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Effects of malting and fermentation treatments on group B-vitamins of red sorghum, white sorghum and pearl millets in Kenya

Ochanda, Simon Oduor[1], Onyango Christine Akoth[1*], Mwasaru Alfred Mwanjallah[1], Ochieng Joy Kagwiria [2], and Mathooko Francis Mutiso[3]

[1]Department of Food Science, Jomo Kenyatta University of Agriculture and Technology. P.O. Box 62000-00200 Nairobi, Kenya; [2]Ministry of Agriculture, Mwingi District Agriculture Office, P.O. Box 31, Mwingi, Kenya; [3]South Eastern University College, P.O. Box 170-90200 Kitui, Kenya.

*Corresponding author: Email cakoth2002@yahoo.co.uk; Cell phone +254 733 730918
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ABSTRACT

Objectives: To enhance the safety and nutritive values and shelf life of sorghums and millet flours through malting and fermentation.

Methodology and results: Malting and fermentation were carried out for a period of seven days with the aim of determining the optimal number of days needed for each of these processing treatments. The quantities of folic acid, niacin, thiamin, pyridoxine and riboflavin (B-vitamins) were then determined by the reversed-phase HPLC method described by Ekinci and Kadakal (2005), modified from Cho et al., (2000) on each successive day of malting and fermentation. Optimum results as determined on the basis of highest increments in the contents of B-vitamins were obtained after malting for 3 days and fermentation for 2 days, at 25°C. Acidity and pH were also altered by these processing techniques leading to improvement of flavour and aroma shown by preliminary sensory evaluation results.

Conclusion and application of findings: Availability of all the selected B-vitamins was significantly enhanced by fermentation by between 71.2 - 94.2%. On the other hand only riboflavin was significantly affected by malting with 44.2% increase, the rest increased by less than 10.5%. The results of this study will be useful for the development of new products from neglected indigenous cereals like sorghum and millet. This will enable value chain development for the benefit of the community through realization of high nutrient foods and better incomes from crops that do well in adverse climatic conditions.

Key words: Malting, fermentation, B-Vitamins, pH, Total titratable Acidity (TTA).

INTRODUCTION

Malting and fermentation treatments have been employed by various communities to enhance organoleptic properties of foods. Malting increases the endogenous phytase activity in cereals, legumes, and oil seeds through de Novo synthesis, activation of intrinsic phytase or both (Egli *et al.,* 2002) leading to reduction of total phytates in the cereals thus enhancing their nutritional value. Another beneficial effect of germination is the increase in the activity of α-amylase enzyme of cereals, especially in sorghum and millet. The complex carbohydrates are hydrolyzed to dextrins

and maltose, reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Gibson *et al.*, 1998).

Fermented food has many beneficial products metabolized by bacteria. These include biomass proteins and amino acids, vitamins, minerals, carbohydrates, flavor and aroma compounds. There are also products of respiratory and biosynthetic pathways such as lactic acid, ethanol, acetaldehyde and pyruvic acid. These alter the pH of foods to levels that reduce the growth of pathogenic microorganisms. Fermentation therefore, not only enhances food safety and shelf life, naturally preserving the food, but also enhances nutritional value (Au and Fields, 1981; Baghel *et al.,* 1985; Steinkraus, 1996; Beaumount, 2002; Deshpande and Salunke, 2002; Yasmine, 2002; Annan *et al.,* 2003).

MATERIALS AND METHODS

Plant materials: Sorghum (*Sorghum bicolor* (L) *Moench*) varieties acquired for the research were white sorghum -KARI *Mtama* 1 (KM1) and the red sorghum-*Seredo*. The pearl millet *Pennisetum glaucum*, variety used was ICMV. The dry cereal grains (moisture content approximately 13%) were obtained from Nguni sub-location, Mwingi District, semi arid zone in Eastern Province, Kenya. Sampling was done three times from three selected sources. The first samples were obtained from farmers' stores from the harvest of the year 2007; six farmers provided 2kg of each sample variety. The second samples were obtained from the same farmers from the harvest of the year 2008. The third samples were obtained from local cereal stores in Nguni market where six (6) shops were randomly selected from which 2kg each sample was purchased. Farmers in these regions do not apply fertilizers but instead use manure made from plant, cattle and poultry wastes. After each sampling, treatment and analysis were done within a period of one month.

The grain samples were first subjected to alkali treatment (*magadi* soda) to reduce the levels of tannins and phytates before the malting and fermentation treatments. This is a practice normally carried out by people living in ASAL communities to reduce bitterness in these cereals. The grains were steeped in a 2% solution of *magadi soda* for 12 hours. This does not interfere with their germinability. The grains were then rinsed in tap water and a portion dried in an air oven at 100°C for analysis while the rest were subsequently subjected to malting followed by fermentation. The changes in nutrient contents, acidity and pH were then analyzed.

Malting and fermentation treatments: Malting was done by first steeping for 24hr at 25ºC; germination was left to take place for a period of one week followed by natural sun drying to a moisture content of 13%. The sprouts were removed by pounding, the grains milled and analysis of B-vitamins done.

Fermentation was done by natural lactic acid method. Flours were mixed with water in a ratio of 1:3 to form slurries which were fermented at room temperatures for a period of one week. Following fermentation, the pH, total titratable acidity (TTA) and B-vitamins were determined.

Selected Group B-vitamins determination

Folic acid, pyridoxine, niacin, and biotin: The reversed-phase HPLC method described by Ekinci and Kadakal (2005), modified from Cho et al*.,* (2000) was used. The sample treatment consisted of SPE with Sep-Pak C_{18} (500mg) cartridges that enabled separation of water-soluble vitamins and removed most of the interfering components. Twenty (20) ml of water was added to 5g of the sample. The mixture was homogenized using a homogenizer (Model KS 250 basic, Germany) at 2000 rpm for 1 min. The homogenized samples were centrifuged for 10 min at 14×10^3g (Japan model H–2000C). To prepare the stationary phase, a Sep-Pak C_{18} (500mg) cartridge was flushed with 10 ml methanol and 10 ml water (pH 4.2) to activate the column. The homogenized and centrifuged samples were then loaded on the Sep-PaK C18 (500mg) cartridges and eluted with 5 ml acidified water (pH 4.2) then 10 ml methanol at a flow rate of 1 ml min^{-1} using a syringe. The eluent was collected in a bottle and evaporated to dryness in a vacuum evaporator-Model Rotavapor R-3000 BUCHT Switzerland. The residue was dissolved in mobile phase (0.1 mol /L KH_2PO_4 (pH 7.0)–methanol, 90: 10) and then filtered through 0.45μm pore size filters. Samples of 20μl each were injected into the HPLC column. The column elute was monitored with a photodiode-array detector at 234 nm for thiamin, 324 nm for pyridoxine, 282 nm for folic acid, and 261 nm for niacin. The vitamins were analyzed in an HPLC

Shimadzu model SCL-10A using a column of inertsil ODS 5µm 4.6 × 250 mm 5LI0101Z with 0.1 mol /L KH_2PO_4 (pH 7.0)–methanol, 90:10 mobile phase (filtered through 0.45µm membrane and degassed by sonication), flow rate of 0.5 ml/min, a photodiode-array detector (Waters 2996 model), oven temp of 25ºC, and a sample volume of 20µl. Identification of compounds was achieved by comparing their retention times and UV spectra with those of standards. Five different concentrations of each standard were used to prepare calibration plots for each vitamin. This was done by plotting concentration (µg/ml) against peak area (mAU). Their correlation coefficients were greater than 0.997. Concentrations of the water-soluble vitamins were calculated from integrated areas of the sample and the corresponding standards. Vitamin content (mg/g) = (y/b) × (dilution factor / weight of sample × 1000), where y is the calculated weight of the vitamin in sample and b is the weight of the sample.

Riboflavin*:* HPLC (AOAC, 1996) method was used to quantify riboflavin. Approximately 1g of ground sample was mixed with 10 ml of distilled water and homogenized. The pH was adjusted to 3.0 using acetic acid glacial (1:1, v/v). The sample was vortexed before centrifugation at 10,000 rpm (Japan model H–2000C) for 20 min and the resulting supernatant transferred into a 25 ml volumetric flask. The sediment was washed with 5 ml of 2% acetic acid solution, the washings combined and centrifuged. The second supernatant was added to the first and volume made to the mark using 2% acetic acid solution. Filtration was done and the sample injected into the HPLC (Japan Shimadzu model C–R7A with chromatopac). Operating conditions were as follows: Column: Inertsil, ODS (C_{18}) 5µm 250 × 4.6 mm, Mobile phase: Methanol: Water: acetic acid (40:59.5:0.5), Detector: UV-VIS (MDS510M-24-F2) at 270 nm and 0.02 sensitivity, Flow rate: 0.5-1.0ml/min, Injection volume: 10 µl. The centrifuge micro-filters size was 0.45 µm. Riboflavin standard stock solution of 1000 ppm in 2% aqueous acetic acid was prepared and serial dilutions of 100 - 600 ppm made. The standards were injected into the HPLC and corresponding peak areas obtained and used to plot a standard curve whose equation was used to calculate the quantity of riboflavin as:

Vitamin content (mg/g) = (y/b) × ([dilution factor / weight of sample] × 1000); where y is the weight of the vitamin in grams and b the weight of the sample.

pH*:* This was done by the method of Ofori and Hahn (1994). Before measurements of pH were taken, the sample slurries were thoroughly stirred to homogenize the mixture and achieve uniformity. The pH electrode was dipped in each of the slurries and measurements were taken when the readings on the pH meter were stable using a TOA pH Meter HM 7B.

Total titratable acidity (TTA): The TTA analysis was done using AOAC (1996) method. Approximately 10 ml of sample was pipetted into a conical flask and 2 drops of phenolphthalein indicator added. Titration was done using 0.1N NaOH to a faint pink colour for at least 1 min compared against a white background. The titre volume was noted and used to calculate TTA which was expressed as percentage lactic acid. TTA was determined and expressed as follows:

% Lactic acid = A × 0.009 × 100/V; where A = ml of 0.1 NaOH required for the titration; and V = ml of sample taken for the test.

Data analysis: Data was analyzed using Microsoft excel and SAS statistical Package (Snedecor and Cochran, 1987). Mean comparisons for treatments were made using Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

B–vitamins quantities: Five B-vitamins are known to increase with malting and fermentation treatments in cereals. The results of the effects of malting and fermentation on B-vitamin contents of the cereals used in this study are in Tables 1-3. Malting had little or no effect on the quantities of these vitamins. Fermentation on the other hand significantly enhanced their quantities ($p \leq 0.05$). The initial values of the B-complex vitamins in the cereals were similar to those obtained by other researchers (USNRC/NAS, 1982; Chauhan *et al.,* 1986). Folic acid range was 0.02-0.02mg/100g, niacin 4.20-5.23mg/100g, thiamin 0.34-0.35mg/100g, pyridoxine 0.25-0.26mg/100g and riboflavin 0.15-0.21mg/100 for the sorghums with malting. While pearl millet had folic acid 0.01-0.02mg/100g, niacin 4.32-5.46mg/100g, thiamin 0.35-0.35mg/100g, pyridoxine 0.27-0.27mg/100g and riboflavin 0.21-0.26mg/100g. For fermentation the sorghums values were folic acid 0.02-0.09mg/100g, niacin 4.22-15.70mg/100g, thiamin0.35-1.05mg/100g, pyridoxine 0.35-1.32mg/100g, and riboflavin 0.15-0.18mg/100g while for pearl millet the values were folic acid 0.01-0.04mg/100g, niacin 4.42-10.24mg/100g, thiamin 0.36-1.60mg/100g, pyridoxine 0.27-0.50mg/100g and

riboflavin 0.21-0.24mg/100g. The increments in the B-vitamin values of the malted cereals were not significant except with riboflavin while the fermented cereals all showed statistically significant differences in all the grains and were in agreement with reported work from other researchers ($p \leq 0.05$). These values were obtained with optimal time periods of 3 days of malting and 2 days of fermentation.

Table 1: Changes in B-vitamins contents of malted and fermented red sorghum (mg/100g)

Treatment	Time (days)	Folic acid	Niacin	Thiamin	Pyridoxine	Riboflavin
Malting	0	0.02	4.20	0.34	0.25	0.16^{d}
	3	0.02	5.23	0.35	0.26	0.19^{c}
	4	0.02	5.25	0.35	0.26	0.21^{b}
	6	0.02	5.30	0.35	0.26	0.31^{a}
	7	0.02	5.34	0.35	0.27	ND
	8	0.02	5.37	0.35	0.27	ND
LSD		<0.01	1.43	0.01	0.17	0.01
Fermentation	0	0.02^{d}	4.22^{d}	0.35^{c}	0.22^{b}	0.16^{b}
	1	0.03^{dc}	5.89^{dc}	1.11^{c}	0.25^{b}	0.18^{b}
	2	0.06^{bc}	13.07^{c}	1.79^{bc}	0.27^{b}	ND
	4	0.09^{ba}	22.85^{b}	5.54^{bac}	0.44^{ba}	0.29^{a}
	7	0.10^{a}	29.91^{ba}	7.22^{ba}	0.50^{ba}	ND
	8	0.11^{a}	33.73^{a}	4.52^{a}	1.06^{a}	ND
LSD		0.03	7.34	4.21	0.68	0.06

Values are means of 3 replicates. Means on the same column followed by different letters are significantly different ($p \leq 0.05$).
*ND=Not determined. LSD= Least significant difference of the mean replicates.

Several researchers have reported increments in some vitamins brought about by malting and fermentation (Aliya and Geervani, 1981; Carter and Carpenter, 1981; Opoku *et al.;* 1981; Malleshi and Desikachar, 1986*)*. Some vitamins in cereals like niacin exist in bound forms which are alkali soluble but biologically unavailable to humans. Such vitamins are available for the growth of the microorganisms and can be accessible to humans by fermentation (Ghosh *et al.,* 1963; Adrian *et al.,* 1970; Carter and Carpenter, 1981, 1982 Wall and Carpenter, 1988).

Table 2: Changes in B-vitamins contents of malted and fermented white sorghum (mg/100g)

Treatment	Time (days)	Folic acid	Niacin	Thiamin	Pyridoxine	Riboflavin
Malting	0	0.02	4.25^{b}	0.35	0.17	0.15^{d}
	1	0.02	4.95^{ba}	0.35	0.18	ND
	3	0.02	5.11^{a}	0.35	0.18	0.21^{cd}
	4	0.02	5.26^{a}	0.35	0.18	0.25^{cb}
	6	0.02	5.30^{a}	0.35	0.18	0.38^{a}
	8	0.02	5.34^{a}	0.35	0.18	ND
LSD		<0.01	0.77	0.01	0.02	0.06
Fermentation	0	0.02^{b}	4.55^{c}	0.35^{c}	0.35^{c}	0.15^{d}
	1	0.03^{b}	8.92^{c}	0.53^{c}	0.53^{bc}	0.18^{cd}
	2	0.03^{b}	15.70^{bc}	1.05^{bc}	1.32^{c}	ND
	4	0.04^{b}	31.56^{ba}	1.32^{bc}	1.05^{bc}	0.28^{bc}
	7	0.07^{ba}	42.26^{a}	1.97^{ba}	1.97^{ba}	ND
	8	0.09^{a}	47.8^{a}	2.51^{a}	2.51^{a}	0.52^{a}
LSD		0.05	20.67	1.19	1.19	0.13

Values are means of 3 replicates. Means on the same column followed by different letters are significantly different ($p \leq 0.05$).
*ND=Not determined. LSD= Least significant difference of the mean replicates.

Malting raised folic acid levels by 9%, niacin by 3%, thiamin by 0.2%, pyridoxine by 10% and riboflavin by 44%. Fermentation raised niacin folic acid by 90%, niacin by 91%, thiamin by 94%, pyridoxine by 86% and riboflavin by 71%. Malting did not significantly enhance the availability of the water soluble B-vitamins mentioned above except riboflavin. This could be because germination which occurs during malting, utilizes nutrients for growth and development of the seedlings while contributing very little in terms of synthesis of new products. Fermentation on the other hand significantly enhanced availability of the water soluble vitamins. This may partly be attributed to microbial activities during fermentation where synthesis and breakdown of substances occur (Hulse *et al.,* 1980).

Table 3: Changes in B-vitamins content of malted and fermented pearl millet (mg/100g)

Treatment	Time (days)	Folic acid	Niacin	Thiamin	Pyridoxine	Riboflavin
Malting	0	0.01	4.32^{b}	0.35	0.27	0.21^{b}
	1	0.01	5.23^{ba}	0.35	0.27	ND
	3	0.02	5.46^{a}	0.35	0.27	0.26^{b}
	4	0.02	5.48^{a}	0.35	0.28	0.33^{a}
	7	0.02	5.57^{a}	0.35	0.28	0.37^{a}
	8	0.02	5.58^{a}	0.35	0.28	ND
	6	0.02	5.58^{a}	0.36	0.28	ND
LSD		<0.01	1.05	0.01	0.17	0.06
Fermentation	0	0.01^{c}	4.42^{c}	0.36^{b}	0.27^{c}	0.21^{c}
	1	0.02^{c}	6.41^{c}	1.25^{ba}	0.37^{bc}	0.24^{c}
	2	0.04^{bc}	10.24^{c}	1.60^{ba}	0.50^{bac}	ND
	4	0.07^{bac}	22.12^{b}	2.23^{ba}	0.67^{bac}	0.34^{b}
	7	0.08^{ba}	28.81^{a}	2.92^{a}	0.83^{ba}	ND
	8	0.11^{a}	34.61^{a}	3.36^{a}	0.97^{a}	0.41^{a}
LSD		0.05	5.86	2.35	0.54	0.05

Values are means of 3 replicates. Means on the same column followed by different letters are significantly different ($p \leq 0.05$).
*ND=Not determined. LSD= Least significant difference of the mean replicates.

Effects of fermentation on pH and TTA of the cereals: The pH of the fermented cereals gradually reduced as fermentation time increased while TTA increased, Figure 1(a and b). There were rapid and significant differences in changes in TTA and pH ($p \leq 0.05$) from day 0 to day 2 in all the cereals, while from day 2 onwards the changes were less rapid but still significant ($p \leq 0.05$).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 and attained the critical value of pH 4 by the end of the second day. Fermentation to pH value of 4 and below is recommended in cereal flours and products meant for making thin porridges for complementary feeding of children. This pH helps in their preservation during storage due to the high acid levels in which many microorganisms cannot survive (Steinkraus, 1996).
The drop in pH is expected to make the fermented mix sour and also enhance the keeping quality since microbial inhibition is effected below pH 4.

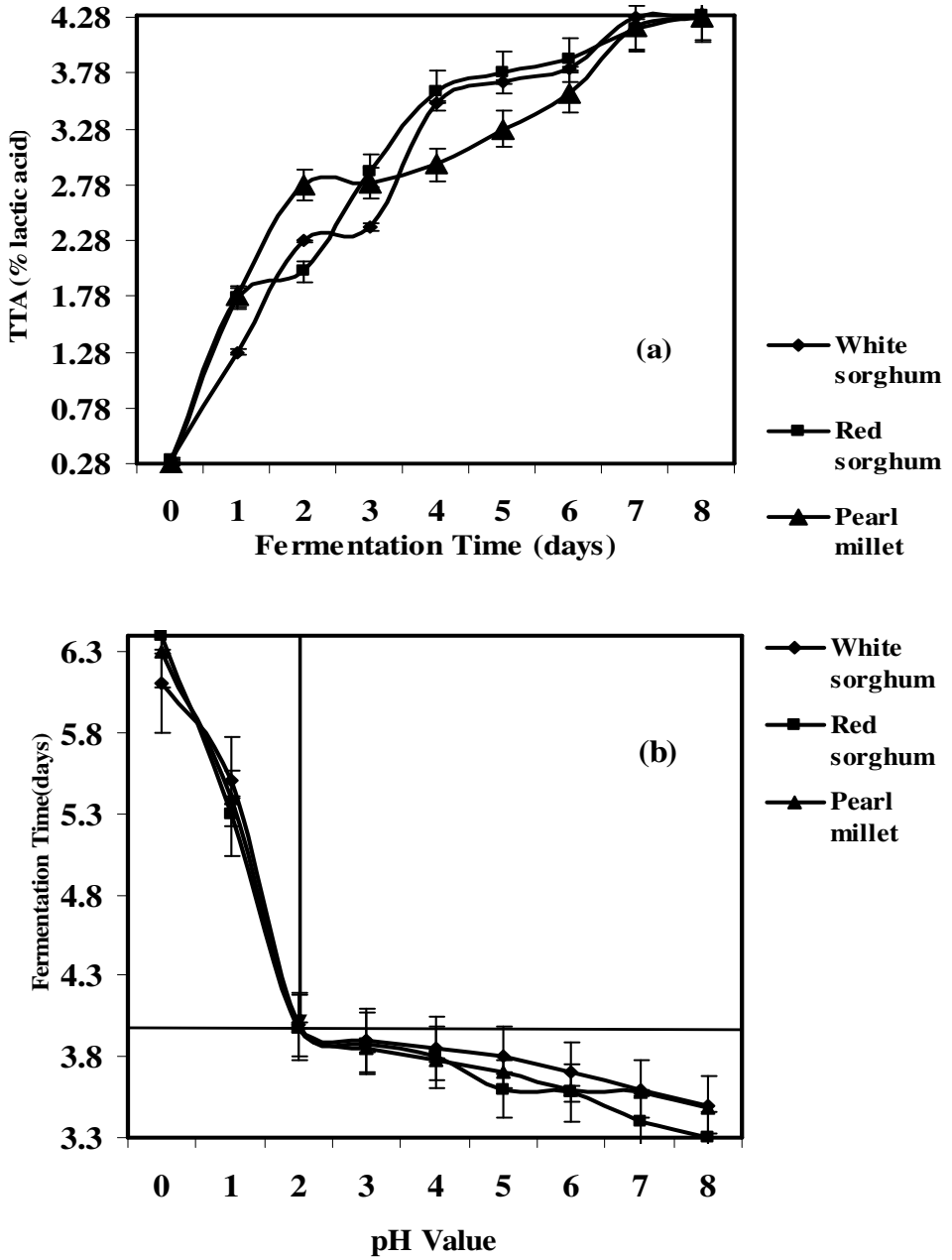

Figure 1: Total Titratable acidity (TTA) (a) and pH (b) of fermented cereal flours over 8 days

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