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Full Length Research Paper

Calcium regulation of carbohydrate modification in sorghum

Bartholomew N. Okolo[1], Anene N. Moneke[1]*, Lewis I. Ezeogu[1] and Francis S. Ire[2]

[1]Brewing Science Laboratory, Department of Microbiology, University of Nigeria, Nsukka, Nigeria.
[2]Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria.

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Two improved Nigeria sorghum cultivars (KSV 8 and ICSV 400) were used to evaluate the effects of steep water Ca^{2+} treatment on carbohydrate modification in sorghum. The response of all the carbohydrate mobilization indicators evaluated [α- and β- amylases, diastatic activity (DP), extract and cold water soluble carbohydrates (CWS-carbohydrates)], to steep water Ca^{2+} treatment was highly significantly ($p \leq 0.001$) cultivar and steep water Ca^{2+} treatment dependent. In contrast to KSV 8, Ca^{2+} treatment generally caused significant repression of α-amylase development in ICSV 400. Development of β-amylolytic activity in KSV 8 was however, significantly repressed by Ca^{2+} treatment. Interestingly, β-amylase activity constituting well over 80% of total diastatic activity was attained in ICSV 400 grains subjected to 100 ppm Ca^{2+} treatment. Hot water extract (HWE) showed statistically insignificant ($p \geq 0.1$) linear variation with Ca^{2+} treatment. Although Ca^{2+} treatment significantly ($p \leq .001$) repressed CWS-carbohydrates in both cultivars, significantly higher CWS-carbohydrates and HWE were released in ICSV 400 for each DP unit than the corresponding DP in KSV 8 malts would permit. Thus, suggesting important roles for factors other than DP, possibly proteolysis, in determining HWE and CWS-carbohydrates. The benefits of reduced kernel growth and malting loss were neutralized by the general repression of carbohydrate modification indices for both cultivars.

Key words: Sorghum malt, steep water Ca^{2+} treatment, amylase, CWS-carbohydrate, diastatic power, 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). Barley is traditionally the cereal chosen for malting in order to develop enzymes (Kuntz and Bamforth, 2007). In Nigeria, where attempts to cultivate barley have met with little success, the high cost of importing barley malt, in conjunction with the rising demand for European-type lager, has forced the use of local cereals particularly sorghum, as a malting and brewing grain. Hence, sorghum has replaced barley in Nigeria as the primary source of extract for brewing.

Several studies have demonstrated that cations, especially Ca^{2+}, Mg^{2+} and K^{+}, play important roles in the regulation of endosperm mobilization during cereal grain germination (Brookes et al., 1976; Bush et al., 1986; Chrispeels and Varner, 1967; Diekman and Jones, 1986; Glennie et al., 1983; Jones and Jacobsen, 1983; Varner and Mense, 1972). Ca^{2+} is believed to exert a strong influence on both barley aleurone and rice scutellum by stimulating selective synthesis and secretion of α-amylase isoforms and other hydrolytic enzymes (Bush et al., 1986; Chrispeels and Varner, 1967; Diekman and Jones, 1986; Jacobsen et al., 1973; Moll and Jones, 1982; Stuart et al., 1986). The site at which Ca^{2+} exerts its influence and the molecular mechanism of its action are still unknown (Beck and Ziegler, 1989; Fincher, 1989). However, it is believed that Ca^{2+} participates in gibberellic acid- induced stimulation of α-amylase synthesis and

*Corresponding author. E-mail: annymoneke@yahoo.com. Tel: +234 803 3357 734

secretion and for other endosperm degrading enzymes in the barley aleurone cells (Okon and Uwaifo, 1985). This plant hormone does not elicit an equivalent response in sorghum aleurones (Aisien et al., 1986; Daiber et al., 1973). Although there are numerous studies on various aspects of the physiology and biochemistry of sorghum malting (Aisien and Muts, 1987; Aisien and Palmer, 1983; Dufour et al., 1992; Etokakpan and Palmer, 1990; Hudson, 1986; Ilori and Adewusi, 1991; Malleshi and Desikachar, 1986; Morrall et al., 1986; Ogbonna et al., 2003; Palmer, 1989; Taylor, 1983), the role of Ca^{2+} in the regulation of sorghum endosperm degradation is not known. Previous studies (Okolo and Ezeogu, 1996) revealed that two improved Nigerian sorghum cultivars ICSV 400 and KSV 8 possess high malting qualities which compared well with barley. Therefore, this study was designed to assess the influence of Ca^{2+} concentration in steep water on carbohydrate mobilization in two improved Nigerian sorghum cultivars ICSV and KSV 8 malts.

MATERIALS AND METHODS

Grains samples

Two improved Nigeria sorghum cultivars ICSV 400 and KSV 8 used in this study were obtained from the National Seeds Service, Zaria, Nigeria. The grains had good germinative energies and were not water sensitive (Ezeogu and Okolo, 1994).

Sorghum malting

Grains were prepared for malting as described previously (Ezeogu and Okolo, 1994). Surface sterilized grains were steeped in double deionized water for 53 h at 30°C and with warm water (40°C) final steep lasting 6 h. The steep cycle was as follows: 8 h wet; 3 h dry, 6 h wet; 3 h dry; 6 h wet; 3 h dry; 6 h wet; 3 h dry; 6 h wet; 3 h dry; 6 h wet. Varying concentrations of $CaCl_2$ in the range 100 - 500 ppm were added to the steep water. A control steep was set up in which $CaCl_2$ was not added to the steep water. At the end of each steep cycle, grains were once again submitted for surface sterilization as described earlier (Ezeogu and Okolo, 1995). Steep out moisture content for all grains was 40 - 42%. Germination was conducted in shallow trays with fine mesh bottom in wooden boxes in an atmosphere of near water saturation at 30°C for four days. At 12 h intervals grains were turned and sprayed with 10 ml of deionized water using an atomizer spray. After germination, grains were dried for 24 h at 50°C in a forced draught oven. Roots and shoots were removed manually. Malts were subsequently milled for two 30 s periods in a cooled Waring blender at high speed and used for analyses.

Analyses

Root lengths and malting loss:

At the end of germination, 20 kernels each of the germinated sorghum were randomly selected and their roots lengths measured using a ruler as described elsewhere (Ezeogu and Okolo, 1994; Ilori and Adewusi, 1991). Malting loss was determined using published procedures (Recommended Methods of Analysis, 1986).

Diastatic power and amylase assays:

Diastatic activity and α-amylase activity of the sorghum malts were determined as described previously (Ezeogu and Okolo, 1994) using the diamylase procedure of Etokakpan and Palmer (1990). β-Amylase activity was calculated as the difference diastatic power and α-amylase activity. One unit of the enzyme activity was defined as any amount of enzyme that is capable of releasing 1 µg glucose equivalent under the assay conditions.

Extract:

Cold water extract (CWE) of the sorghum malts was measured using Holmes (1991) modification of the Recommended Methods of Analysis (1986). Hot water extract (HWE) was determined according to the decantation method described by Etokakpan (1992) where the enzymic wort is separated, then re-introduced into the gelatinized and cooled sorghum malt mash. The cold water soluble carbohydrate (CWS-carbohydrate) that is, the soluble carbohydrate fraction of the total cold water soluble (CWE) was determined according to the procedure outlined by Holmes (1991). Samples of malt (1.250 g) in snap-top 30 ml polythene centrifuge tubes containing deionized water were mixed with 2.5 ml each of the malt extractant and enzyme de-activant solution containing $ZnSO_4$ (0.2 N) and Ba $(OH)_2$ (0.2 H), respectively. Extraction was conducted with continuous shaking for 1 h in a laboratory box shaker at ambient temperature. The soluble fraction in the clear extract after centrifugation at 3000 g for 10 min was thereafter determined by the specific gravity method (Recommended Methods of Analysis, 1986).

Statistical analysis

The effects of $CaCl_2$ concentrations of the steep liquor on the carbohydrate related malt properties examined were analysed by two way analyses of variance (ANOVA) and the Kruskal-Wallis test (Cohen, 1988). Correlation analyses were also performed to determined how these malt properties varied in relation to level of calcium ion in steep liquor. Means that differed significantly were identified by the t-test and least significant difference (LSD) tests at $p \leq 0.001$. Results are presented as means of triplicate experiments.

RESULTS AND DISCUSSION

The effects of different concentrations of calcium ion (Ca^{2+}) in steep liquor on α-amylase development in the two sorghum cultivars ICSV 400 and KSV 8 were investigated. The results are depicted in Table 1 where it can be seen that the pattern of α-amylase activity response to Ca^{2+} treatment differed clearly in both cultivars. Highest α-amylase activity in ICSV 400 (99.0 units) was exhibited on exposure of grains to 300 ppm steep liquor Ca^{2+} concentration. This amylase activity was significantly lower than the activity obtained for control malts (153.7 Units). Thus, Ca^{2+} treatment apparently caused a general repression of α-amylase development in ICSV 400. At the best, a 35% reduction in α-amylase activity relative to control values was obtained for this cultivar. However, α-amylase activity in ICSV 400 increased progressively as the concentration of Ca^{2+} in

Table 1. Effects of $CaCl_2$ concentration of steep water on the α-amylase activity of sorghum cultivars ICSV 400 and KSV 8 Malts (means, n = 3).

Cultivar	α-Amylase activity (µg glucose equivalent) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	153.7	28.2	42.4	99.0	56.6	84.9
KSV 8	113.1	56.6	160.6	70.7	96.32	84.9

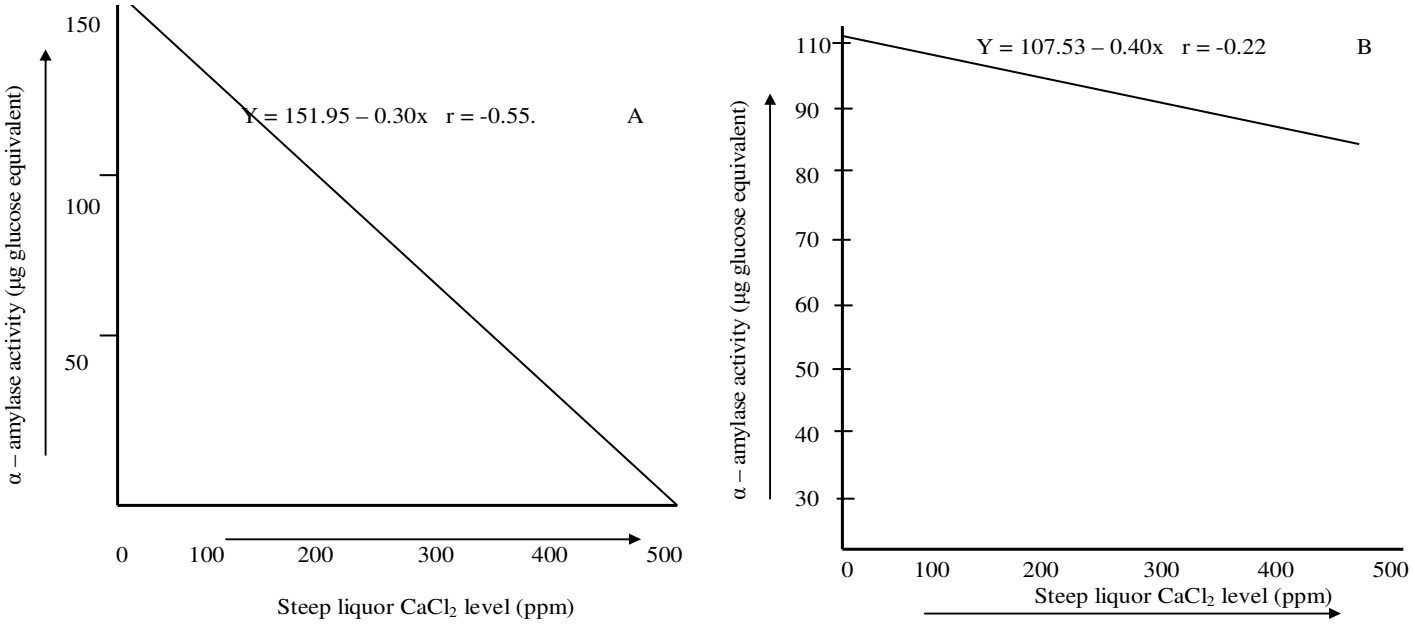

Figure 1. Correlation of steep liquor $CaCl_2$ concentration with malt α -amylase for sorghum cultivars (A) ICSV400 and (B) KSV8.

the steep liquor was increased from 100 to 300 ppm. Thereafter, further increases in Ca^{2+} treatment elicited obvious reductions in enzymes activity. Nevertheless, a second but lower high point of α-amylase activity was observed on exposure of the grains to 500 ppm Ca^{2+} treatment. For KSV 8, no definite trend in amylase development was observed. Highest activity was recorded on exposure of grains to 200 ppm steep liquor Ca^{2+} concentration with a second high point occurring at 400 ppm Ca^{2+} treatment. Steeping KSV 8 in liquor containing 100 ppm Ca^{2+} caused about 50% reduction in α-amylase activity compared to control values. It is however striking that a highly significant ($p < 0.001$) stimulation of α-amylase development was observed for KSV 8 grains subjected to 200 ppm steep liquor Ca^{2+} level at 169.6 units. This value represents the highest α-amylase activity for KSV 8 and a 50% enhancement, over control values. These observations indicate that the response of α-amylase development to Ca^{2+} treatment is cultivar related. However, correlation analyses confirm a poor relationship between the two factors for both cultivars (Figure 1). The relevance of steep treatment in the development of the diastase enzymes during cereal grain malting is well documented (Aisien and Palmer, 1983; Axcell et al., 1983; Filgueira, 1976; Jones and Jacobsen, 1983; Pollock, 1962). Specifically, the use of calcium chloride in steeps to regulate malt α-amylase synthesis in barley has been well researched (Bush et al., 1986; Jacobsen et al., 1970; Jones and Jacobsen, 1983). The differences in the response of sorghum ICSV 400 and KSV 8 α-amylase to Ca^{2+} treatment may reflect highly significant differences in grain physiology and biochemistry; perhaps arising from the inability of certain cultivars to synthesize particular enzyme isoforms when subjected to specific treatments or the inability of the grains to activate their α-amylases by degrading α-amylase inhibitor substances. Several authors (Bush et al., 1986; Jones and Jacobsen, 1983; Varner and Mense, 1972) have reported that Ca^{2+} selectively stimulated synthesis of the high P1 α-amylase isoforms in barley. Besides, the capacity of Ca^{2+} to promote synthesis of this particular α-amylase isoform in barley seems to be closely related to the presence of gibberellic acid (GA_3) within the endosperm of the grains (Bush et al., 1986). A phenomenon which appears to suggest that Ca^{2+} induced enhancement of α-amylase synthesis in barley is due to improved aleurone activity stimulated by the presence of gibberellic acid. This may also explain the good

Table 2. Effects of $CaCl_2$ concentration of steep water on the β-amylase activity of sorghum cultivars ICSV 400 and KSV 8 Malts (means, n = 3).

Cultivar	β-Amylase activity (µg glucose equivalent) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	100.3	155.6	28.3	42.4	56.6	28.3
KSV 8	127.29	56.6	0.0	56.6	110.4	84.9

correlation between level of Ca^{2+} in steep liquor and starch solubilising capacity in barley grain during germination (Bush et al., 1986).

Unlike in the case of barley, the scutellum is the major site of de Novo enzyme synthesis in sorghum (Aisien, 1982; Aisien and Palmer, 1983; Aisien et al., 1986; Hill and MacGregor, 1988; Koehler, 1981). Contributions from the aleurone account only for very little amount usually < 10% of total α-amylase synthesis in sorghum during germination (Hill and MacGregor, 1988). Therefore, the poor correlation established between α-amylase activity and steep liquor Ca^{2+} level, for both cultivars (Figure 1) confirm the inability of Ca^{2+} treatment to stimulate production of satisfactory levels of α-amylase activity by the aleurone cells of sorghum. Since the role of Ca^{2+} in seed α-amylase synthesis is closely related to GA_3 stimulation of cereal grain aleurone cells (Jacobsen and Higgins, 1982; Jacobsen et al., 1970; Jones and Jacobsen, 1983), the inability of Ca^{2+} to promote satisfactory α-amylase activity in our sorghum varieties might be related to absence of GA_3 stimulation of aleurone cells in sorghum and is consistent with previous reports (Aisien, 1982; Aisien and Palmer, 1983; Aisien et al., 1983; Koehler, 1981).

Furthermore, the differences noted in the pattern and extent of α-amylase development in ICSV 400 and KSV 8 may be a reflection of the responsiveness of the respective aleurones of these grains to Ca^{2+} treatment and invariably GA_3 stimulation. Another plausible explanation for the observed $CaCl_2$ inhibition of α-amylase development in both sorghum cultivars would be that proteolytic enzyme activity is repressed leading perhaps to reduce rate of activation of dormant amylases through post-translational processing. It is also possible that the grains lose the capacity to degrade endogenous α-amylase inhibitor substances as a result of steep treatment. Activation of α-amylase through proteolytic processing of post translational products has been demonstrated in barley (Hill and MacGregor, 1988). The occurrence of two high points of α-amylase activity in the sorghum cultivars presumably suggests the possibility of production by the aleurones of at least two Ca^{2+} dependent α-amylase isoforms. It is also possible that the second high point of α-amylase activity might have resulted from improved stability of secreted α-amylase due to Ca^{2+} treatment. Similar opinions have been advanced for barley by various workers (Hill and MacGregor, 1988; Jacobsen et al., 1970; Jones and Jacobsen, 1983).

In a similar investigation, the effect of steep water calcium concentration on β-amylase development was evaluated. Data illustrated in Table 2 reveal that as for α-amylase development, β-amylase activity in the grains was highly significantly ($P < 0.001$) influenced by cultivar and steep water Ca^{2+} level as well as their pairwise interactions. The saccharifying activity ranged between 25 - 85% and 0 - 55% of total diastatic activity for ICSV 400 and KSV 8, respectively. Highest β-amylase activity was attained for ICSV 400 when grains were subjected to steeping in liquor containing 100 ppm $CaCl_2$. For KSV 8 however, the control steep that is, without $CaCl_2$ gave higher β-amylolytic activity than all the other steeps treated with Ca^{2+}. Moreover, the pattern of development of the saccharifying enzyme differed highly significantly in both cultivars. For instance, development of β-amylolytic activity in KSV 8 was completely repressed in steeps treated with 200 ppm Ca^{2+}. Further increases in Ca^{2+} beyond this level however, led to a resurgence of the enzyme activity with highest activity of 111.4 µg glucose equivalent attained on exposure of grains to 400 ppm Ca^{2+} treatment. Though β-amylolytic activity was similarly severely repressed at 200 ppm Ca^{2+} treatment for ICSV 400, an activity of 28 units still remained. These observations indicate that development of β-amylolytic activity in sorghum is both cultivar and steep Ca^{2+} related. Furthermore, no definite trend in β-amylolytic activity was observed with variation in steep water Ca^{2+} concentration for both cultivars. This observation is supported by the poor correlation between β-amylolytic activity development and Ca^{2+} treatment as shown in Figure 2 for both cultivars (ICSV 400 $r = - 0.68$; KSV 8 $r = - 0.007$).

In an earlier communication (Okolo and Ezeogu, 1995) the possibility of occurrence of at least two β-amylase enzyme forms in sorghum was proposed. The occurrence of two high points of β-amylolytic activity for both cultivars across the range of Ca^{2+} treatment evaluated in this study lends credence to this conclusion. It would appear therefore that for ICSV 400 and KSV 8, one of the β-amylase isoforms is optimally expressed at low Ca^{2+} treatment while the other is maximally secreted at high steep water Ca^{2+} level. Similar observations have been reported for barley Malleshi et al. (1986). Recently, several workers (Diekman and Jones, 1986; Etokakpan, 1992; Etokakpan and Palmer, 1990; Morrall et al., 1986) have demonstrated the possibility of producing significant levels of β-amylolytic activity in sorghum malts through careful modulation of malting physiology. In actual fact, in

Table 3. Effects of $CaCl_2$ concentration of steep water on the diastatic activity of sorghum cultivars ICSV 400 and KSV 8 malts (means, n = 3).

Cultivar	Diastatic activity (µg glucose equivalent) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	254.0	183.7	70.7	141.4	113.1	113.1
KSV 8	240.4	113.1	160.6	127.3	200.7	169.7

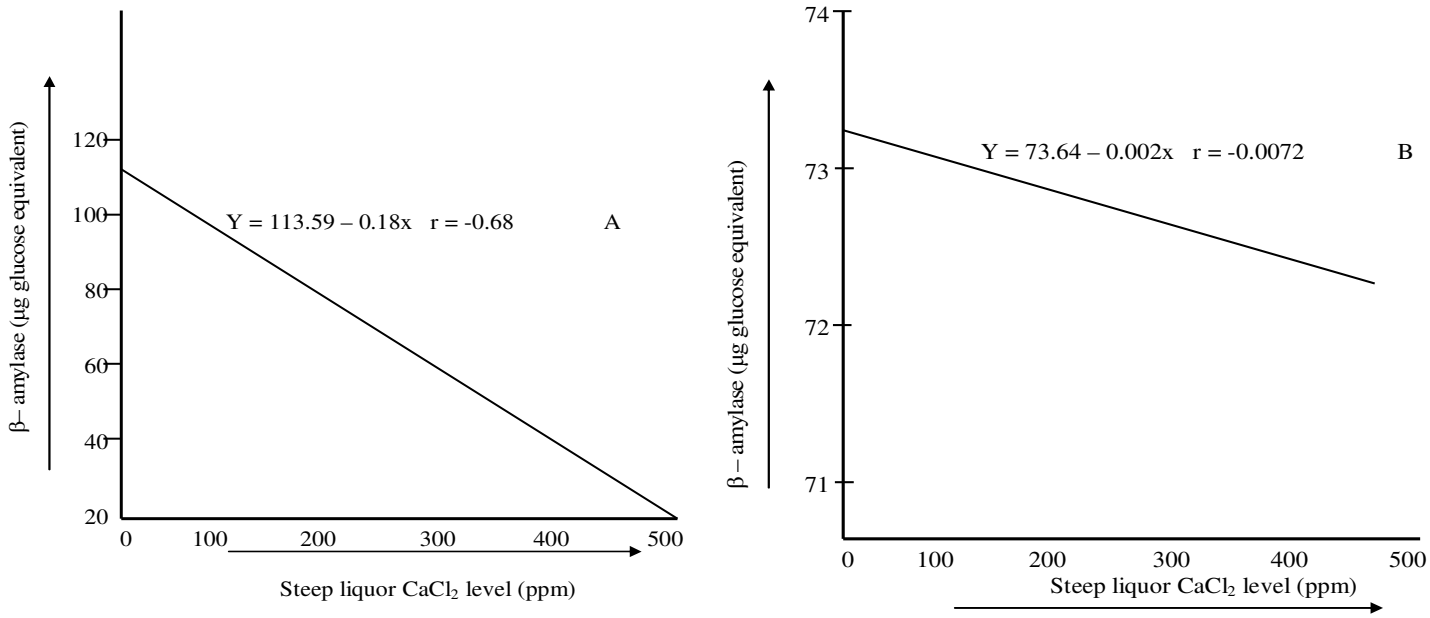

Figure 2. Correlation of steep liquor $CaCl_2$ concentration with malt β -amylase for sorghum cultivars (A) ICSV400 and (B) KSV8.

a preceding paper, β-amylase activity accounting for as much as 73% of total diastase activity was reported for ICSV 400 subjected to alkaline steeping. It is therefore not surprising that β-amylolytic activity in excess of 84% of total diastatic activity was obtained for ICSV 400 treated to 100 ppm Ca^{2+} in steep water.

The response of sorghum malts diastatic power of level of $CaCl_2$ in steep water is presented in Table 3. Highest diastatic activity was attained for ICSV 400 and KSV 8 on exposure of grains to steep liquors containing 100 and 400 ppm Ca^{2+}, respectively. However, these activities were lower than the diastatic activity recorded for control malts. There seemed to be no obvious trend in DP development as concentration of steep water $CaCl_2$ was increased for both cultivars. Both cultivars also showed two high points of diastatic activity (100 and 300 ppm Ca^{2+} treatment for ICSV 400 and 200 and 500 ppm Ca^{2+} treatment for KSV 8). In fact, KSV 8 in general exhibited higher diastatic activity than ICSV 400 in this study. This observation though at variance with previous DP levels obtained for malts from both cultivars supports our earlier opinion on the existence of differences in the malting physiology of the two cultivars (Ezeogu and Okolo, 1994; Ezeogu and Okolo, 1995; Okolo and Ezeogu, 1995). Two-way analyses of variance confirmed that cultivar and steep treatment plus their pairwise interactions are highly significant factors at P = 0.001 in diastatic power development in these sorghum cultivars. A highly significant linear variation of malt diastatic power with steep Ca^{2+} treatment was established by Kruskal-Wallis tests thus, confirming highly significant roles for these factors. Both cultivars exhibited unsatisfactory inverse correlation between steep Ca^{2+} treatment and malt diastatic activity development at 65 and 14%, respectively for ICSV 400 and KSV 8 (Figure 3).

Cold water extract is one of the most useful indicators of malt modification. It represents the soluble products of the enzymic hydrolysis from the malting process. These are readily available sugars and amino acids located in the endosperm of malts (Holmes, 1991; Irvine, 1985). The well modified malt value is usually about 20%. As shown in Table 4, cold water extract development was affected highly significantly at p = 0.001 by cultivar and Ca^{2+} content of steep liquor as well as their pairwise interactions. Cold water extract of the control malts for both cultivars were significantly higher than values obtained for malts derived from Ca^{2+} treated steeps. This clearly indicates that addition of Ca^{2+} to steep liquor

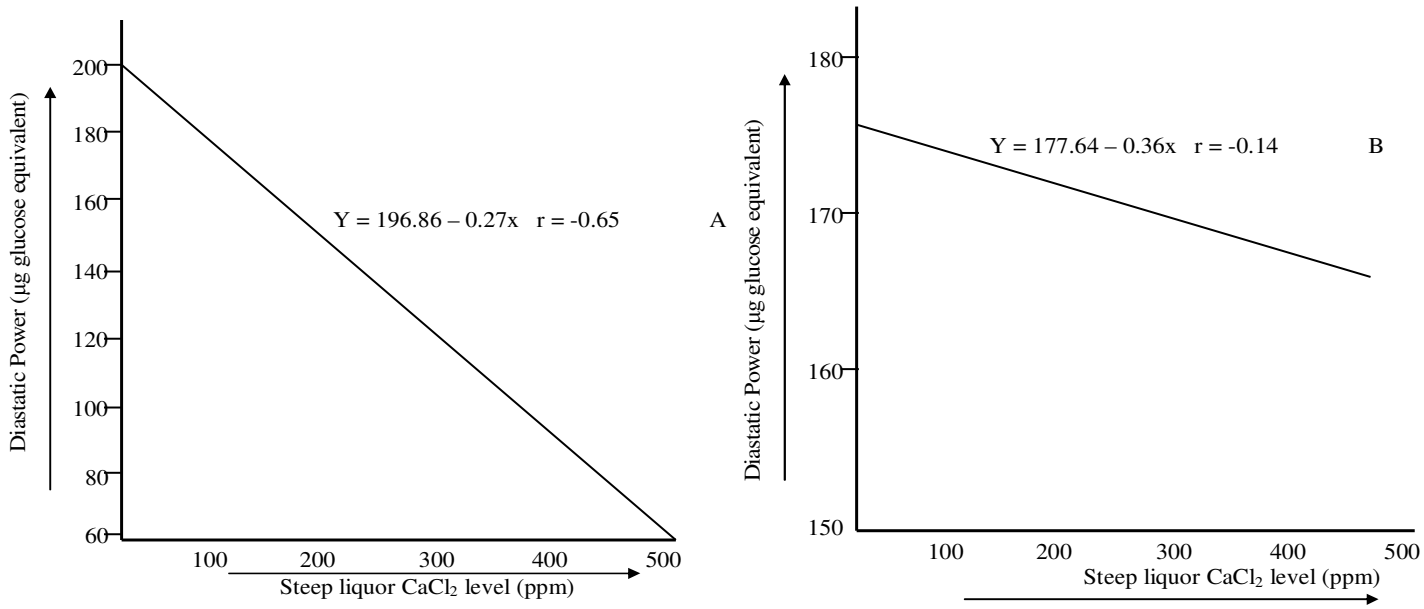

Figure 3. Correlation of steep liquor $CaCl_2$ concentration with diastatic Power activity of malt from sorghum cultivars (A) ICSV400 and (B) KSV8.

Table 4. Effects of $CaCl_2$ concentration of steep water on the cold water extract of sorghum cultivars ICSV 400 and KSV 8 malts (means, n = 3).

Cultivar	Cold water extract (%) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	33.8	25.0	20.8	22.9	18.7	14.1
KSV 8	24.5	16.7	19.3	23.4	16.2	13.0

significantly repressed development of total cold water soluble during malting. The extent of this repression, in general, appeared to increase as the concentration of Ca^{2+} in the steep water was increased. This observation is supported by the highly significant negative linear variation obtained between the two factors on application of Kruskal-Wallis test (Table 4). The effect of Ca^{2+} treatment on CWE however seemed to be more correlated in ICSV 400 (r = - 0.93) than KSV 8 (r = - 0.70) malts (Figure 4). A progressive increase in CWE as the Ca^{2+} concentration in steep water was increased from 100 to 300 ppm was observed in KSV 8 malts. This was followed by a fall in CWE level to a final low level of 13%. In contrast, ICSV 400 exhibited no definite pattern of CWE development with increasing Ca^{2+}treatment. These observations were confirmed by analyses of variance data which revealed that CWE was highly significantly influenced by grain variety, steep water Ca^{2+} treatment plus, their pairwise interactions.

As for most other malt properties evaluated, the hot water extracts of the sorghum malts were affected by cultivar and steep liquor Ca^{2+} concentration as well as their pairwise interactions (Table 5) in a highly significant ($p < 0.001$) manner. However, unlike others, HWE showed a statistically insignificant linear variation at $p > 0.1$ with Ca^{2+} treatment. In fact, the HWE obtained did not reflect reduced diastatic activities recorded for the malts at almost all levels of Ca^{2+} treatment. For example, HWE for ICSV 400 malts were generally higher than the values for KSV 8 and at all levels of treatment despite the higher DP, α- and β-amylase activities exhibited by KSV 8 malts. These observations are supported by the poor correlation between the two factors for ICSV 400 (r = 0.56) and KSV 8 (r = - 0.36) (Figure 5).

Holmes (1991) proposed the use of the proportion of total carbohydrates solubilised during malting as an indicator of malting and brewing potential of barley malts. The soluble carbohydrate fraction of the cold water extract was therefore determined in an attempt to verify if the proportion of carbohydrates solubilised during malting of sorghum would be related to the diastatic activity development across various steep water calcium ion concentrations. As for DP development, cold water soluble carbohydrates (CWS-carbohydrate) development in both sorghum cultivars was significantly ($P \leq 0.001$) repressed by Ca^{2+} treatment (Table 6). CWS-carbohydrate of malts

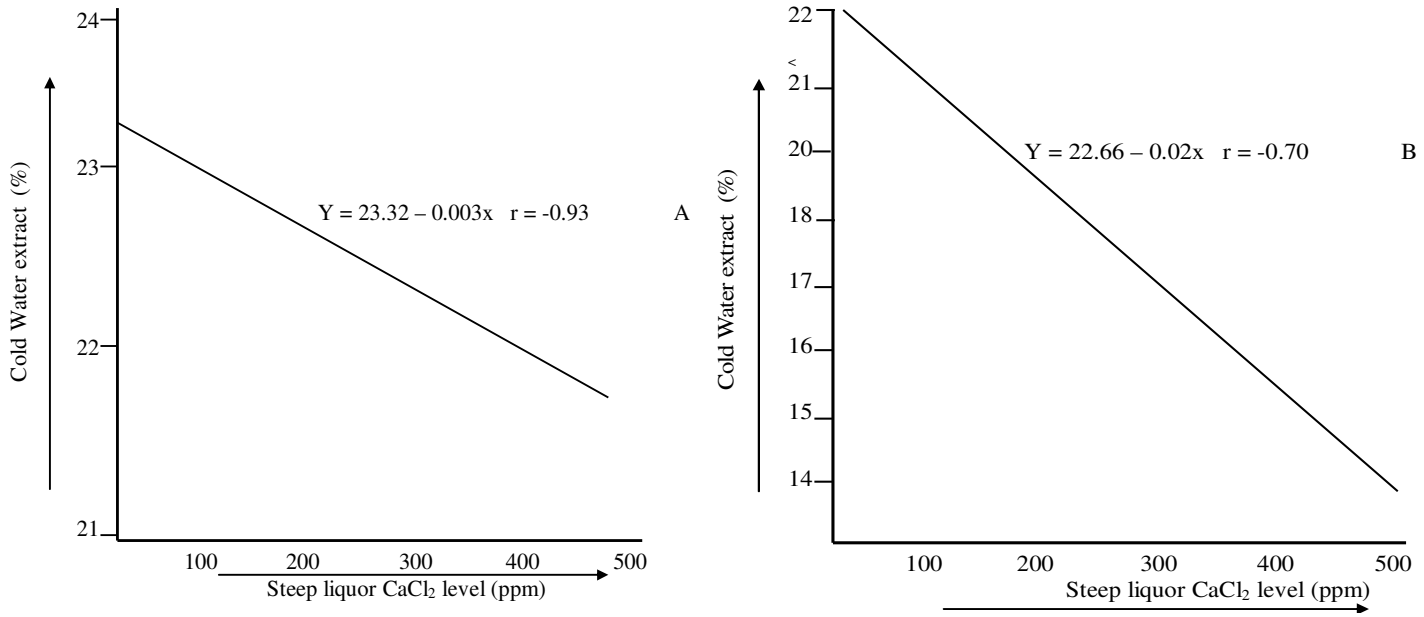

Figure 4. Correlation of steep liquor $CaCl_2$ concentration with Cold Water extract (CWE) for sorghum cultivars (A) ICSV400 and (B) KSV8.

Table 5. Effects of $CaCl_2$ concentration of steep water on the hot water extract of sorghum cultivars ICSV 400 and KSV 8 malts (means, n = 3).

Cultivar	Hot water extract (%) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	336.6	346.6	347.6	354.6	340.6	353.6
KSV 8	348.6	354.6	342.6	322.1	345.1	345.1

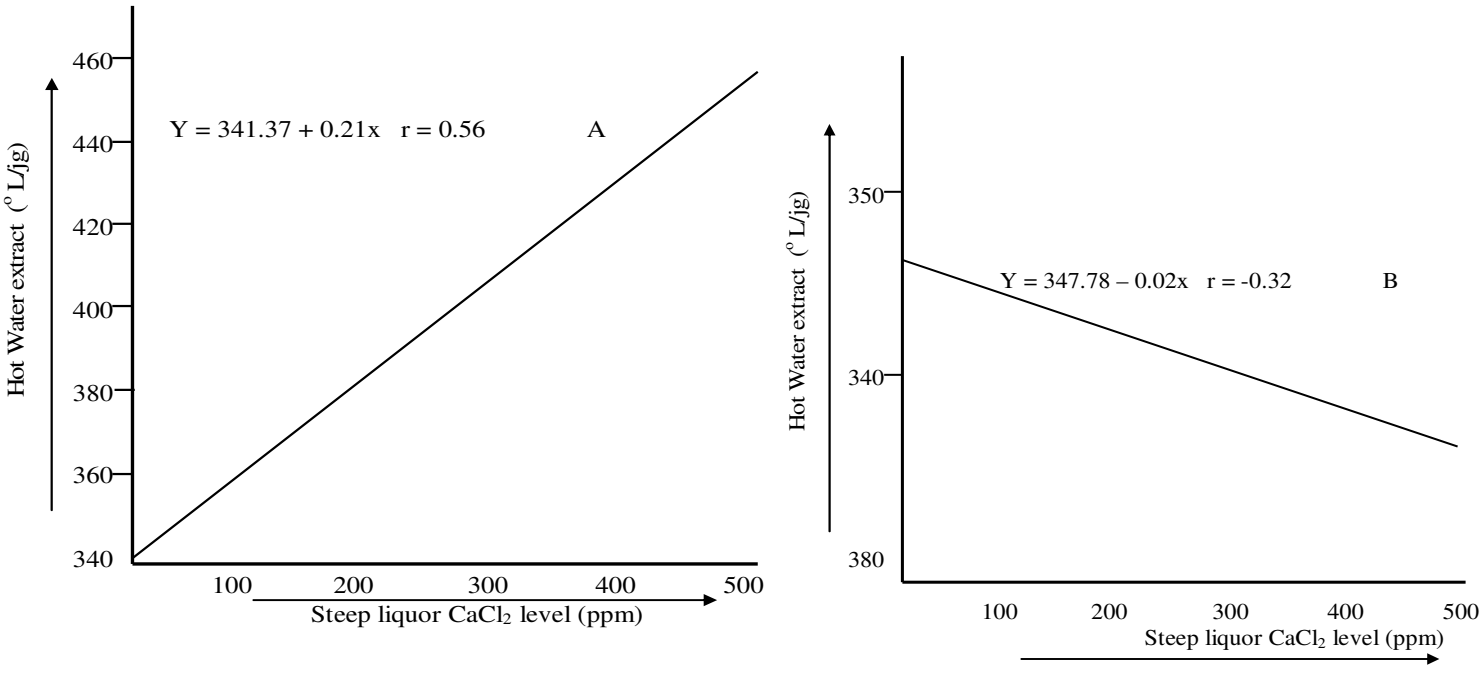

Figure 5. Correlation of steep liquor $CaCl_2$ concentration with Hot Water extract (HWE) for sorghum cultivars (A) ICSV400 and (B) KSV8.

Table 6. Effects of $CaCl_2$ concentration of steep water on the cold water soluble carbohydrate of sorghum cultivar ICSV 400 and KSV 8 malts (means, n = 3).

Cultivar	Cold Water soluble carbohydrate (%) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	19.3	215.1	13.0	13.5	12.5	12.0
KSV 8	18.7	13.0	12.0	7.7	6.4	4.7

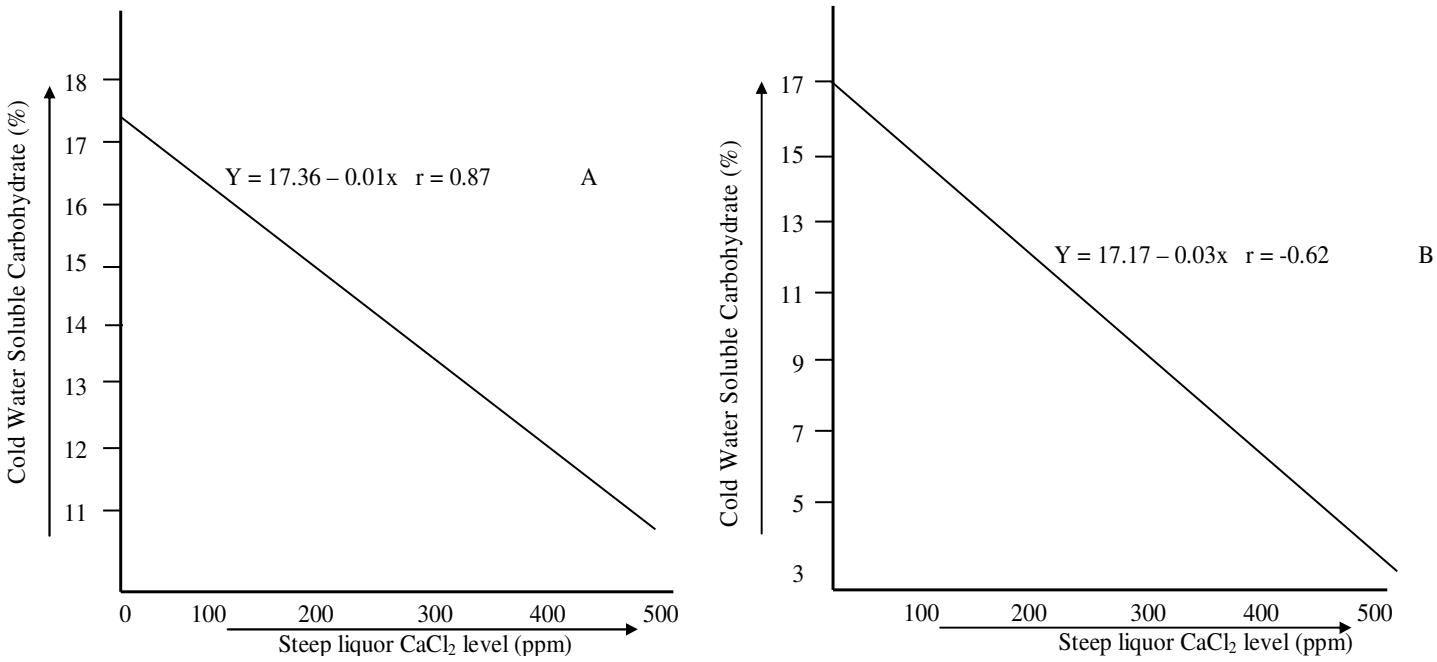

Figure 6. Correlation of steep liquor $CaCl_2$ concentration with Cold Water Soluble Carbohydrate (CWS-carbohydrate) for sorghum cultivars (A) ICSV400 and (B) KSV8.

decreased progressively as concentration of Ca^{2+} in steep liquor was increased across the range of Ca^{2+} treatment applied. The effect of Ca^{2+} treatment however, appeared to be more obvious in KSV 8 than ICSV 400. A second high point in CWS-carbohydrate of ICSV 400 at 300 ppm Ca^{2+} treatment appeared to correspond to similar behaviour in DP development on exposure to same conditions. However, CWS-carbohydrate exhibited by ICSV 400 malts seemed more significantly correlated to steep Ca^{2+} treatment at r = 0.87 (Figure 6) than DP (r = - 0.65). Besides, correlation analyses revealed a relationship represented as follows:

CWS-carbohydrate = 10.477 + 0.029 DP with r = 0.32 for ICSV 400.

This indicates that though grain CWS-carbohydrate increased with increasing DP, a poor relationship existed between malt DP and CWS-carbohydrate. Similarly, for KSV 8, a DP to CWS-carbohydrate relationship represented thus:

CWS-carbohydrate = 4.01 + 0.038 DP with r = 0.34 established.

Although, similar relationships between DP and CWS-carbohydrate were obtained for both sorghum cultivars, the significant positive displacement of the intercept in ICSV 400 compares to KSV 8 presumably indicates that for similar DP values ICSV 400 would probably release much more extract than KSV 8 malt. This implies that ICSV 400 would under the circumstance yield significantly higher CWS-carbohydrate than its DP value would predict.

The CWS-carbohydrate as a proportion of total cold water soluble of malts from both sorghum cultivars was also evaluated at all Ca^{2+} treatment levels. Data depicted in Table 7 indicate that this ratio generally was enhanced progressively as the level of Ca^{2+} treatment was increased across the range of Ca^{2+} examined for ICSV 400. Addition of $CaCl_2$ to steep water clearly enhanced CWS-carbohydrate/CWE ratio over the control values for ICSV 400 and at all levels of treatment. Highest CWS-

Table 7. Effects of $CaCl_2$ concentration of steep water on the CWS-carbohydrate/CWE ratio of sorghum cultivar ICSV 400 and KSV malts (means, n = 3).

Cultivar	CWS- carbohydrate/CWE ratio (%) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	56.9	60.4	62.4	59.1	66.5	85.4
KSV 8	76.6	77.9	62.3	34.6	39.4	36.0

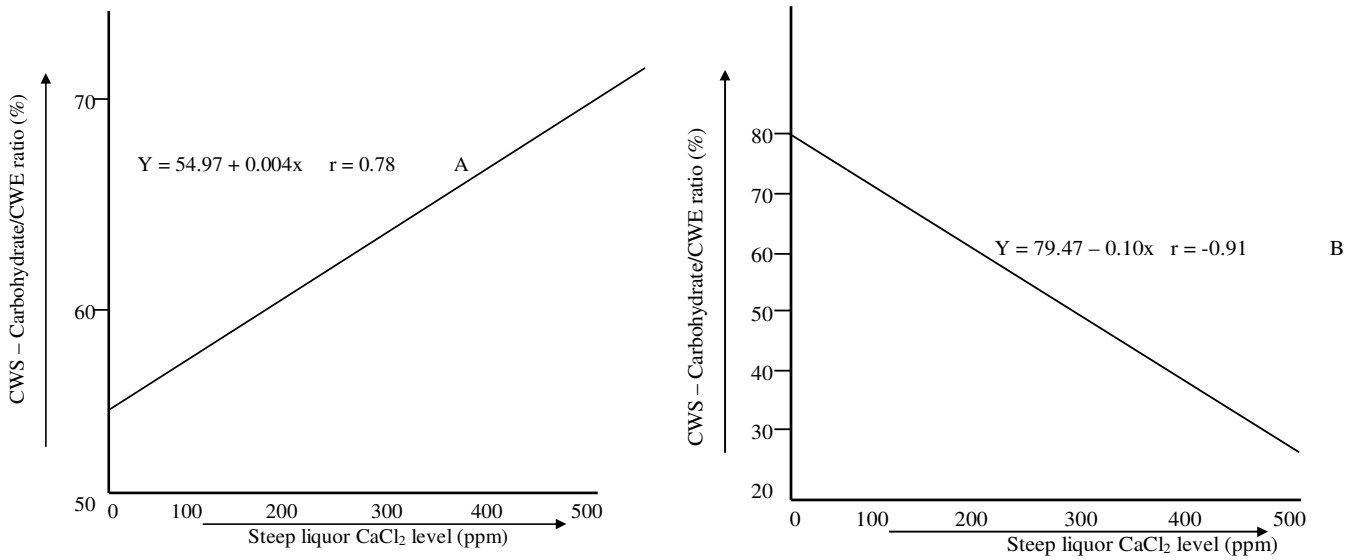

Figure 7. Correlation of steep liquor $CaCl_2$ concentration with malt CWS – carbohydrate/CWE ratio for sorghum cultivars (A) ICSV400 and (B) KSV8.

carbohydrate/CWE ratio for this cultivar was attained on exposure of grains to 500 ppm Ca^{2+} treatment. CWE-carbohydrate/CWE ratio for KSV 8 responded rather, differently. Ca^{2+} treatment obviously caused declining values of this ratio across the range of Ca^{2+}, 100 to 500 ppm in steep water examined. These observations are consistent with our earlier assertions on the significant varietals differences in the physiological responses of sorghum cultivars during malting (Okolo and Ezeogu, 1995; Okolo and Ezeogu, 1996). Carbohydrases seemed therefore responsible for most (up to 85%) of the solubilised extract in ICSV 400 malts treated to 500 ppm Ca^{2+} during steeping while for KSV 8, other enzymes perhaps proteases would increasingly become important in extract release as steep $CaCl_2$ level was increased from 100 to 500 ppm. Indeed, this might explain the consistently higher level of proteinase enzymes obtained for KSV 8 compared to ICSV 400 (Okolo and Ezeogu, 1996). Unlike ICSV 400 (r = 0.78), KSV 8 showed a highly significant inverse relationship between CWS-carbohydrate/CWE ratio and level of Ca^{2+} treatment at r = - 0.91 (Figure 7).

The ratios CWS-carbohydrate/HWE for the two sorghum cultivars were also determined. As shown in Table 8, CWS-carbohydrate/HWE ratio was highly significantly affected by steep water Ca^{2+} treatment in both cultivars. A progressive repression of this ratio for both cultivars was observed as the level of Ca^{2+} treatment was increased across the range of Ca^{2+} concentration examined. CWS-carbohydrate/HWE values for ICSV 400 were consistently higher than those obtained for KSV 8 and at all levels of treatment. This presumably suggests that higher proportion of total extract was degraded in ICSV 400 during malting than for KSV 8. Furthermore, the data reveal that the proportion of cold water soluble carbohydrate in relation to total extract (HWE) formed in the grains during malting decreased as the Ca^{2+} treatment was increased. Thus, suggesting that higher Ca^{2+} treatment during steeping promotes better extract release in both malts during mashing apparently due to Ca^{2+} stabilization of α-amylase activity. These findings are supported by correlation analyses (Figure 8) which revealed a highly significant inverse relationship between steep liquor $CaCl_2$ level and CWS-carbohydrate/HWE ratio for ICSV 400 at r = - 0.86 and KSV 8 (r = - 0.98). Correlation of CWS-carbohydrate with HWE of the malts gave the following relationships:

For KSV 8, HWE = 334.4 – 0.8 CWS-carbohydrate, r = - 0.39

Table 8. Effects of $CaCl_2$ concentration of steep water on the CWS-carbohydrate/HWE ratio of sorghum cultivar ICSV 400 and KSV 8 malts (means, n = 3).

Cultivar	CWS- carbohydrate/HWE ratio (%) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	5.7	4.4	3.7	3.8	3.7	3.4
KSV 8	5.4	3.7	3.5	2.4	1.8	1.4

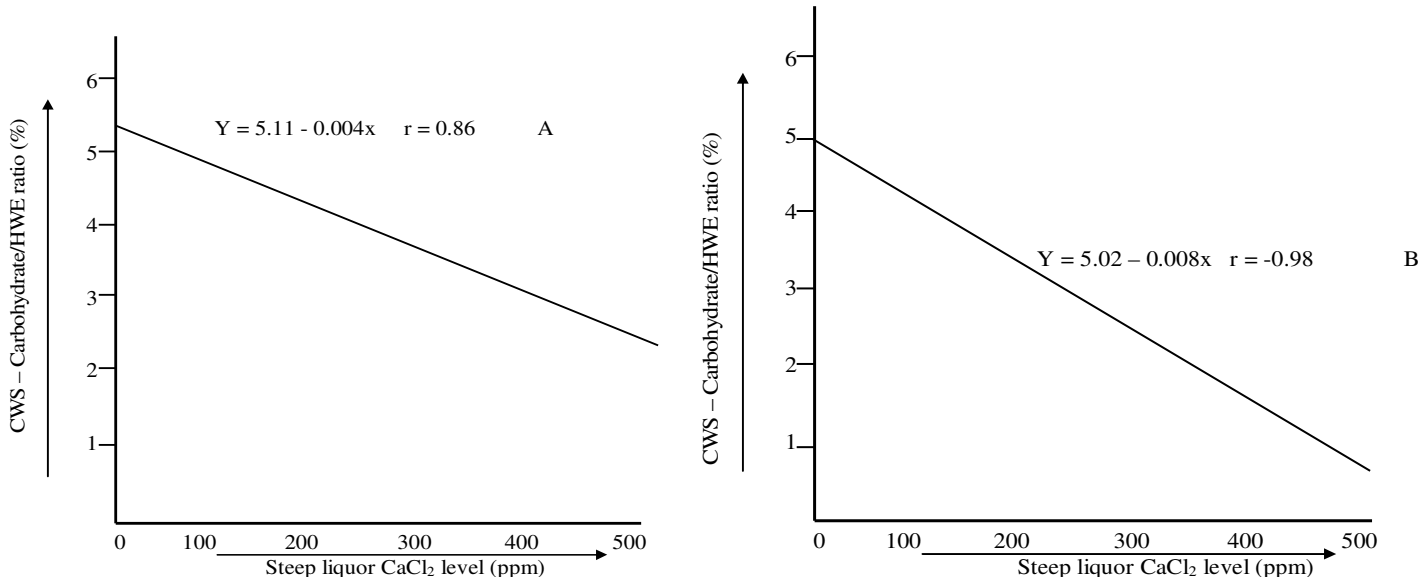

Figure 8. Correlation of steep liquor $CaCl_2$ concentration with malt CWS – carbohydrate/HWE ratio for sorghum cultivars (A) ICSV400 and (B) KSV8.

For ICSV 400, HWE = 367.7 – 1.5 CWS-carbohydrate, r = - 0.61

The more significant positive displacement of the intercept in ICSV 400 compared to KSV 8 suggests that the former would under the prevailing conditions yield higher HWE than its CWS-carbohydrate values would predict. Moreover, the inverse correlation established between CWS-carbohydrate and HWE in ICSV 400 also suggest that higher CWS-carbohydrate solubilization during malting would result in lower HWE release during mashing. When HWE of the malts were correlated with DP, a poor linear relationship was established for KSV 8 (HWE = 332.4 – 0.06 DP; r = 0.27) while a relatively good inverse correlation was established between the two factors for ICSV 400 at HWE = 355.3 – 0.067 DP; r = - 0.74. Their relationships presumably suggest that significantly more HWE was released in ICSV 400 for each unit of diastatic activity than a corresponding DP value would permit in KSV 8 malts. Besides, the differences in the slopes of the two cultivars further confirm the significance of varietals differences in physiological behaviour of sorghum grains on exposure to changes in malting schedule. The lowered correlation coefficient (r = 0.27) obtained for KSV 8 also seemed to suggest important roles for factors other than DP, possibly including grain proteolytic activity and structure, in determining the rates and extent of extract (HWE) release in this cultivar. Data obtained in this study strongly suggest that, unlike in the case of barley (Holmes, 1991) where extract release is closely correlated with malt diastatic activity, the ability of sorghum malts to release HWE during mashing is not entirely dependent on the diastase enzymes. Furthermore, the degree of this independence seemed to be strongly related to cultivar. In fact, the development of high HWE in grains treated with high $CaCl_2$ steep liquor (300 – 500 ppm Ca^{2+}) irrespective of the low DP, CWE and CWS-carbohydrate probably suggests Ca^{2+} stabilization of enzyme action during hot water extraction. Similar results have been reported on the effect of Ca^{2+} on extract release during sorghum mashing (Taylor and Daiber, 1988).

A major problem in sorghum malting is its large outlay of malting loss resulting from excessively lengthy roots and shoots (Ezeogu and Okolo, 1994; Ezeogu and Okolo, 1995; Ilori, and Adewusi, 1991; Morrall et al., 1986; Nout and Davies, 1982; Okon and Uwaifo, 1985).

Table 9. Effects of $CaCl_2$ concentration of steep water on main root length development of sorghum cultivar ICSV 400 and KSV malts (means, n = 3).

Cultivar	Average main root length (cm) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	2.6	2.3	2.0	0.9	0.5	0
KSV 8	2.4	2.1	1.2	0.6	0	0

Data illustrated in Table 9 show the effects of Ca^{2+} concentration of steep liquor on the average main root development of the two sorghum cultivars. Obviously, Ca^{2+} seemed a significant determining factor for grain average main root development. Main root length for both cultivars clearly decreased progressively as the level of Ca^{2+} treatment was increased across the range of Ca^{2+} concentration examined. In ICSV 400, steeping in liquor containing 100 and 200 ppm $CaCl_2$ caused 11.5 and 23% reductions in main root lengths respectively relative to control values. Further increases beyond this level of Ca^{2+} treatment seemed to elicit more severe main root length sensitivity. This is evident from the occurrence of sharper rates of decrease in grain main root lengths, at 65, 80 and 100% reductions during 300, 400 and 500 ppm Ca^{2+} treatments, respectively. A similar trend was observed for KSV 8 except that this grain clearly exhibited a higher sensitivity to Ca^{2+} steep treatment compared to ICSV 400. These observed differences in the degree of sensitivity of the sorghum rootlets to Ca^{2+} challenge perhaps reflect fundamental differences in the physiologies of the two cultivars. This view is supported by analyses of variance data which revealed highly significant ($P < 0.001$) influences on grain main root development by cultivar and steep treatment plus, their pairwise interactions. Correlation analyses established a significant inverse relationship between the two factors for both grains at r = - 0.98 (Figure 9). Although data depicted in Table 9 does not permit an authoritative explanation of the mechanism of Ca^{2+} regulation of main root development in sorghum, it is conceivable that root suppression might have resulted from retardation of certain aspects of cell metabolism, particularly enzyme synthesis and secretion, responsible for root initiation and elongation within the growing region of the young seedling. Similar views have been advanced for other steep treatments (NH_4OH and $KBrO_4$) which tend to impair rooting in cereal seeds (Brookes et al., 1976; Ilori and Adewusi, 1991; Jones, 1969).

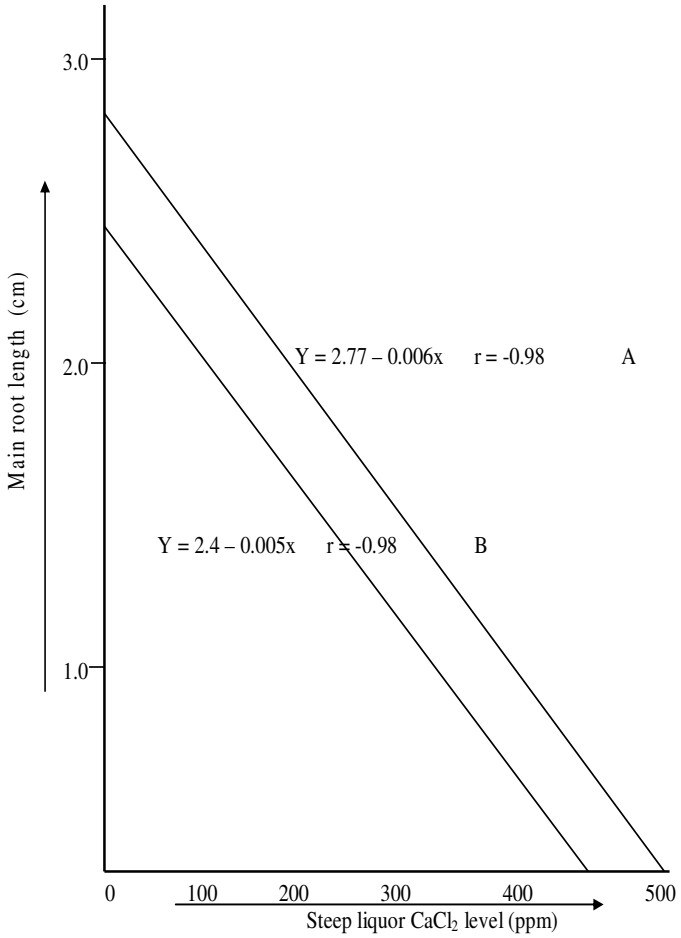

Figure 9. Correlation of steep liquor $CaCl_2$ concentration with average main root length for sorghum cultivars (A) ICSV400 and (B) KSV8.

The malting loss patterns for ICSV 400 and KSV 8 malts followed the same trend as grain main root development. Table 10 shows malting loss values obtained for the two sorghum cultivars. Evidently Ca^{2+} treatment highly significantly ($p < 0.001$) affected malting loss in each of the cultivars. A progressive reduction in malting loss was noticed with exposure to increasing concentrations of steep water $CaCl_2$ for both cultivars. However, reductions in grain malting loss were observed to be generally more enhanced for KSV 8 than ICSV 400 at all Ca^{2+} treatment levels. Two-way analyses of variance revealed that the factors of cultivar and Ca^{2+} treatment as well as their pairwise interactions highly significantly ($p < 0.05$) affected malting loss in both ICSV 400 and KSV 8. In fact, significant linear relationships were established between malting loss and Ca^{2+} treatment for both cultivars form Kruskal-Wallis tests. As shown in Figure 10 for both cultivars, an inverse correlation between malting loss and Ca^{2+} treatment was obtained (ICSV 400, r = - 0.98; KSV 8, r = - 0.94).

Table 10. Effects of $CaCl_2$ concentration of steep water on malting loss of sorghum cultivar ICSV 400 and KSV malts (means, n = 3).

Cultivar	Malting loss (%) by $CaCl_2$ concentration (ppm)					
	0	100	200	300	400	500
ICSV 400	56.9	60.4	62.4	59.1	66.5	85.4
KSV 8	76.6	77.9	62.3	34.6	39.4	36.0

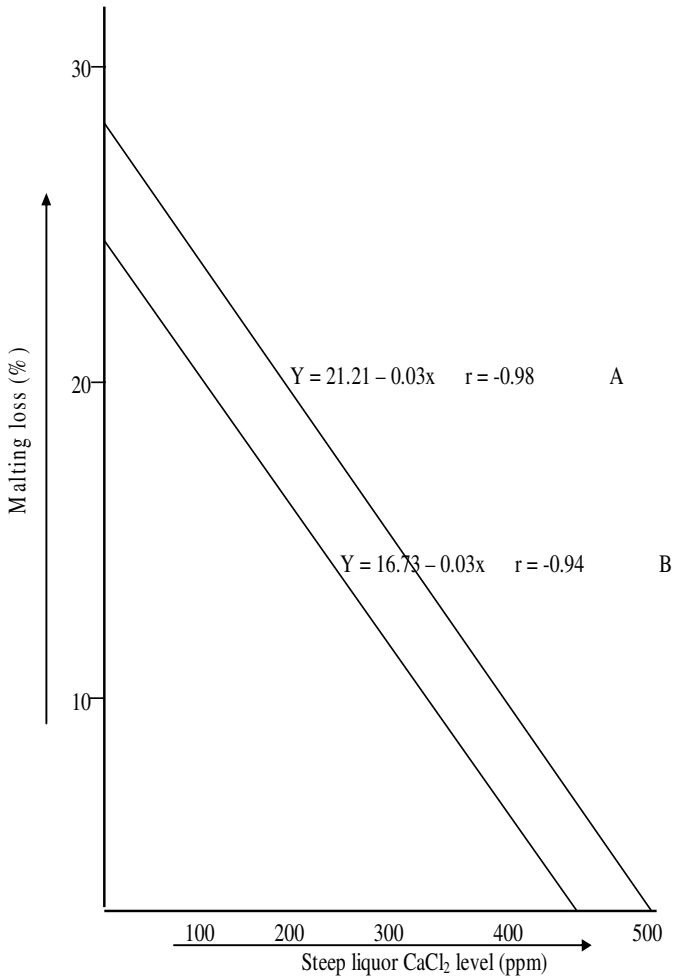

Figure 10. Correlation of steep liquor $CaCl_2$ concentration with malting loss for sorghum cultivars (A) ICSV400 and (B) KSV8.

Conclusion

Data presented in this study reveal that Ca^{2+} treatment of sorghum steeps would be highly beneficial in reduction of excessive root development and enormous malting losses characteristics of sorghum malts. However, these obvious gains are severely contradicted by the repression, sometimes at highly significant levels ($p < 0.001$), of the development of important endosperm carbohydrate modification indices.

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