *et al.* (2001) found that β -amylase activity was a better predictor of diastatic power (DP) than α -amylases in barley grains, and increased markedly during germination. This paper therefore describes the effects of alkaline steeping on the development of diastatic power in three sorghum malt varieties.

Material and Methods

Sorghum varieties: Three improved Nigerian sorghum varieties, SK5912, KSV8 and ICSV400, were obtained from the National Seeds Service, Zaria, Nigeria. The grains had good germinative energies and were not water sensitive.

Sorghum washing: Grains for malting were cleaned by winnowing and sorting to remove dust, broken kernels and foreign materials. Thereafter, sorted samples, in triplicates of 200 grain batches, were surface sterilized by immersion for 40 min in hypochlorite solution having 1% (v/v) available chlorine. Subsequently, the grains were drained and washed severally in tap water (Morrall *et al.*, 1986).

Steeping of grains: Two hundred grain batches of each sorghum sample, with good moisture contents were steeped in 400 ml distilled water, 0.01M sodium hydroxide and 0.01M calcium hydroxide, respectively. A steep cycle of 6 h wet; 3 h dry for 45 h was applied. In another regime, continuous (con) steeping, grains were continuously steeped in distilled water, sodium hydroxide and calcium hydroxide with liquor being changed after 9h.

Germination and kilning: At the end of steeping, grains were again surface-sterilized prior to germination and processed for analyses as described previously (Ezeogu and Okolo, 1995). Germination lasted for 5 days in an atmosphere of near saturation at 30°C while samples were killed daily in a forced draught oven at 50°C (Novellie, 1960; Narziss *et al.*, 1973).

Moisture content: Moisture contents of the grains were determined at 0, 12, 24, 30, 36, 40 and 45 h of steeping, using the methods of the Association of Official and Analytical Chemists (AOAC, 1980).

Analyses

Root lengths and malting loss: At the end of germination, 20 kernels each of the malted sorghum were randomly selected and their root lengths measured using a ruler as previously described (Ezeogu and Okolo 1995). Malting loss was determined according to published procedure (Aniche and Palmer, 1990).

Diastatic power and amylase activity: Diastatic power (total reducing activity) and α -amylase activity were determined by the diamylase procedure of Etokakpan and Palmer (1990) in which α -amylase activity was calculated as the difference between diastatic power and β -amylase activity. One unit of enzyme activity was defined as any amount of enzyme capable of releasing 1 μ g glucose equivalent per minute.

Cold and hot water extracts: Cold water extracts were determined as described by Ezeogu and Okolo (1995). Hot water extracts were determined according to the procedure described by Etokakpan (1992) in which enzymic wort is separated and then

re-added to the gelatinized and cooled sorghum starch.

Results and Discussion

The sorghum cultivars used in this study showed good germinative properties as shown in their high viabilities. There were no mould growths on the sorghum cultivars. The major objective of malting is to promote the development of hydrolytic enzymes which are not present in the non-germinated grain. Sorghum malt quality is assessed primarily in terms of diastatic power and free amino nitrogen. Diastatic power is a measure of the joint activity of α - and β -amylases. The diastatic power of the malts increased with increasing germination time until about the 5th day (Fig. 1) in air rest steeping for SK 5912. Figures 2 and 3 depict the diastatic profile of SK 5912 and ICSV 400 during continuous steeping, where decline in diastatic power were noticed on the 3rd and 4th day of germination, respectively.

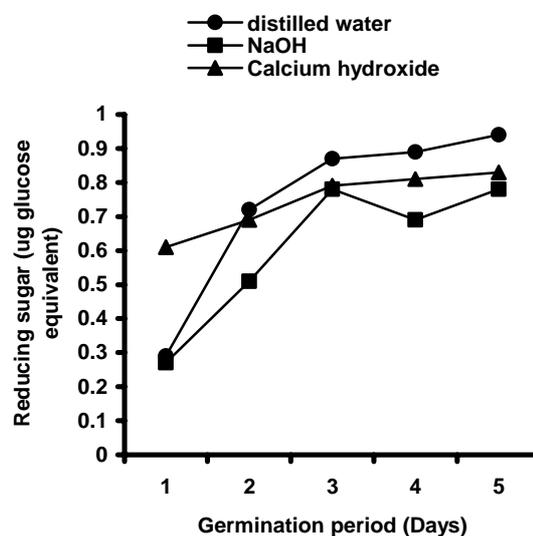


Fig. 1: Diastatic power of SK 5912 for air rested

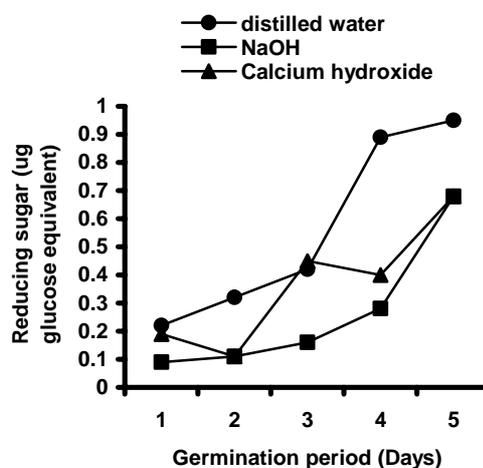


Fig. 2: Diastatic power for SK 5912 continuous steeping

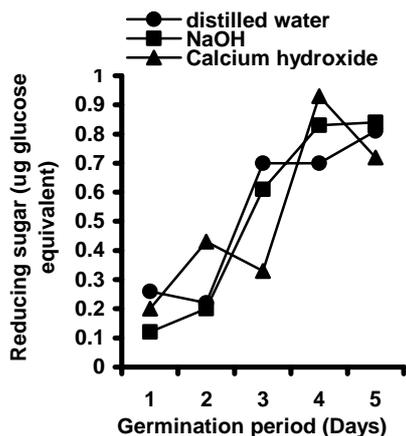


Fig. 3: Diastatic power for ICSV 400 continuous steeping

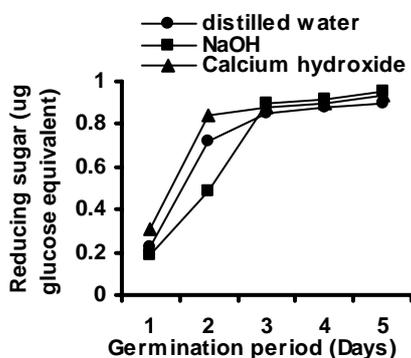


Fig. 4: Diastatic power for ICSV 400 air rested

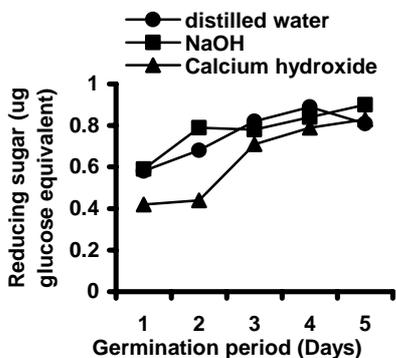


Fig. 5: Diastatic power for KSV 8 continuous steeping

This is in agreement with the reports of Dewar (1999). In the alkali treatment study, it was found that SK 5912 and ICSV 400 when steeped in $\text{Ca}(\text{OH})_2$ gave higher values of diastatic power while KSV 8 had peak values in NaOH (Figures 4 and 5). This finding supports what was reported by Okolo and Ezeogu (1996) that alkaline steeping improved the diastatic power of malts. Lefyedi and Taylor (2006) reported that steeping sorghum grain in dilute NaOH gave malts with improved diastatic power, reduced malting loss and enhanced carbohydrate mobilization without any adverse effects on the grain.

Okungbowa *et al.* (2002) showed that malt α -amylase level is critically correlated with environmental conditions of steeping and kilning. They observed that the low β -amylase activity of sorghum malts has been identified as one of the most serious obstacles to its use as replacement for barley malts. As shown in Figures 6 and 7, β -amylase gave least value when compared with α -amylase. Okokon (2004) has equally observed that sorghum malt amylases are less stable than those of barley malt. The results from this study equally reflect this observation as can be seen from Figures 8 and 9. Hence our result is consistent with previous work (Okokon, 2004). Abiodun (2002) stated that diastatic activity was positively correlated with the cold water and hot water extracts. The cold water extract increased as the diastatic activity increased for all the cultivars (Figures 10 and 11) and enzyme activities are responsible for both hot and cold water extracts. In cold water extract, malts that underwent air-rest treatment gave higher values when compared to malts that underwent continuous steeping treatment. This is consistent with previous reports that the ability to solubilize endosperm reserve materials depends on aeration, good contact between enzyme and substrate-complex of the grains (Okolo and Ezeogu, 1996).

Bush *et al.* (1986) reported that Ca^{2+} stabilized the activities of hydrolytic enzymes such as α -amylase. This view was consistent with our result where it was observed that Ca^+ stabilized the activities of both α and β amylases (Figure 12 and 13). $\text{Ca}(\text{OH})_2$ -treated malts gave higher values when compared to other alkaline liquor and distilled water control malts. In hot water extract development, distilled water control malts recorded high values in the three cultivars (data not shown). In this study, malts subjected to continuous steeping regimes gave higher values in the three cultivars when compared with those of air-rested malts. This is in contrast to the report of Ezeogu and Okolo (1995) which showed that air-rest favoured increase in germination and extract generation.

Cold water soluble-carbohydrate development was significantly ($p < 0.01$) affected by steep liquor treatment and duration of germination. Grains steeped in alkali gave higher values than distilled water-steeped grains. This is in agreement with the work of Okolo and Ezeogu (1996b) which states that dilute alkaline enhanced general malt quality by enhancing seed coat modification, increasing water uptake and promoting improved hydrolytic enzyme synthesis and activity. This indicates that an important factor in determining the cold water soluble-carbohydrate is the steep liquor treatment.

Conclusion: In this study, the effects of different dilute alkali steeping liquors and different steeping regimes on the cold water extract, hot water extract, cold water soluble carbohydrate and diastatic power of three different malted sorghum cultivars were investigated. The results indicated that steeping of grains in dilute alkali produced good quality malt.

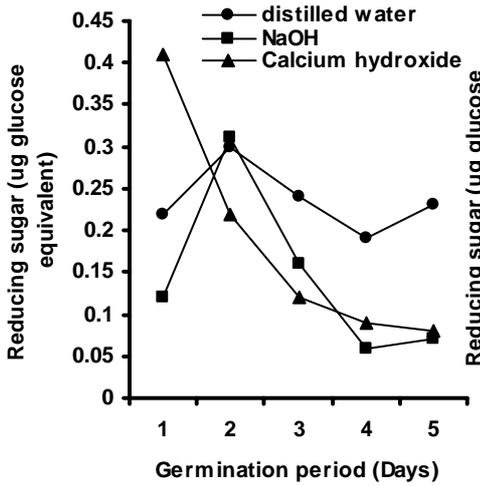


Fig. 6: Beta amylase activity for SK 5912 air rested

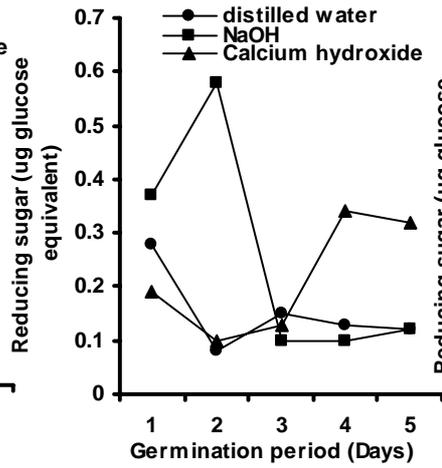


Fig. 7: Beta amylase activity for KSV 8 continuous steeping

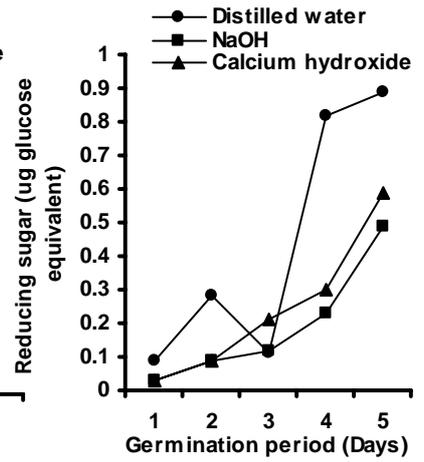


Fig. 8: Alpha amylase activity for SK 5912 continuous steeping

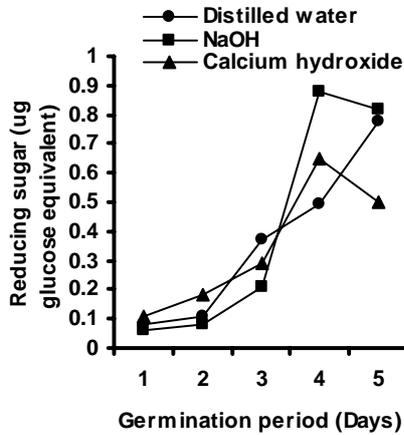


Fig. 9: Alpha amylase for ICSV 400 continuous steeping

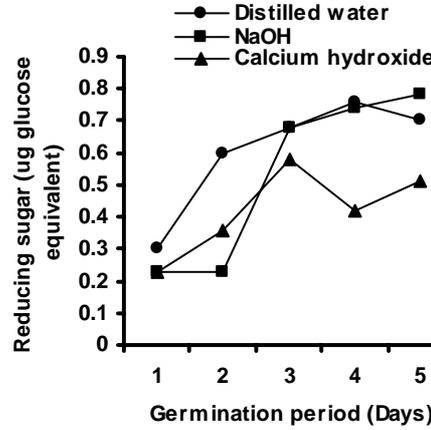


Fig. 10: Alpha amylase activity for KSV 8 continuous steeping

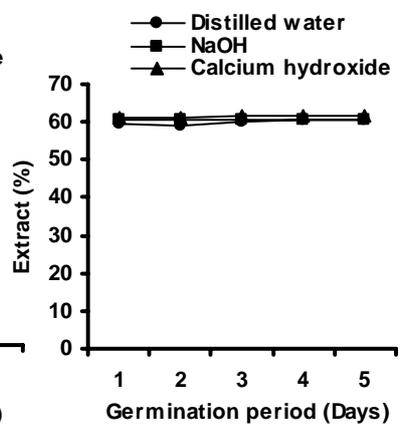


Fig. 11: Cold water extract for ICSV air rested

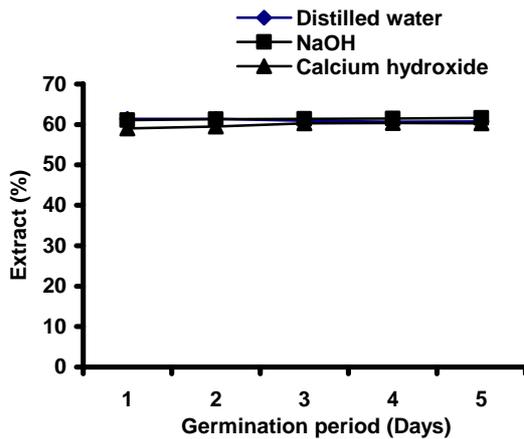


Fig. 12: Cold water extract for KSV air rested

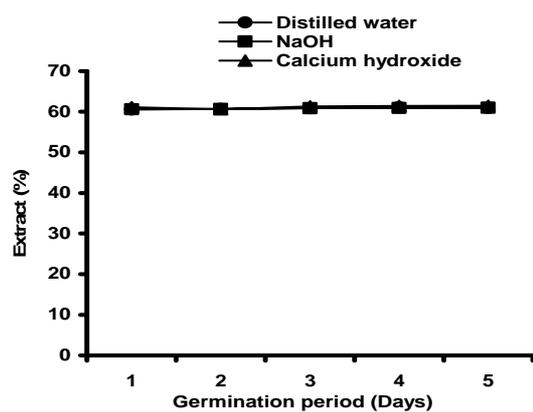


Fig. 13: Cold water extract for SK 5912 air rested

The results obtained from this study also indicated that grains steeping using air rest regime influenced the development of diastatic power than grains subjected to continuous steeping regime.

Thus, suggesting that diastatic power may be strongly dependent on the variety of the sorghum cultivar, steep liquor and germination period. This confirms the reports of other workers which show that air-rest steeping resulted in increase of extract.

Therefore, proper selection of sorghum cultivar and steep liquor will enhance the development of diastatic power of malts.

References

- Agu, R. C. and Palmer, G. H. (1996). Enzymatic breakdown of endosperm proteins of sorghum at different malting temperatures. *Journal of the Institute of Brewing*, 102: 415 – 418.
- Abiodun, A. (2002). The effect of kernel size and texture in the malting properties of sorghum. *The Journal of Food Technology in Africa*, 7(3): 78 - 81.
- Aniche, G.N. and Palmer, G.H. (1990) Development of amylolytic activities in sorghum malt and barley malt. *Journal of the Institute of Brewing*, 96: 377 – 379.
- Association of Official and Analytical Chemists (1980) Official Methods of Analysis. The Association Washington DC, 1980.
- Badau, M. Y., Yideani, I. A. and Nkama, I. (2006). Amylase activities and values in hot and cold water extract of pearl millet. *J. Appl. Glycosci.*, 53: 1 – 6.
- Baxter, E. D. (1981). Hordein in barley and malt – a review. *Journal of the Institute of Brewing* 87: 173–176.
- Beta, T., Rooney, L. W. and Waniska, R. D (1995). Malting characteristics of sorghum cultivars. *Cereal Chemistry*, 72: 633 – 538.
- Bush, D. S., Cornejo, M. J., Huang, C. N. and Jones, R. L. (1986). Ca^{2+} stimulated secretion of amylases during developments in barley aleurone protoplasts. *Plant Physiology*, 82: 566
- Chen, J., Dai, F., Wei, K. and Zhang, G. (2006). Relationship between malt qualities and β -amylase activity and protein content as affected by timing of nitrogen fertilizer application. *J Zhejiang Univ Science B*, 7(1): 79 – 84.
- Dewar, J. (1999). Influence of malting on Sorghum protein quality: A report submitted to CSIR *Environmentek South Africa*, pp. 1-6
- Doggeth, H. (1988). *Sorghum*. 2nd edition. Longman Scientific and Technical. London, pp. 450-453.
- Etokakpan, O.U. (1992) Amylolytic potentials and wort fermenting components of Nigerian Sorghums and barley. *World Journal of Microbiology and Biotechnology*, 8: 287 – 289.
- Etokakpan, O.U. and Palmer, G.H. (1990) A simple diamylase procedure for the estimation of alpha and beta-amylase. *Journal of the Institute of Brewing*, 96: 89 – 91.
- Evans, D. E., Lance, R. C. M., Elington, J. K., Logue, S. J. and Barr, A. R. (1995). The influence of β -Amylase Isoform Pattern on β -Amylase Activity in Barley and Malt. *Proc. 45th Austr. Cer. Chem. Conf.*, Adelaide, p.357-364.
- Ezeogu, L.I. and Okolo, B.N. (1995) Effects of air rest periods on malting sorghum responses to final warm water steep. *Journal of the Institute of Brewing*, 101: 39 – 45.
- Georg-Kraemer, J.E., Mundstock, E.C., Cavalli-Molina, S., (2001). Developmental expression of amylase during barley malting. *Journal of Cereal Science*, 33:279-288.
- Kanauchi, M. and Bamforth, C.W. (2008). The relevance of different enzymes for the hydrolysis of β -glucans in malting and mashing. *Journal of the Institute of Brewing*, 114(3): 224-229
- Lefyedi, M.L. and Taylor, J.R.N. (2006). Effect of dilute alkaline steeping on the microbial contamination, toxicity and diastatic power of sorghum malt. *Journal of the Institute of Brewing*, 112(2): 108-116.
- Morrall, P., Boyd, H.K., Taylor, J.R.N. and Vander-Walt, W.H. (1986) Effect of germination time, temperature and moisture on malting sorghum. *Journal of the Institute of Brewing* 92: 439–445.
- Muoria, J.K., Linden, J.C. and Bechtel, P.J. (1998). Diastatic power and α -amylase activity in millet, sorghum and barley grains and malts. *J. Am. Soc. Brew. Chem.*, 56(4):113-135.
- Narziss, L., Rusitka, P. and Stippler, K. (1973) Effect of the drying process on the development of malt enzymes and several other groups of components. *Proceedings of the European Brewery Convention Congress*, Salzburg, 85–98.
- Novellie, L. (1960). Kaffircorn malting and brewing studies. V: occurrence of b-amylase in kaffircorn malt. *Journal of the Science of Food and Agriculture* 11: 475–460.
- Ogu, E. O., Odibo, F. J. C., Agu, R. C. and Palmer, G. H. (2004). Malting studies of some selected sorghum varieties. *Tech. Q. Master Brew. Assoc. Am.*, 41(4): 386–389.
- Okokon, U.A (2004). Changes in sorghum malt during storage. *Journal of the Institute of Brewing*, 110(3): 189 – 192.
- Okolo, B.N. and Ezeogu, L.I. (1996). Enhancement of amylolytic potential of sorghum malts by alkaline steep treatment. *Journal of the Institute of Brewing*, 102: 79-85.
- Okolo, B.N. and Ezeogu, L.I. (1996b). Promoting sorghum reserve protein mobilisation by steeping in alkaline liquor. *Journal of the Institute of Brewing*, 102: 277-284
- Okungbowa, J., Obeta, J.A.N. and Ezeogu, L.I. (2002). Sorghum β -amylase production, relationship with grain cultivar, steep regime, steep liquor composition and kilning temperature. *Journal of the Institute of Brewing*, 108(2): 362-370.
- Pierce, J.S. (1982) The Margaret Jones Memorial Lecture: amino acids in malting and brewing. *Journal of the Institute of Brewing* 88: 228–233.
- Taylor, J.R.N. and Belton, P.S. (2002). Sorghum. In: *Pseudocereals and Less Common Cereals*. Belton, P.S and Taylor, J.R.N (eds). Springer-Verlag Berlin. pp 55-59.
- Taylor, J.R.N. and Dewar, J. (2001). Developments in sorghum food technologies. *Advances in Food and Nutrition Research*, 43: 217-264.