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Effects of alkali treatment on tannins and phytates in red sorghum, white sorghum and pearl millet

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ABSTRACT
Objective: Tannins and phytates anti-nutrients are abundant in sorghum and millet; they reduce the nutrient value and organoleptic properties of foods. Alkali treatment was therefore employed to detoxify them.
Methodology and results: Grain samples were sourced from the selected site (Nguni, Ukambani Kenya). Preliminary physical and chemical analysis were carried out to determine the indicators of ant-nutrients (colour and presence of testa) followed by actual levels of anti-nutrients (quantities of tannins and phytates) and the suitability of the grains for use in other treatments like germination which are normally employed in the utilization of the cereals (Shull *et al*., 1987). Following the physical and chemical analysis, alkali treatment was done to reduce the levels of the anti-nutrients and the most effective alkali identified. The quantities of the alkali application was based on a concentration that retained germinability of the grains, had no negative effect on consumer acceptability and had acceptable residual values of any potentially harmful chemical on the grains. Four alkalis were used including ash from pigeon pea pods, magadi soda, ammonia and sodium bicarbonate. The cereals were steeped in the alkali solutions of varying concentrations from 0-10% at varying time periods of between 0-7days. The reduction in tannins and phytates were analyzed. The overall best alkali was magadi soda applied at 1% for 2 days of steeping, this reduced tannins and phytates by 68-75% and 14-29%, respectively. The grains had acceptable organoleptic properties, over 91% germinability and the residual value of sodium from the magadi soda was 98.09 mg/100g which was acceptable (Shull *et al*., 1987; Makokha *et al*., 2002).
Conclusion and application of results: The different alkalis proved effective in the detoxification of tannins and phytates, each of them reducing statistically significant amounts of the anti-nutrients ($p \leq 0.05$). The most effective of them was magadi soda, followed by ash, ammonia and sodium bicarbonate. This information is useful to the communities using these cereals since they can use locally available compounds like magadi soda and ash from the legumes they use to remove the anti-nutrients associated with the cereals thus improving their quality and nutritive value. If the alkalis are used in the right quantities as shown in this study, germinability of the grains and organoleptic properties can still be retained. Therefore alkali treatment can be used in combination with other processing techniques like malting and even fermentation to add value to sorghum and millet.
Key words: Tannins, phytates, detoxification, alkali treatment.

INTRODUCTION

More than 95% of total food use of sorghum occurs in countries of Africa and Asia. Data from FAO (1995) indicate that despite an increase in total food use there has been a decline in the utilization of sorghums and millets since early 1960s in both Asia and Africa. This decline in per capita consumption in many countries was due in part to shifts in consumer habits brought about by a number of factors including the rapid rate of urbanization, the amount of time and energy required to prepare food based on sorghums and millets, inadequate domestic structure, poor marketing facilities and processing techniques, unstable supplies and relative unavailability of sorghum and millet products.

Sorghums and millets are arid and semi-arid land (ASAL) crops that are used as staples in many parts of the country. This study will concentrate on one aspect that has led to low utilization of the cereals- the anti-nutrients tannins and phytates. The anti-nutrients interfere with mineral absorption and palatability of the cereals so detoxification is vital to enhance their nutrient value and organoleptic properties. Several detoxification methods are available, including decortication, malting, fermentation and alkali treatment (Muindi and Thomke, 1981; Daiber and Taylor, 1982; Ibrahim *et al*., 1988; Osuntogun *et al*., 1989; Banda-Nyirenda and Vohra, 1990). Among these methods, alkali treatment was employed in this study for detoxification purposes. In their application precautionary measures were put in relation to the way these impacted on other possible and subsequent treatments like malting, and also on the organoleptic properties of the cereals as well as on the possible health hazards caused by any residues contained in the alkalis.

MATERIALS AND METHODS

Cereal samples: The varieties of the sorghum, *Sorghum bicolor* (L) Moench acquired were white sorghum -KARI *Mtama* 1 (KM1) and the red sorghum-*Seredo* while that of pearl millet, *Pennisetum glaucum* was the ICMV variety (Plates 1-3).

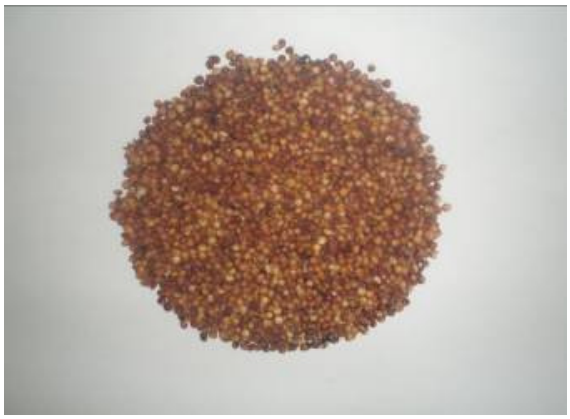

Plate 1: Red sorghum grains, *Sorghum bicolor* (L) Moench-*Seredo* variety

Plate 2: White sorghum grains, *Sorghum bicolor* (L) Moench-KARI *Mtama* 1 variety

Plate 3: Pearl millet grains, *Pennisetum glaucum*-ICMV variety

The cereals were obtained from Mwingi District, Eastern province of Kenya. Sampling was done three times from different sources. The first batch of the cereal samples was obtained from farmers' stores of the harvest of the year 2007; the second batch of samples was obtained from the harvest of year 2008 and the third batch samples was obtained from local stores in Mwingi town (These were a mixture of the harvests of years 2007 and 2008).

Initial determination of the levels of the major anti-nutrients of concern, the tannins and phytates was carried out before the detoxification process. The preliminary detoxification process involved making solutions of high concentration of up to 10% of the four alkalis, ash from pigeon pea pods, *magadi soda,* sodium bicarbonate and ammonia then steeping the grains in them for a period of 12 hours. The grains were then washed, dried, ground and analyzed for tannins and phytates. The alkali having the best detoxification effect was then chosen. Optimization studies of the detoxification process involved soaking in lower alkali concentration of the most effective alkali with as low as 0.1% to a maximum of 2% alkali treatment with longer steeping time periods. The chosen alkali (*magadi* soda) was the most effective in detoxifying the grains. The maximum concentration of the alkali chosen was determined by the extent of the interference of the alkali with germinability of the steeped grains and the residual quantities of potentially harmful elements from the alkali. At 1% alkali treatment germinability was still over 90% which is recommended as suitable for grains that are to be further processed by malting (Shull *et al*., 1987) while the sodium ion residue was at acceptable levels (Makokha *et al.,* 2002).

Presence or absence of testa: The grain kernel was scraped with a surgical blade to remove the pericarp. The testa was indicated by the presence of a pigmented layer covering the endosperm (Shull *et al*., 1987).

Grain colour: Cereal grains were packed in transparent polythene bags for the purpose of analysis. Tristimulus colorimeter was used to take color measurements (Simple Spectrophotometer NF 333-Model 99061, Nippon Nenshoku Ind., Tokyo, Japan). The instrument expresses color measurement in the CIELAB (L*, a*, b*) form. The instrument was first calibrated using standard black and white plates (with transparent papers placed on the standard plates). After calibration colour measurements were randomly taken in triplicates. The hue angle of difference (HD) which describes the visual sensation according to an area which appears to be similar to one or proportions of two of the perceived colours, red yellow, green, and blue was calculated according to the formula given below.

Hue angle of difference (HD) = $[\tan^{-1}(`b^*`_x / `a^*`_{x)} - \tan^{-1}(`b^*`_o / `a^*`_o)]^{0.5}$

Where $`L^*`_o$, $`a^*`_o$, and $`b^*`_o$ are values at the time zero (time for calibration) and $`L^*`_x$, $`a^*`_x$ and $`b^*`_x$ are at time x (of object analysis).

Tannin determination: This was done by the Vanillin-Hydrochloric acid method (Burns, 1963; Price *et al*., 1978). Approximately 0.25g of ground sample was weighed into Erlenmeyer flask. Ten (10) ml of 4% HCl in methanol was pipetted into each of the flasks and the flask sealed with parafilm. The flasks were gently shaken for 20 min on a shaker (German model KS 250 basic); the resulting extracts were centrifuged for 10

min at 4500 rpm (Japan model H–2000C). The supernatant aliquots were transferred to 25ml volumetric flasks. Second extractions were done by adding 5ml of 1% HCl in methanol to the residue from the first extraction and repeating the extraction process. The aliquots of the first and second extracts were combined and made up to 25ml volume. Approximately 1ml of each extract was pipetted to a corresponding labeled test tube. A set of catechin standard solutions was prepared ranging from 100 to 1000ppm using methanol as the solvent. Approximately 1ml of each respective standard and sample extract were pipetted into test tubes and 5ml of freshly prepared vanillin-HCl reagent added. Sample blanks were prepared by adding 5ml of 4% HCl in methanol to 1ml of the aliquots of the extracts pipetted into the test tubes. The absorbance of the standard solutions, sample extracts and blanks were read in a UV-VIS spectrophotometer (Shimadzu, UV mini 1240 model) and at 500nm 20 min after adding Vanillin-HCl reagent to the samples and standards.A standard curve was prepared from the readings of the catechin standard solutions.

The blank absorbances were subtracted from the samples absorbances and the corrected absorbance substituted into the regression equation (y = a + bx) in order to calculate the concentration of the sample extracts.

The concentration in µg per ml was converted in to mg catechin per ml. The percent catechin equivalents (% CE) were calculated as follows:

% CE = (CC × VM) / (VE × Wt) × 100

Where: CC = catechin concentration (mg/ml); VM = volume made up (25ml); VE = volume of extract (1ml); and Wt = weight of sample (250mg).

Phytate determination: This was done by HPLC analysis of phytic acid according to Camire and Clydesdale (1982). Approximately 50mg of sample was weighed into a 125ml Erlenmeyer flask and 10ml of 3% H_2SO_4 added. The flasks were placed on a shaker (German model KS 250 basic) at a moderate speed of 1500rpm for 30 min at 25ºC and filtered using Whatman no.1 filter paper (12.5cm Diameter). The filtrate was transferred to a boiling water bath (BWB) for 5min and 3ml of a $FeCl_3$ solution (6mg ferric iron per ml in 3% H_2SO_4) added. A second BWB heating was done for 45min to complete precipitation of the ferric phytate complex. Centrifugation followed at 2500 rpm (Japan model H–2000C) for 10 min and the supernatant discarded. The precipitate was washed with 30 ml distilled water, centrifuged and the supernatant discarded. Three (3) ml of 1.5N NaOH were added to the residues and the volume brought to 30ml with distilled water. Heating was done for 30min in a BWB to precipitate the ferric hydroxide. Cooled samples were centrifuged and the supernatant transferred into a 50ml volumetric flask. The precipitate was rinsed with 10ml distilled water, centrifuged and the supernatant added to the contents of the volumetric flask.

Samples of 20µl of the supernatant were injected into a HPLC (Shimadzu model C-R7A plus) fitted with a 50377 RP-18 (5µm) column Cat. at an oven temperature of 30ºC and RID-6A detector model. The mobile phase was 0.005N sodium acetate in distilled water, at a flow rate of 0.5µl min^{-1}.

A stock solution of the standard containing 10mg /ml of sodium phytate (Inositol hexaphosphoric acid $C_6H_6(OPO_3Na_2)_6+H_2O$) in distilled water was prepared. Serial dilutions were made for the preparation of a standard curve. Results of phytate content were obtained as per the calculations of Vohra *et al*. (1965). The equation of the standard curve line was also obtained (y = bx + c) and used for calculating the phytate values as;

Phytate content (mg/g) = (y/b) x (dilution factor / weight of sample)

Where: y = Peak area; and b = Concentration

Germinative energy: A hundred intact grains of each cereal variety were placed on a filter paper moistened with 4ml of distilled water in a Petri-dish. The Petri-dishes were placed inside an incubator set at a relative humidity of 100% and a temperature of 25°C for 48 hrs (Constant temperature humidity chamber, Model TD-384KN, Thermo, Tec, Tokyo, Japan). All grain kernels that showed any distinct sign of germination were counted and the percentage germinative energy calculated from the proportion of the germinated grain in relation to the number of incubated grains (Dewar *et al*., 1995).

Sodium metal residue analysis: Five grams of sample were weighed in crucibles and transferred to hot plates in a fume hood chamber where they were charred to clear all the smoke from the carbonatious material before transferring them to the muffle furnace. The charred materials were then incinerated at 550ºC until they were reduced to white ash. The ashes were cooled, 15ml of 6N HCL was added to each of them in the crucibles before transferring them to 100ml volumetric flasks. Distilled water was used to top them up to the mark before mineral analysis (AOAC, 1995). Atomic Absorption Flame Emission Spectrophotometer was used for the sodium metal residue analysis of the

alkali treated samples (Model A A-6200, Shimadzu, Corp., Kyoto, Japan).

Data analysis: Each determination was carried out on three separate samples, on dry weight basis and analyzed in triplicate; the figures were then averaged using Microsoft Excel. Data was assessed using Analysis of Variance (ANOVA) with the Statistical Analysis Software (SAS) statistical package (Snedecor and Cochran, 1987). Mean comparisons for treatments were made using Duncan's Multiple Range Tests (Steel and Torrie, 1980). The mean values were displayed with standard deviation (S.D) of the means.

Standard deviation (S.D) is given by: $\{\Sigma (x-x')^{2,}\}/ (n-1)$. Where Σx is the sum of the sample, x is sample mean, x' is population mean and n is the number of sample in the population. Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Tannin and phytate content: Tannin content ranged from 1.2 in white sorghum to 4.4% C.E in the red sorghum (table 1). The tannin contents of the different cereals as shown in Table 1 were significantly different from each other ($p \leq 0.05$). Red sorghum genetically has higher condensed tannin compared to white sorghum and pearl millet. The tannin values were similar to those reported in similar studies (Gomez *et al.,* 1997; Beta *et al.,* 1999) Phytate values were similar to those reported previously by Wang *et al.,* (1959), Sankara Rao and Deosthale (1983) and Chauhan *et al*. (1986). There were significant differences in the, tannin and hue values of the cereals before detoxification treatment but no significant differences ($p<0.05$) were observed in the phytate contents of red and white sorghums and pearl millets. Tannins impart a bitter taste to the grains making them unpalatable and also interfere with protein digestibility (Harris and Burns, 1970). Phytates interfere with mineral absorption especially calcium and zinc (Doherty *et al*., 1982). The high quantities of these anti-nutrients in the cereals reduce their food value hence causing low utilization unless processed prior to use (Rao and Deosthale, 1983; Rao and Deosthale, 1988; Mukuru, 1992).

Table 1: Raw material color, tannins, phytates, and presence of testa

Parameter	Red sorghum	White sorghum	Pearl millet	LSD
Hue Difference (Colour)	$1.4^{b}\pm0.37$	$2.2^{a}\pm0.58$	$1.5^{b}\pm0.59$	0.5
Tannin Content (%C.E)	$4.2^{c}\pm0.04$	$1.1^{a}\pm0.02$	$1.4^{b}\pm0.03$	0.1
Phytate Content (mg/100g)	$130.5^{a}\pm0.44$	$125.2^{a}\pm0.01$	$127.4^{a}\pm0.39$	0.4
Testa	Present	Present	Present	NA

Value =Mean ± S.D. Each value is a mean of 3 replicates Means on the same row followed by the same letter are not significantly different ($p \leq 0.05$) S.D=Standard deviation. Hue difference is a measure of color purity. %C.E= Percentage Catechin Equivalents. LSD= Least significant difference of the mean replicates. NA= Not applicable.

Phytate values were similar to those reported previously by Wang *et al.,* (1959), Sankara Rao and Deosthale (1983) and Chauhan *et al*. (1986). There were significant differences in the, tannin and hue values of the cereals before detoxification treatment but no significant differences ($p<0.05$) were observed in the phytate contents of red and white sorghums and pearl millets. Tannins impart a bitter taste to the grains making them unpalatable and also interfere with protein digestibility (Harris and Burns, 1970). Phytates interfere with mineral absorption especially calcium and zinc (Doherty *et al*., 1982). The high quantities of these anti-nutrients in the cereals reduce their food value hence causing low utilization unless processed prior to use (Rao and Deosthale, 1983; Rao and Deosthale, 1988; Mukuru, 1992).

Alkali treatment was done to reduce tannins and phytates. Four different alkalis were used and there were significant reductions in tannins in the treated cereals at different concentrations of alkalis used, but there were no significant differences between the different alkalis used in Fig.1 a-e ($p>0.05$). However the alkali of preference for use in reducing tannins in the cereals was *magadi* soda which gave the lowest value for anti-nutrient reduction. Besides its effectiveness in reducing the anti-nutrients *magadi* soda is readily available and affordable in rural communities so it can be easily accessed and used for detoxification purposes.

A consideration of the alkali detoxification effects on the high tannin red sorghum illustrates its effectiveness. The red sorghum had a tannin content of 4.4% C.E. At 1% concentration of alkali treatment, this was reduced to 1.1, 1.2, 2.2 and 2.2% C.E, by *magadi* soda, ash, ammonia and sodium bicarbonate, respectively.

Tannin reduction increased as the concentration of alkali and soaking time increased. There were significant differences in tannin content in the red sorghum between the days of soaking and between the low ranges of *magadi* soda concentrations of 0-1% ($p \leq 0.05$). In choosing the concentration of *magadi* soda to use in the detoxification treatment process, the concentration that gave the least tannin mean with 0-1% *magadi* soda was taken. This corresponded to the highest concentration of the low ranges which was 1% *magadi soda*. A two day optimal soaking time period gave satisfactory reduction in tannin levels within a reasonable time period since further steeping from day 2 onwards would negatively affect germination of the cereals by delaying it. This was revealed during preliminary germinative energy tests. Germinability was considered because most of the communities who utilize these grains usually use them for fermented porridge production. If the germinability of the grains is affected then the detoxification with the alkali would be unsuitable.

Phytate analysis was also carried out after alkali treatments on grains. There were significant differences in phytate reduction between different alkalis at different concentrations in Fig.2a – e ($p \leq 0.05$). The best alkali for use in reducing phytates was also identified as *magadi* soda followed by ash, ammonia and bicarbonate of soda.

There were significant differences in phytate reductions with increases in the concentration of *magadi* soda used in Fig.2e ($p \leq 0.05$). However, there were no significant differences in phytate reduction with the increase in steeping time ($p > 0.05$). The highest concentration of the *magadi* soda alkali was therefore chosen for use. The appropriate steeping period remained 2 days since there were no significant differences in phytate reduction levels regardless of an increase in the number of steeping days.

The residual sodium metal content of the cereals after the 1% *magadi* soda alkali treatment was determined and found to be 98.09 mg/100g. This value was in the range reported by other researchers which showed that the grains were safe for consumption (Makokha *et al.,* 2002). Again germinability and palatability of the cereals were not adversely affected by the alkali treatment at this concentration.

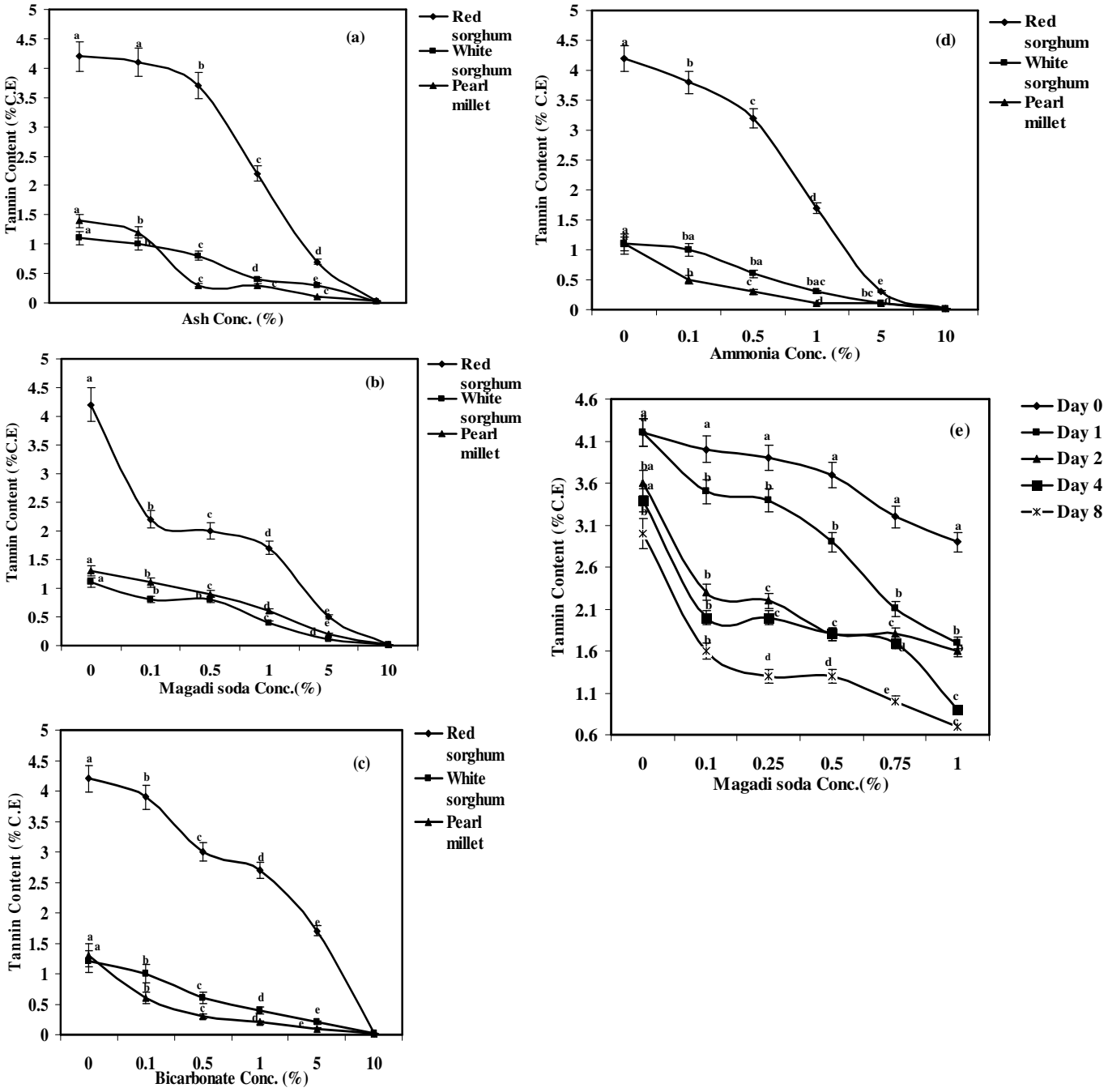

Figure 1: Tannin content (%C.E) of the cereals treated with (a) Ash, (b) *Magadi* soda, (c) Sodium bicarbonate, (d) Ammonia and (e) Reduction of Tannins (%C.E) in red sorghum treated with *magadi* soda at different concentrations. %C.E= Percent Catechin equivalent. The vertical bars represent S.D of the mean of 3 replicates. When absent the S.D value is <0.01 and falls within the dimensions of the symbol S.D= Standard deviation. Points on the same curve with similar letter(s) have means that are not significantly different ($p \leq 0.05$).

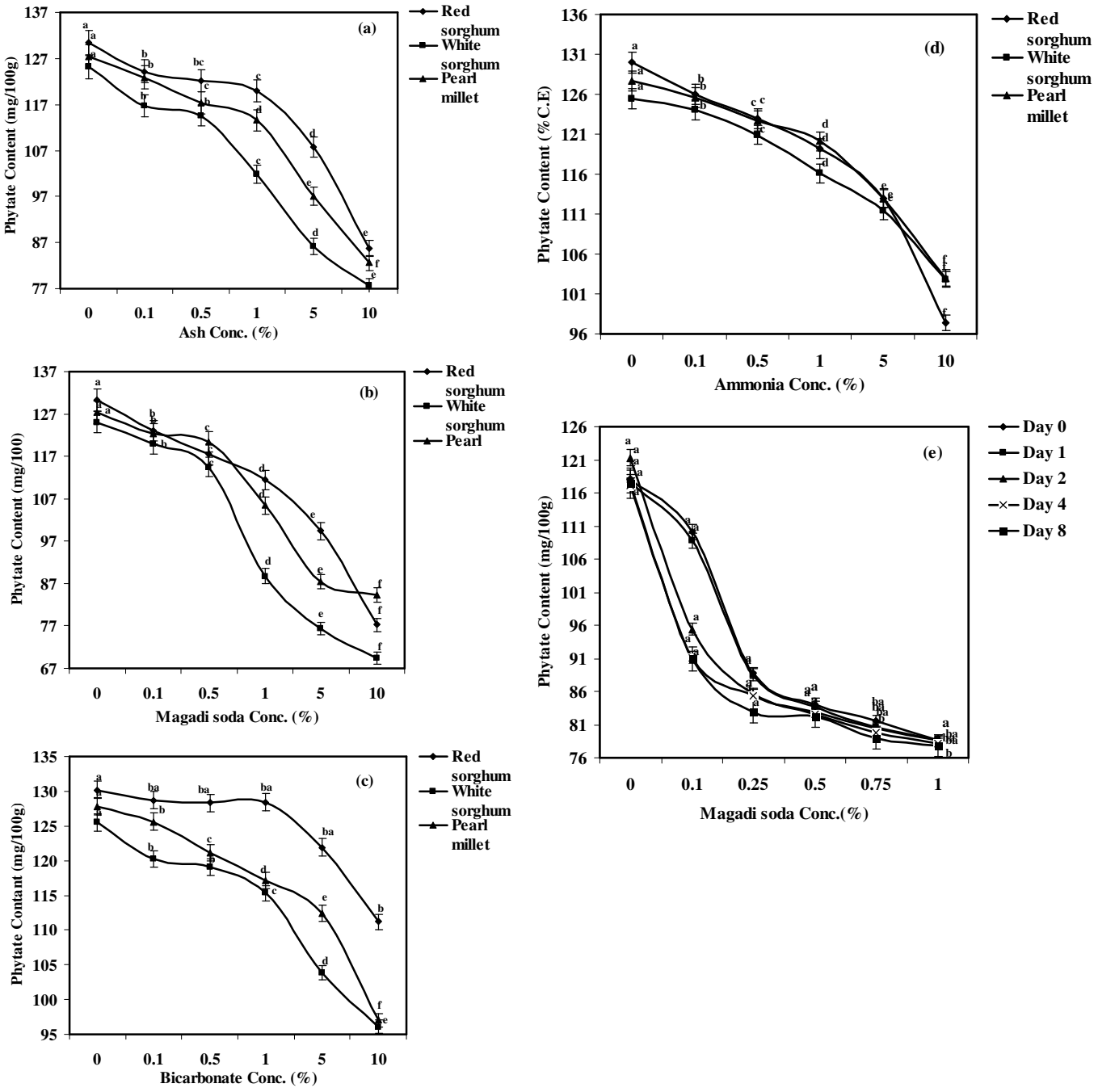

Figure 2: Phytate content (mg/100g) of the cereals treated with (a) Ash, (b) *Magadi* soda, (c) Sodium bicarbonate, (d) Ammonia and (e) Reduction of phytate (mg/100g) in red sorghum treated with *magadi* soda. The vertical bars represent S.D of the mean of 3 replicates. When absent the S.D value is <0.01 and falls within the dimensions of the symbol S.D= Standard deviation. Points on the same curve with similar letter(s) have means that are not significantly different ($p \leq 0.05$).

CONCLUSION

Magadi soda exhibited the highest detoxification effect on tannins and phytates. After optimization tests on appropriate quantities and steeping time period for alkali application 1% *magadi* soda concentration was chosen for application for a period of 2 days. These analyses prove that the nutritive value of foods can be improved by using simple techniques such as alkali treatment to reduce the anti-nutrients present in the foods. Therefore consumers can improve the nutritive value of these cereals by using inexpensive locally available material such as *magadi* soda, ash from legume pods and sodium bicarbonate for detoxification purposes. These simple treatments can be used in combination with other treatments like malting and fermentation to further enhance the nutritive value of the cereals since at moderate concentrations they do not affect germinability, palatability of leave harmful residual elements in the grains as in the case of *magadi* soda applied at 1% for 2days.

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