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Effects of Malting and Fermentation on Anti-Nutrient Reduction and Protein Digestibility of Red Sorghum, White Sorghum and Pearl Millet

C. A. Onyango[1], S. O. Ochanda[1], M. A. Mwasaru[1], J. K Ochieng[2], F. M. Mathooko[3] & J. N. Kinyuru[1]

[1] Department of Food Science, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

[2] Ministry of Agriculture, Mwingi District, Agriculture Office, Mwingi, Kenya

[3] South Eastern University College, Kitui, Kenya

Correspondence: C. A. Onyango, Department of Food Science, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya. E-mail: cakoth2002@yahoo.co.uk

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Abstract

Sorghum and millet and their products require specialized treatment in order to improve their nutritive value, organoleptic properties and shelf-life. They contain anti-nutrients which are the major phytochemicals which negatively affects their nutritive values. The phytochemicals of concern include tannins and phytates, which interfere with mineral absorption, palatability and protein digestibility. Malting and fermentation treatments were applied to reduce the anti-nutrients, improve protein digestibility, and acidity to increase the products shelf life. The effects of malting and fermentation on the cereals nutritive value and anti-nutrient reduction were studied and evaluated for a period of 8 days. A treatment combining malting for 3 days and fermentation for 2 days respectively both at room temperatures (25°C) was employed. Tannins and phytates were significantly reduced ($p \leq 0.05$) by malting and fermentation. Protein digestibility was significantly ($p \leq 0.05$) improved by malting and fermentation treatments; malted cereals digestibility ranged between 34.5-68.1% while the fermented flours protein digestibility range was 97.4-98.3%. The pH values were lowered to below 4.0, a level at which they could effectively inhibit spoilage microorganisms at the end of the fermentation period. A combination of optimum time treatments of malting and fermentation for 3 days and 2 days respectively were effective in reducing tannins and phytates and improving protein digestibility of the cereals.

Keywords: phytates, tannins, malting, fermentation, protein digestibility

1. Introduction

Sorghum and millet provide nutrition to many people living in arid and semi-arid lands (ASAL) according to FAO (1995). This is because these crops do well both in areas with adequate rain as well as in ASAL regions. More than 95% of total food use of sorghum occurs in Africa and Asia (FAO, 1995; Doggett, 1988). FAO (1995) indicated that despite of the increase in total food use, there has been a decline in the utilization of sorghum and millet since early 1960s in both Asia and Africa. This decline in per capita consumption is partly due to shifts in consumer habits brought about by poor palatability of some varieties, low digestibility of protein, low nutritive value of the grains among other factors (Butler, 1984; 1988). Palatability can be improved by reducing tannin levels in affected varieties while digestibility and nutrient availability of grains can be improved by malting and fermentation processes (Dassenko, 1980; Ejeta, 1987). Many research findings have shown that fermentation imparts several invaluable attributes to food products. Fermented foods have many beneficial products metabolized by bacteria and fungi including biomass proteins, amino acids, vitamins, minerals, flavor and aroma compounds among others. (Annan et al., 2003; Au & Fields, 1981; Baghel et al., 1985; Beaumount, 2002; Carter & Carpenter, 1981; 1982; Deshpande & Salunke, 2002; Steinkraus, 1996; Yasmine, 2002). Malting and fermentation may decrease anti-nutrients and increase protein digestibility (Dhankher & Chauhan, 1987; Gibson, 1994; Mosha, 1994; Ochanda et al., 2010; Steinkraus, 1983, Udayasekhara et al., 1988; West et al., 2002).

1.1 Germination

Germination increases endogenous phytase activity in cereals, legumes, and oil seeds through *De Novo* synthesis, activation of intrinsic phytase, or both. Tropical cereals such as maize and sorghum have a lower endogenous

phytase activity than do rye, wheat, triticale, buckwheat, and barley (Egli et al., 2002). Certain tannins and other polyphenols in legumes such as *Vicia faba* and red sorghum may also be reduced during germination as a result of the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides (Camacho et al., 1992). Such reductions in polyphenols may facilitate iron absorption (Hurrell, 2004).

1.2 Fermentation

Natural fermentation induces phytate hydrolysis via the action of microbial phytase enzymes originating from the microflora on the surface of cereals and legumes, thereby reducing the phytates (Sandberg et al., 1991; Hurrell, 2004). Fermentation imparts other invaluable attributes of food products. Fermented food has many beneficial products metabolized by bacteria and these include biomass proteins, amino acids, vitamins, minerals, flavor and aroma compounds and carbohydrates. Products of respiratory and biosynthetic pathways such as lactic acid, ethanol, acetaldehyde and pyruvic acid are also produced which alters the pH of foods to levels that they control the growth of pathogenic microorganisms. This therefore enhances food safety and shelf life thus aiding in food preservation (Annan et al., 2003; Au & Fields, 1981; Baghel et al., 1985; Beaumount, 2002; Deshpande & Salunke, 2002; Steinkraus, 1996; Yasmine, 2002).

2. Materials and Methods

2.1 Materials

The varieties of the sorghums, Sorghum bicolor (L) Moench acquired were white sorghum -KARI Mtama 1 (KM1) and the red sorghum-Seredo. The pearl millet Pennisetum glaucum variety was ICMV. The sorghum and millet varieties were obtained from Mwingi district one of the Arid and semi-arid Land areas of Kenya. The cereals were sampled form batches of two different years, 2007 and 2008 which were provided by farmers from their cereal stores. Grains (2 kgs) from several, farmers were acquired and mixed to form uniform representative samples for analysis.

2.2 Sample Preparation

Malting of the cereals was done by steeping for 24 hrs at 25ºC and germination for 72 hrs at 25ºC. Samples were sun-dried to a moisture content of 13%. The rootlets were removed by pounding, the grains milled and tannin, phytates and protein digestibility assays done.

Fermentation was through the natural way by allowing environmental microflora to colonize the cereal flours and cause lactic acid fermentation. Grains were milled and flours mixed with water at a ratio of 1:3 to form slurries which were fermented at room temperatures of 25ºC for 48 hrs. Following fermentation, tannin and phytate determination, protein-digestibility, pH and acidity levels were determined.

2.3 Determination of Tannins

This was done according to vanillin-hydrochloric acid method (Burns, 1963) modified by Price et al., (1978). Approximately 0.25 g of ground samples were weighed into Erlenmeyer flasks. Ten (10) ml of 4% HCl in methanol was pipetted into each of the flasks and closed with parafilm. The flasks were gently shaken for 20 min in a shaker (Model KS 250 basic, Germany) and the resulting extracts centrifuged for 10 min at 4500 rpm (Model H–2000C, Kokusan Corp., Tokyo, Japan). The supernatant aliquots were transferred to 25 ml volumetric flasks. Second extractions were done by adding 5 ml of 1% HCl in methanol to the residue from the first extraction and the extraction process repeated. The aliquots of the first and second extractions were combined and made up to 25 ml volume. Approximately 1 ml of each extract was pipetted to a corresponding labeled test tube. A set of catechin standard solutions was prepared ranging from 100 to 1000 ppm using methanol. Approximately 1 ml of each respective standard and sample extract were pipetted into test tubes and 5 ml of freshly prepared vanillin-HCl reagent added. Sample blanks were prepared by adding 5 ml of 4% HCl in methanol to 1 ml of the aliquot extracts in test tubes. The absorbencies of the standard solutions, sample extracts and blanks were read in a UV-VIS spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto Japan) at 500 nm 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 absorbance was subtracted from the samples absorbance and the corrected absorbance substituted into the regression equation ($y = 0.0004x$, $R=0.9972$) 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:

$$\% CE = (CC \times VM) / (VE \times Wt) \times 100$$

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

2.4 Determination of Phytates

This was done by HPLC method of phytic acid according to Camire and Clydesdale (1982). Approximately 50 mg of sample was weighed into a 125 ml Erlenmeyer flask and 10 ml of 3% H_2SO_4 added. The flasks were placed on a shaker at a moderate speed for 30 min at room temperature and filtered using a fast filter paper (Shaker Model KS 250 basic, Germany). The filtrate was transferred to a boiling water bath (BWB) for 5 min and 3 ml of $FeCl_3$ solution (6mg ferric iron per ml in 3% H_2SO_4) added. A second BWB heating was done for 45 min to complete precipitation of the ferric phytate complex. Centrifugation followed at 2500 rpm for 10 min and the supernatant discarded (Centrifuge Model H–2000C, Shimadzu Corp., Kyoto, Japan). The precipitate was washed with 30 ml distilled water, centrifuged and the supernatant discarded. Three (3) ml of 1.5 N NaOH were added to the residues and the volume brought to 30 ml with distilled water. Heating was done for 30 min in a BWB to precipitate the ferric hydroxide. Cooled samples were centrifuged and the supernatant transferred into a 50 ml volumetric flask. The precipitate was rinsed with 10 ml distilled water, centrifuged and the supernatant added to the contents of the volumetric flask.

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

A stock solution of the standard containing 10 mg/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 obtained (y = 263.13x, R= 0.9938) and used for calculating the phytate values as follows;

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

Where y is the y intercept of obtained from the standard curve of phytates, and b is the peak area of the injected sample.

2.5 Determination of Protein Digestibility

Protein digestibility was determined according to the method described by Mertz et al. (1984). This method involved determination of the protein content of sample before and after pepsin enzyme digestion. Malted, fermented samples and a sample combining both malted and fermented were assayed.

The pepsin digestion involved weighing 0.2 g of ground sample that was passed through a 0.4 mm screen and adding 35 ml of 0.1 M phosphate buffer: pH 2 containing 1.5 mg pepsin/ml. Incubation of the pepsin-sample mixture was done at 37ºC for 2 hrs with continuous gentle shaking. The suspension was then centrifuged at 4800 rpm (Centrifuge Model H–2000C, Shimadzu Corp., Kyoto, Japan) at 4ºC for 20 min. The supernatant was discarded and the residue washed with 15 ml of 0.1 M phosphate buffer: pH 7 followed by centrifugation as previously done. The supernatant was again discarded and the residue washed on Whatman's No. 3 filter paper in a Buchner funnel. The filter paper containing the undigested protein residue was folded, placed in a digestion tube and dried for 2 hrs at 80ºC.

Control samples of non-fermented and non-germinated cereals and blanks prepared, pepsin digested and protein content determined using the AOAC (AOAC, 1995, Method 20.87-32.1.22) method.

Calculation: Percentage protein digestibility = (A−B)/A

Where: A = % protein in the sample; and B = % protein factor after pepsin digest

2.6 Determination of pH

This was done by the method of Ofori and Hahn (1994). The pH meter (TOA pH Meter HM–7B, Tokyo, Japan) was standardized using buffer solutions of acidic and basic values of 4.01 and 9.08 at 25°C. The electrode was rinsed with distilled water before taking measurements. The fermented samples, (slurry mixtures of flour and water in the ratio of 1:3) were homogenized by stirring to achieve uniformity. pH readings were taken by dipping the electrode in the fermented mix and measurements taken from the display screen when the readings stabilized.

2.7 Determination of Total Titratable Acidity (TTA)

TTA analysis was done using AOAC (1995) method. Approximately 10 ml of sample was pipetted into a conical flask and 2 drops of phenolphthalein indicator used. Titration was done using 0.1N NaOH to a faint pink colour

for at least one min (compared against a white background). The titre volume was noted and used for calculations of TTA which was expressed as percentage lactic acid. Calculations of TTA was determined and expressed as follows:

$$\% \text{ Lactic acid} = A \times 0.009 \times 100/V$$

Where: A = ml of 0.1 NaOH required for the titration; and V = ml of sample taken for the test. 0.009 is a Constant.

2.8 Data Analysis

Variations between malting and fermentation treatments with respect to tannin, phytates and protein digestibility among the cereals was determined using ANOVA and means separated using Duncan's Multiple range test. SAS statistical package was used. Results are given as mean ± SD (Snedecor & Cochran, 1987).

3. Results and Discussion

3.1 Effect of Malting and Fermentation on Tannins and Phytates

There were significant differences in anti-nutrient reduction with malting and fermentation ($p \leq 0.05$). Phytate reduction range was 20-21% with 2 days of fermentation and 19-33% with 3 days of malting (Figures 1 (a) and (b); Figures 2 (a) and (b)). High tannin content inhibits phytase activity. This renders fermentation less effective in reducing anti-nutrients in cereals such as bulrush millet and red sorghum (Sandberg, 1991). For fermentation to be effective in reducing the anti-nutrients it should be carried on grains with low levels of the anti-nutrients. These could be varieties with low anti-nutrient levels or decorticated grains; alkali treated grains or malted grains. At low anti-nutrient levels, fermentation induces phytate hydrolysis to lower inositol phosphates which have a negative effect on zinc and iron absorption (Lönnerdal et al., 1989).

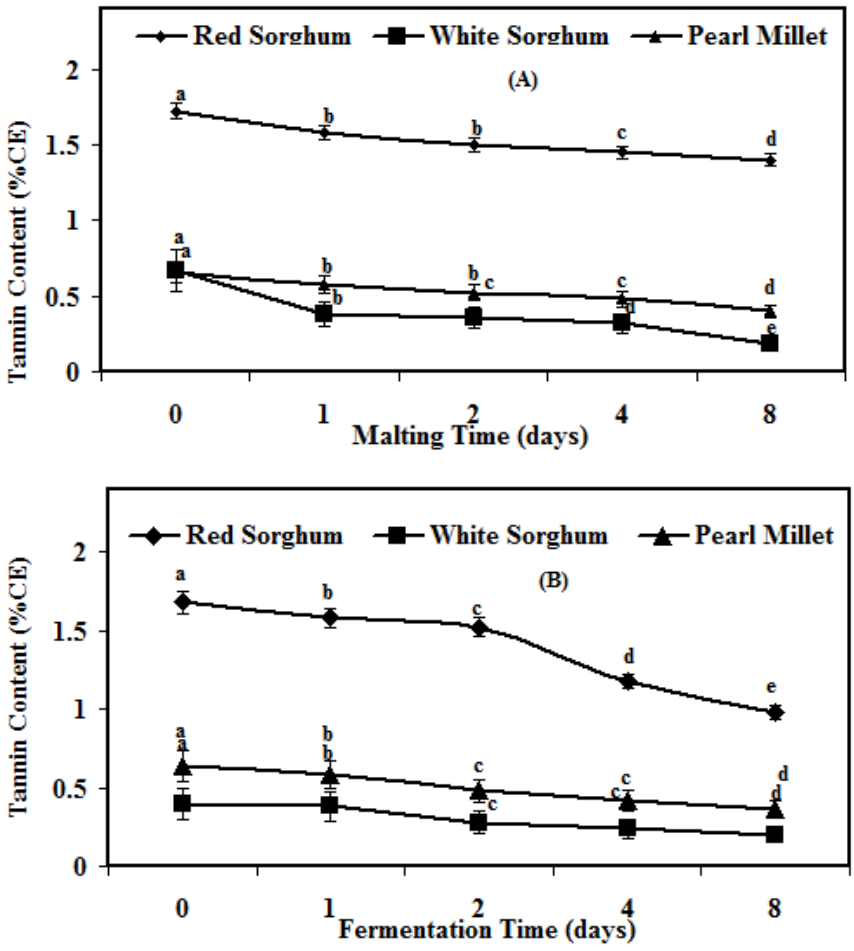

Figure 1. Effect of malting (a) and fermentation (b) on tannin content of cereals. Points on the same curve with similar letter(s) have means that are not significantly different ($p \geq 0.05$)

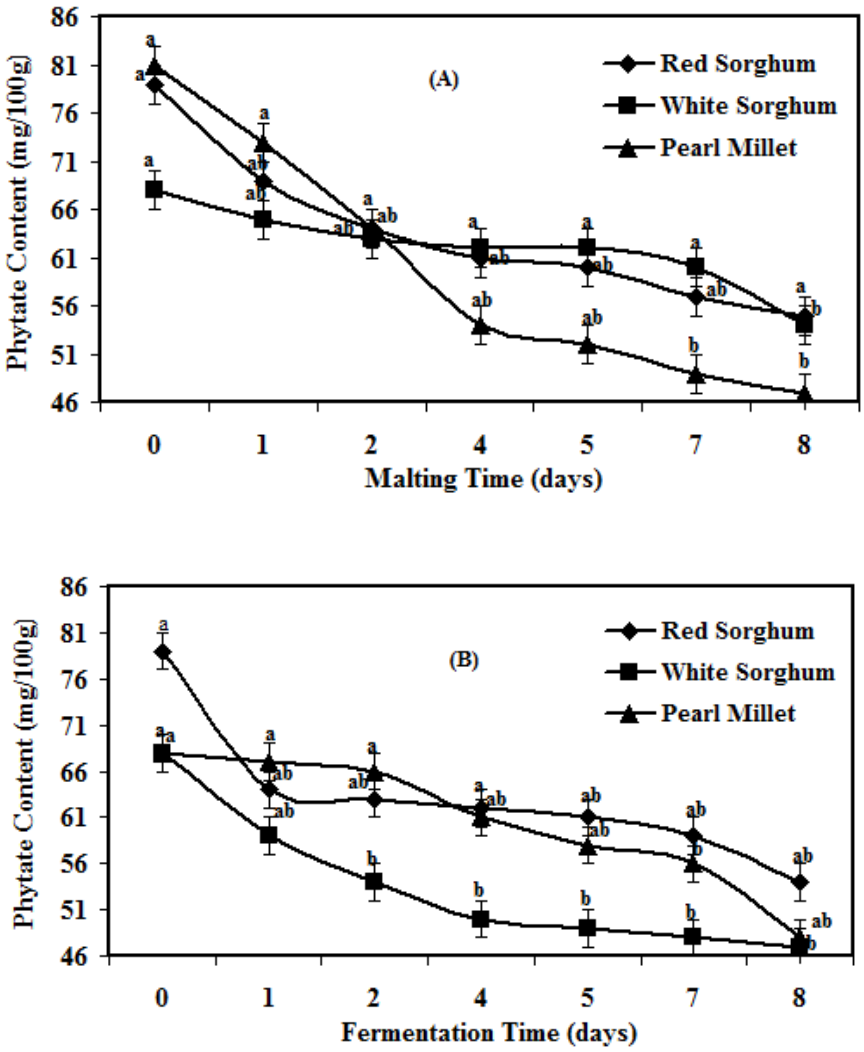

Figure 2. Effect of malting (a) and fermentation (b) on phytate content of the cereals. Points on the same curve with similar letter(s) have means that are not significantly different ($p \geq 0.05$)

3.2 Effect of Malting and Fermentation on Protein Digestibility

Figure 3 shows percentage protein digestibility of malted and fermented cereals compared to the control. There were significant differences ($p \leq 0.05$) in the protein digestibility of the control, malted and fermented cereals. Pearl millet had the least digestibility (21.5%) among the three cereals while white sorghum had the highest digestibility (51.7%). These findings were in agreement with work by other similar research (Aliya & Geervan, 1981). Malting for 24 hrs significantly ($p \leq 0.05$) increased protein digestibility of the cereals. Pearl millet's digestibility increased from 21.5% to 34.5% and red sorghum from 48% to 68.1%. Fermentation also significantly increased the protein digestibility of the cereals ($p \leq 0.05$). The range was from 97.4% to 98.3% in red and white sorghum respectively. A combination of malting and fermentation produced highly digestible flours which were significantly ($p \leq 0.05$) different from the control and malted flours but not from fermented flours. The cereals used were detoxified using alkali treatment followed by malting and then fermentation.

3.3 Effects of Fermentation on pH and Total Titratable Acidity (TTA)

The TTA of the fermented gruels increased as the pH of the ferments gradually reduced with increase in fermentation time, Figures 4(a) and (b). There were significant increments in TTA and pH ($p \leq 0.05$) from day 0 to day 2 in all the cereals. The changes from day 2 onwards were less rapid though they were still significant ($p \leq 0.05$).

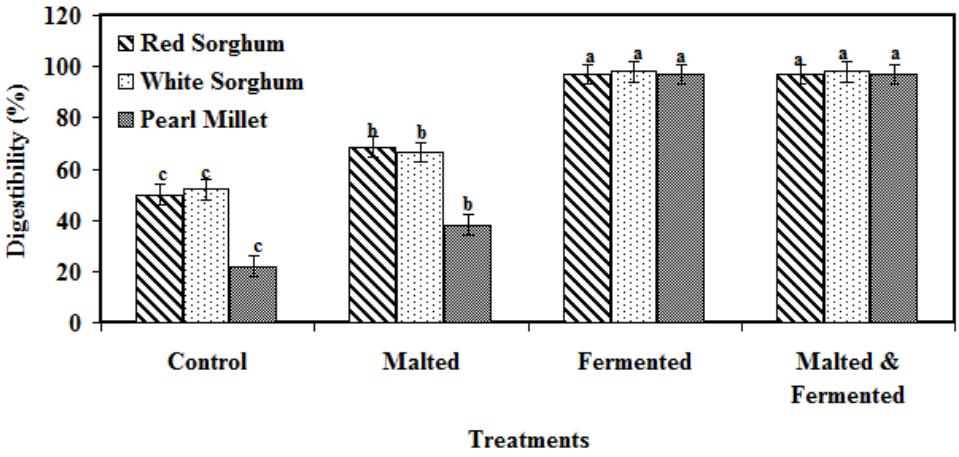

Figure 3. Changes in protein digestibility after malting and fermentation of selected cereals for 24 hrs (Cereals represented by the same letter(s) in the legend are not significantly different at $p \leq 0.05$)

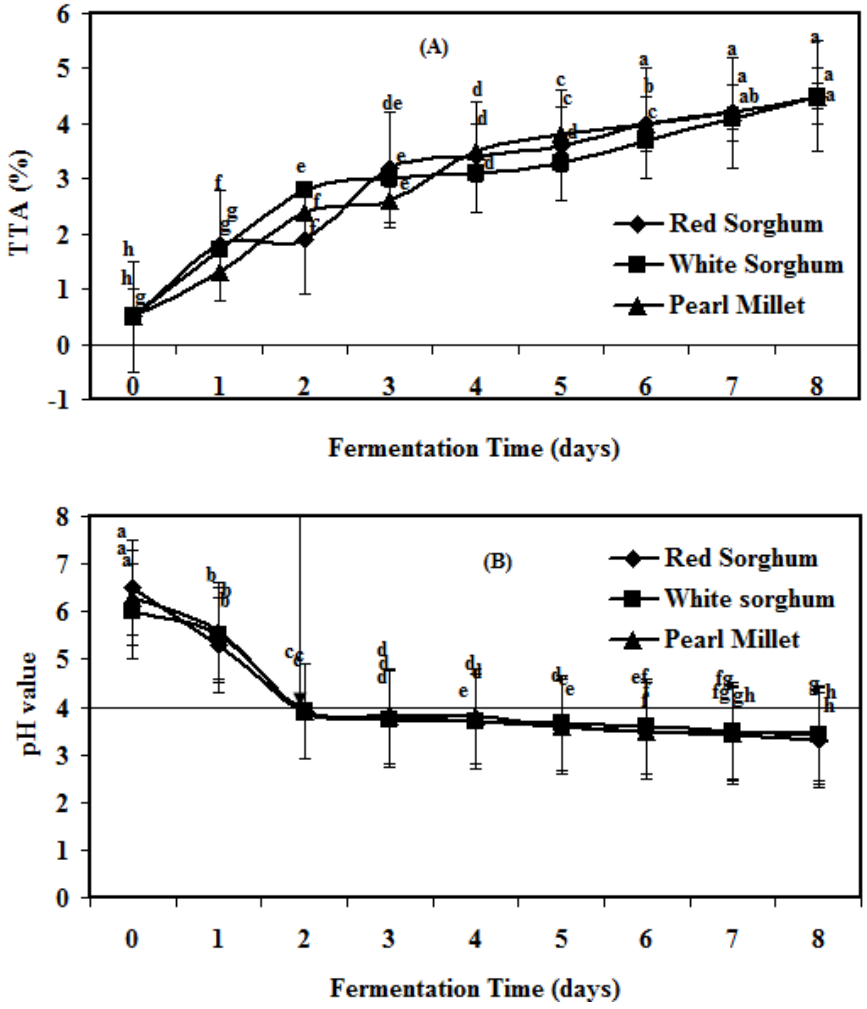

Figure 4. TTA (a) and pH (b) of cereal flours during fermentation for a period of 8 days

The vertical bars represent S.D of the means of 3 replicates. S.D= Standard deviation. The horizontal line cutting across the chart shows the point where the pH values of 4 lies on the pH scale. The arrow indicates the time when pH value of 4 was attained. Points on the same curve with similar letter(s) have means that are not significantly different ($p \leq 0.05$).

Pearl millet and red sorghum exhibited the highest reduction in pH from day 0 of fermentation. The pH drop in all the cereals was fast during the fermentation period of 8 days and the critical pH value of 4.0 was attained by the end of the second day. Fermentation to pH value of 4.0 and below is recommended in cereal flours and products meant for making thin porridges for complementary feeding of children. This helps in their preservation during storage due to the low pH levels which many microorganisms cannot withstand (Mbugua & Njenga, 1991; Steinkraus, 1996). Further drop in pH serves the purpose of lowering the acidity and making the fermented mix more sour further altering the organoleptic properties of the ferments.

Similarly the TTA of the pearl millet and red sorghum cereals were correspondingly high. High acidity was thus critical and responsible for the low microbial load in the products during the storage period as seen later on in the microbial analysis section of the results. A secondary effect was in the alteration of the organoleptic properties of the cereal products that is imparting the sour acidic taste and aroma compounds (Steinkraus, 1983).

4. Conclusion

Malting and fermentation had several desirable effects on cereals. The processes decreased anti-nutrient levels and increased protein digestibility. Malting and fermentation were optimally done at 25ºC for 3 days and 2 days respectively. Malting increased protein digestibility but to a significantly lower level than that of fermentation ($p \leq 0.05$). Malting and fermentation treatments can therefore be used singly or in combination as anti-nutrient detoxification treatments and at the same time nutrient enhancers by households and processors who use sorghum and millet.

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