African Journal of Food Science and Technology (ISSN: 2141-455) Vol. 14(7) pp. 01-08, August, 2023

DOI: http:/dx.doi.org/10.14303//ajfst.2023.031

Available online @https://www.interesjournals.org/food-science-technology.html

Research Article

Flavour Compound Production during the Fermentation of a Sorghum Beverage

Sanchia Moodley*

Department of Food Science and Technology, University of Pretoria, Pretoria, South Africa

E-mail: sanchia.moodley@gmail.com

Abstract

Traditionally fermented products are developed by local inhabitants of a region and the production method is handed down through generations. These methods result in products with variable quality and a short shelf-life. Motoho is a traditionally produced fermented sorghum beverage indigenous to the Basotho people of southern Africa. The current research aims to determine the effects of using a defined starter culture comprising of *Lactobacillus fermentum* and *Lactobacillus plantarum* compared to the traditional production, on the sensory qualities and shelf-life of motoho. Three different sorghums were used to produce batches A (traditional), J (modified) and M (modified) of motoho respectively. Samples were subjected to 4ºC and 37ºC storage for the shelf-life study while a sensory panel assessed the acceptability of each motoho variety. The microbiological counts for A, J and M incubated at 4ºC were 1 log cfu/ml, 1.48 log cfu/ml and 1.3 log cfu/ml on day 5 respectively. The counts for A, J and M incubated at 37ºC were 3.48 log cfu/ml, 3.18 log cfu/ml and 3.01 log cfu/ml on day 5 respectively. The largest amounts of ethyl and methyl esters were produced during the fermentation of J which could account for the preference in aroma and flavour by sensory panellists. Aldehydes were found in the fermentation of A while acetoin and butanediol were produced during the fermentation of M. Panellists also preferred the appearance of J but the mouth feel of M. The results from this study could be applied during upscaling of the production of motoho.

Keywords: Motoho, Volatile organic compounds, Sensory analysis, Starter cultures, Shelf-life, Sorghum fermentation

INTRODUCTION

Traditionally fermented products are indigenous to certain regions and have been developed by indigenous people using locally sourced raw materials and age-old techniques as an essential part of food security strategies (Aka, et al., 2014) and for commercial use. Fermentation is a cheap method of preserving food as well as increasing the nutritional and sensory quality milk and cereal fermentations to produce beverages which promote health are indigenous to many Asian, European, South American, middle Eastern and African states. where energy and capital usage for food production and preservation is limited (Akinrele, 1970).

Received: 04-November-2021, Manuscript No. AJFST-23-46704; **Editor assigned:** 09-November-2021, PreQC No. AJFST-23-46704 (PQ); **Reviewed:** 23-November-2021, QC No. AJFST-23-46704; **Revised:** 19-July-2023, Manuscript No. AJFST-23-46704 (R); **Published:** 16-August-2023

Citation: Moodley S (2023). Flavour Compound Production during the Fermentation of a Sorghum Beverage. AJFST: 031.

In the food industry, the functional beverage market globally is growing and consumers increasingly want foods to improve their well-being and decrease the risk of disease. Motoho is a traditionally fermented sorghum beverage produced by the Basotho people of southern Africa and can be consumed by the whole family and is prepared daily or weekly. Regular sorghum is used to manufacture motoho by spontaneous fermentation which is an uncontrolled process that depends upon microorganisms which originate from the sorghum, utensils or added as a traditional starter culture. This results in products with variable quality and sensory attributes (Campbell-Platt, 1984). This can be overcome by isolating and purifying the predominant organisms found in an acceptable product and using said organism as a starter culture to initiate the fermentation.

Previous research on motoho included isolating and characterizing Lactic Acid Bacteria (LAB) namely *Lactobacillus fermentum* and *Lb. plantarum* which were selected as potential starter cultures for the optimization process of motoho. *Lactobacillus plantarum* was also identified in Bushera and in many other fermented and cooked products (Chaves-Lopez, et al., 2014) as well as in maize products like and is dominant towards the culmination of many spontaneous cereal fermentations possibly due to high acid tolerance. Lactobacillus. fermentum was isolated during the fermentation of a Sudanese sorghum flatbread called kisra, a Beninese fermented maize dough called mawe (Chen, et al., 2010) and also in a Beninese fermented and malted sorghum food called gowe (Curioni, et al., 2002). It was assumed that the use of starter cultures to produce motoho could decrease the variability of the end product. In order to reproduce the desired traits of traditional beverages for mass production, appropriate starter cultures need to be selected.

The microbes sourced from these beverages have been adapted to their environment over many years and will be able to function at the appropriate conditions like temperature and salt concentration (Efiuvwevwere, et al., 1995). Strain selection also must ensure the correct balance of texture, speed of fermentation, the optimal amount of organic acids, aroma, flavour, vitamins and minerals of the final product (Halm et al., 1993). The aim of this research was to determine the effect of the selected LAB strains on the shelf-life and sensory acceptability of motoho produced with 3 varieties of sorghum and by using traditional and modified methods of production. This research could contribute to the body of knowledge regarding motoho as well as to assist in future upscaling and possible commercialization of the product.

MATERIALS AND METHODS

Sorghum

Commercial pure grain sorghum (King Korn Mabele, King Food Corporation, Potchefstroom, South Africa) was purchased locally and used for the control motoho.

Two varieties (Rakodzi and SC Smile) of brown, high tannin sorghums were used to produce modified motoho and were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Matapos research station, Bulawayo, Zimbabwe.

Microbial strains

Lactobacillus fermentum was isolated during previous research on motoho and stored at -20ºC in glycerol. *Lactobacillus plantarum* ATCC culture was purchased from microbiologics. To obtain pure colonies these bacteria were sub-cultured thrice onto MRS (Hounhouigan, et al., 1993) (Oxoid, Basingstoke, UK) agar which was incubated for 48 h at 37˚C.

Pure bacterial cultures were inoculated into 10 ml of MRS broth (Merck, Darmstadt, Germany) and incubated for 24 h at 37˚C, centrifuged at 4500 rpm (Beckman Coulter, Brea, California, U.S.A) for 10 min and the supernatant was discarded. The cell pellets were re-suspended in 0.85% NaCl solution to produce a stock of 107 cfu/ml. For the starter culture for the control fermentation, commercial sorghum was added to regular tap water (1:10 w/v). Five grams of commercial brown sugar was then and the mixture was incubated for 24 h at 30˚C. *Lactobacillus plantarum* and *Lb. fermentum* (1:1 v/v) were used as the starter culture for the production of the modified motoho (J and M).

Production of motoho

The production of both the traditional motoho (A) as well as the motoho produced using Rakodzi sorghum (J) and SC Smile sorghum (M) is represented in Figure 1.

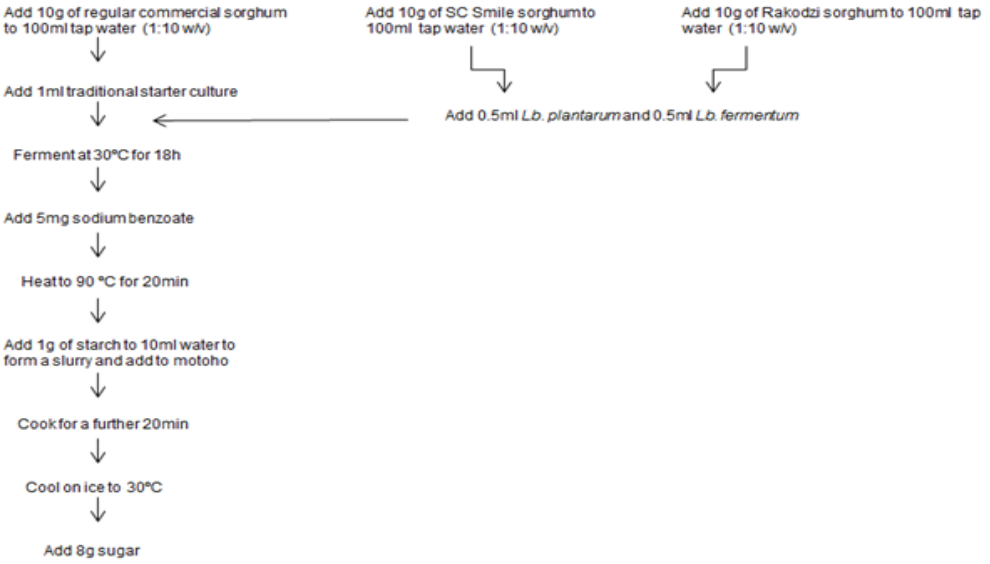

Figure 1. Flow chart outlining the production of the traditional motoho (A) and variations of this method (J and M) using defined starter cultures. Ten replicates of each motoho were produced.

Microbial analyses

Five millilitres of each motoho batch were diluted into 45 ml of sterile Maximum Recovery Diluent (MRD) (1 g bacteriological peptone (oxoid) and 8.5 g NaCl per litre of distilled water). Total Plate Counts (TPC), lab counts and yeast and mould counts were determined by surface spreading 0.1 ml of serially diluted motoho in duplicate. Tryptone soya agar (Merck) plates were used to test for all viable mesophilic aerobic organisms and MRS agar (Merck) was used for LAB detection.

Both TSA and MRS plates were incubated aerobically for 48 h at 30˚C. Yeasts and moulds were quantified on Potato Dextrose Agar (PDA) (Merck) which was incubated for 120 h at 25˚C. The MPN method was used to test for the presence of *Escherichia coli* (*E.coli*) by using test tubes containing Lauryl Tryptose Broth (LTB) (Oxoid). The tubes were incubated at 37°C for 24 h and checked for the presence of gas production.

Shelf-life

A composite of the 10 replicates of A, J and M were made respectively. Each composite was tested in triplicate for Volatile Organic Compounds (VOC's). Five hundred milligrams (mg) of each sample was added to 2 ml of 99.9% Dichloromethane (DCM) (Sigma Aldrich, St. Louis, Missouri and U.S.A), sonicated at room temperature for 10 min and then filtered.

The analysis was conducted on a Leco (St. Joseph, Michigan, United States) GC x GC-TOF low resolution mass spectrophotometer equipped with a BPX-5 column type with a length of 28.838 m, internal diameter 250 µm and film thickness of 0.25 µm with a maximum temperature of 360ºC. Samples (2 µL) were injected and a splitless mode was activated. The flow rate was 1.4 ml/min at constant flow while the front inlet temperature was 280ºC. The initial oven temperature of 50ºC was maintained for 4 min and ramped at 15ºC/min to 300ºC and held at this temperature for 10 min. The transfer line temperature was 300ºC. The aroma compounds of motoho were determined using a mass range of between 35 m/z to 450 m/z. Probability based matching with mass spectra from the NIST (NIST ver. 2.1 2009 mass spectra library, Gaithersburg, Maryland, USA) library was used for further identification.

Volatile organic compounds

A composite of the 10 replicates of A, J and M were made respectively. Each composite was tested in triplicate for Volatile Organic Compounds (VOC's). Five hundred milligrams (mg) of each sample was added to 2 ml of 99.9% Dichloromethane (DCM) (Sigma Aldrich, St. Louis, Missouri, and U.S.A), sonicated at room temperature for 10 min and then filtered.The analysis was conducted on a Leco (St. Joseph, Michigan, United States) GC x GC-TOF low resolution mass spectrophotometer equipped with a BPX-5 column type with a length of 28.838 m, internal diameter 250 µm and film thickness of 0.25 µm with a maximum temperature of 360ºC. Samples (2 µL) were injected and a splitless mode was activated. The flow

rate was 1.4 ml/min at constant flow while the front inlet temperature was 280ºC.

The initial oven temperature of 50ºC was maintained for 4 min and ramped at 15ºC/min to 300ºC and held at this temperature for 10 min. The transfer line temperature was 300ºC. The aroma compounds of motoho were determined using a mass range of between 35 m/z to 450 m/z. Probability based matching with mass spectra from the NIST (NIST ver. 2.1 2009 mass spectra library, Gaithersburg, Maryland, USA) library was used for further identification.

Sensory analysis

An untrained acceptability panel in the food science department of the university of Johannesburg in South Africa was used to evaluate the sensory properties of the three varieties of motoho. The appearance, aroma, mouth feel, flavour and overall like or dislike of the 3 motoho samples were assessed by using a 9-point hedonic scale to rate the products (Johansson, et al., 1995) (9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely). This study was approved by the university of the Witwatersrand human research ethics committee (medical). (Clearance certificate number: M180751). Written informed consent was obtained from all participants in this study.

Statistical analyses

Data was analyzed by one way Analysis of Variance(ANOVA) (Kunene, et al., 2000) with a (significance level of $p<0.05$ and Principal Component Analysis (PCA) using the PRINCOMP procedure from the SAS statistical software.

RESULTS

Microbiological counts of produced motoho and shelf-life

Microbiological counts for the produced motoho were all <10 cfu/ml for TPC, lab counts and yeasts and moulds. Figure 2 shows the mean TPC counts (log cfu/ml) during the 5 day shelf-life study. *E.coli* was absent throughout the 5 day shelf-life study for all the batches of motoho.

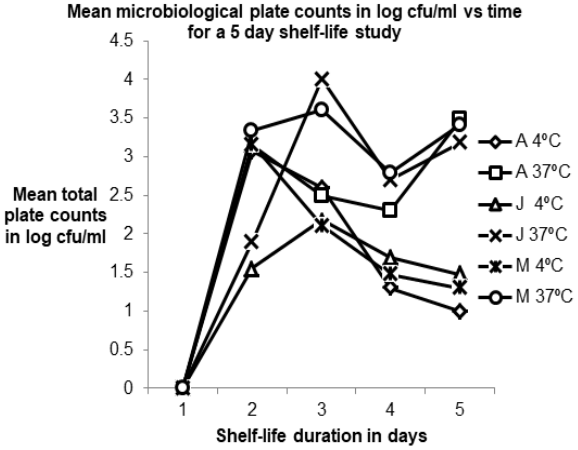

Figure 2. Total bacterial plate counts (log cfu/ml) obtained traditional motoho (A) and variations of this method (J and M) using defined starter cultures, incubated at 4ºC and 37ºC during a 5 day shelf-life study. N (number of replicates)=3.

Volatile organic compounds

The number and relative percentages of the Volatile Organic Compounds (VOC) found in the 3 different varieties of motoho are represented in Table 1.

Table 1. Percentage area of the volatile organic compounds found in 3 different varieties of motoho (A, J, M). Data represented are the means of triplicate values is not detected.

Compound type	Variables	% Area A	% Area J	% Area M
Aldehyde	9,12-Octadecadienal	3.88	-	-
	Octanal, 7-methoxy-3,7-dimethyl-	3.77	-	-
Ketone	Acetoin	-	-	5.43
Esters	9,12-Octadecadienoic acid, methyl ester, (E,E)-	4.71	9.92	9.57
	Acetic acid, bis((trimethylsilyl)oxyl)-, trimethylsilyl ester	3.74	-	-
	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	6.4	-	-
	Undecanoic acid, methyl ester	4.86	-	6.5
	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	3.8		
	9-Octadecenoic acid (Z)-, methyl ester	-	38.38	-
	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	-	3.69	-

	Tridecanoic acid, methyl ester	-	3.91	-
	11-Octadecenoic acid, methyl ester	-	-	12.88
	Dodecanoic acid, methyl ester	-	-	3.39
Hydrocarbon	4-Decene, 2,2-dimethyl-, (Z)-	4.03	3.33	-
	Phosphinous chloride, (chloromethyl)(1,1-dimethylethyl)-	3.77	5.91	5.95
Fatty acid	1, E-11, Z-13-Octadecatriene	-	3.86	-
Alkane	3,5-Dithiahexanol 5,5-dioxide	-	5.43	-
Alcohol	2,3-Butanediol, (S-(R^*,R^*))-	-	-	14.56

Table 2. Sensory analysis of 3 different varieties of motoho. Data represented are means and standard deviations with n=65.

Sample	Aroma	Flavour	Appearance	Mouth feel	Overall quality
A	5.80 ± 2.10	5.91 ± 2.17	6.17 ± 2.0	5.66 ± 1.76	6.00 ± 1.94
J	6.74 ± 1.86	6.82 ± 1.81	6.42 ± 1.9	5.97 ± 2.17	6.50 ± 1.98
M	6.69 ± 1.55	6.74 ± 1.92	6.32 ± 1.7	6.00 ± 1.93	6.69 ± 1.72

A total of 18 volatile compounds whose area were greater than or equal to 3%, were identified in this study. They were largely categorized into 7 groups which included an alcohol (1), aldehydes (2), esters (10), hydrocarbon (2), alkane (1), fatty acid (1) and ketone (1). Out of the 18 volatile compounds, five unique compounds occurred in A and J respectively while 4 occurred in M. The remaining 4 compounds were common between A, J and M. The ester 9, 12-octadecadienoic acid, methyl ester, (E,E)- and organochloride phosphinous chloride, (chloromethyl) (1,1-dimethylethyl)- were common to A, J and M while the hydrocarbon 4-decene, 2,2-dimethyl-, (Z)- was common to A and J.

The ester undecanoic acid, methyl ester was found in both J and M. Esters accounted for 60.34% of the total volatile organic compounds found in A, with cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-being the most predominant. An organochloride (9.68%), aldehydes (19.64%) and a hydrocarbon (10.34%) were also found. The volatile compounds of J were for the most part made up of esters (75.10%) with 9-octadecenoic acid (Z)-, methyl ester being the major ester. Other organic compounds found in J were a fatty acid (5.19%), an alkane (7.30%), a hydrocarbon (4.47%) and an organochloride (7.94%).

In M, 55.49% of the volatile organic compounds primarily constituted esters (55.49%) with 9,12-octadecadienoic acid, methyl ester, (E,E)- being the most predominant. An alcohol made up 25% of the total while a ketone and an organochloride made up 9.3% and 10.21% respectively.

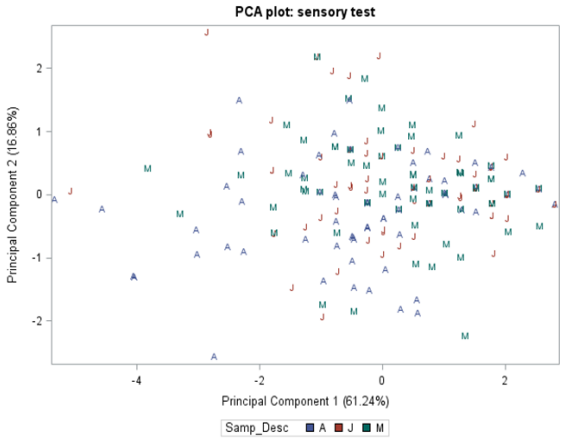

Figure 3. Principal component analysis of the sensory analysis of 3 different varieties of motoho using 65 panellists who rated the motoho using a 9 point hedonic scale. The traditional motoho is represented by A and was produced using spontaneous fermentation while J and M represent the modified motoho produced with starter cultures.

Sensory analysis

The results from the sensory analysis of the 3 varieties of motoho are represented in Table 2. There were significant differences ($p<0.05$) between the motoho varieties in terms of aroma and flavor with panelists preferring the aroma and flavor of J. The appearance, mouth feel and overall differences between the 3 varieties of motoho were not significant ($p>0.05$). Figure 3 shows the Principal Component Analysis (PCA) of the sensory properties of the 3 different varieties of motoho.

The variable system included flavor, mouth feel, appearance and aroma. Principal Component 1 (PC1) accounts for 61.24% of variance across the samples while PC2 accounts for 16.86% of the variance.

DISCUSSION

The flavour of food is subject to the balance of the volatile compounds which are found in foods or by those which are made during the production of foods (Kutyauripo, et al., 2009). Developed a cereal based fermented functional food which was a combination of pearl millet, wheat and white and red sorghum and used Pichia kudriavzevii OG32 as the probiotic. Esters accounted for 32.37% and acids accounted for 32.21% of the VOC's in this functional food and are mainly responsible for the pleasant aromatic notes adding to fruity and floral odours of fermented foods. The smallest proportions of VOC's were alkanes and aromatic compounds which made up 6.02% of the total. In the motoho batches A, J and M, no acids were detected. However, methyl and ethyl esters were the predominant VOC's with the highest area percentage of 55.9% esters found in J, followed by M (25.84%) and A (23.51%). Similarly to the results obtained by (Lennerz, et al., 2015), one of the smallest concentrations of VOC's of motoho were alkanes (5.43%).

Esters were predominant in the production of pito and burukutu, fermented sorghum alcoholic beverages from Nigeria (Linton, et al., 1993). Ethyl esters of straight chain fatty acids are common in cheese and could be formed enzymatically by bacteria when ethanol is condensed with a carboxylic acid. They impart a fruitiness to Italian type cheeses but they could also impart a fruity off-flavour if found in high concentrations. While methyl esters are formed by the methanolysis of acyl-CoA which is made during the synthesis or degradation of fatty acids and affect the sensory properties of a variety of fermented foods (Karovicova, et al., 2007).

The found esters in sorghum beer and found 9 esters in fermented stinky tofu. Batch J of motoho had the highest concentration of esters, which could explain the preference of the flavour of J by the panellists conducting the sensory analysis. Butanediol is an alkanol which was predominant in the fermentation of Pito and Burukutu as well as in batch M of motoho. Alkanols impart an alcohol like aroma and property which is often linked to indigenous beverages, consequently giving beer its warm mouth feel.

Low alcohol concentrations impart fruity-type aromas in foods while increased concentrations are responsible for "hot" aromas which are not desirable to the consumer. Ethanol was not identified in Pito or Burukutu, Sorghum beer or all 3 batches of motoho. This is in contrast to Campbell-Platt who found ethanol to be the main source of alcohols present in fermented cereals. The lack of ethanol could be the result of the quick conversion of ethanol to the intermediary products by alcohol dehydrogenase which is found in fermenting microorganisms.

The fermentation conditions and time of fermentation could also contribute to the lack of ethanol. Aldehydes and ketones are typically found in alcoholic beverages to which they contribute objectionable organoleptic properties. The degradation of lipids and amino acids during microbial fermentation results in ketone formation which strongly impacts the odour of foods. Pito and Burukutu contained 3 aldehydes, namely 3-methyl butanal, acetylaldehyde, 2-methyl and propanal which may have impacted the odour. Aldehydes (9,12-octadecadienal and octanal, 7-methoxy-3,7-dimethyl-) were found in batch A of motoho while acetoin, a ketone was found in batch M, also discovered acetoin during the fermentation of bushera, a non-alcoholic traditionally fermented sorghum beverage from Uganda.

Diacetyl and acetoin are the result of LAB using pyruvate and citrate. Acetoin-like products in cereals are associated with bacterial instead of yeast-dominated flora. This is consistent with the fermentation of batch M of motoho since lab starter cultures were used for the fermentation. The lack of aldehydes or ketones in batch J could explain the preference of the aroma of this batch. Out of the 3 batches of motoho, only J contained a fatty acid (1, E-11, Z-13-octadecatriene).

Fatty acids can impart cheesy, fruity, rancid and fatty notes and are important in establishing the equilibrium in wines because they prevent the hydrolysis of the corresponding ester and their presence is important in the aroma complexity. The presence of the fatty acid in batch J of motoho could have also contributed to the preferred aroma.

Traditionally fermented African cereal products have a limited shelf-life of between 3-5 days which can be attributed to unpleasant off flavours or over souring which results from the sustained microbial activity after the product has been made. This was also apparent in the shelf-life study of motoho where cell counts of the motoho samples incubated at 37ºC continued to increase over the course of the 5 day shelf-life study. Overall, the microbiological counts for A, J and M which were incubated at 37ºC increased while the counts for A, J and M samples incubated at 4ºC decreased over the 5 day shelf-life study. Microbial populations decreased when samples were pasteurized and refrigerated and a shelf-life of 24 days was achieved with Kunan-zaki. Lab is thermoduric which allows them to survive at pasteurization temperatures and are hence found in heat treated samples.

The microbes present are constrained by low storage temperatures. This could account for the lower microbial numbers which were found during the storage of motoho at 4ºC.

The rapid decrease in the shelf-life of many African beverages is extensively recognized and is of great interest. High microbial loads and diverse microorganisms are the main causes of spoilage encountered by producers and consumers of preservative free and unpasteurised traditionally made products. Sodium benzoate is commonly used as a preservative for foods and soft drinks and was also used during the production of motoho. The maximum acceptable microbial load for fermented foods at any given point during the products shelf-life is 105 cfu/g. This is in line with the counts obtained for the shelf-life of motoho with the highest counts of 104 cfu/ml obtained by J stored at 37ºC on day 3 of the shelf-life study. This implies that for all the samples in the study, microbial counts were well below the recommended range and that the motoho produced in this study has shown to have an extended shelf-life possibly due to the use of sodium benzoate during the production of motoho. Many traditionally fermented African cereal based products spoil rapidly and are unsatisfactory to consumers within 1 to 4 days of being produced. However the results of this study show acceptable microbial loads even at day 5 of storage.

CONCLUSION

The shelf-life study showed that the motoho microbial counts were dependent on temperature, irrespective of the traditional or defined starter cultures which were used for motoho production. However, even at an accelerated temperature, the microbial counts were below the recommended microbial load for beverages, even at day 5 of the shelf-life. although it is recommended that motoho is generally consumed within 3 days.

Overall, a sensory panel preferred motoho which was produced using the defined starter culture and brown high tannin sorghum Rakodzi. The high percentage of esters found during the production of this motoho could have accounted for the preference in aroma and flavour. This sorghum, along with the selected LAB strains could be used for further research on motoho in order to upscale the production and possibly commercialize the product as it has shown to be a highly favourable product with an extended shelf-life.

REFERENCES

Aka S, Konan G, Fokou G, DJE KM, Bonfoh B (2014). Review on African traditional cereal beverages. Am J Res Commun. 2: 103-153.

Akinrele IA (1970). Fermentation studies on maize during the preparation of a traditional African starch-cake food. J Sci Food Agri. 21: 619-625.

Campbell-Platt G (1994). Fermented foods a world perspective. Food Res Int. 27: 253-257.

Chaves-Lopez C, Serio A, Grande-Tovar CD, Cuervo-Mulet R, Delgado-Ospina J, et al. (2014). Traditional fermented foods and beverages from a microbiological and nutritional perspective: The Colombian heritage. Comprehes Rev Food Sci Food Safety. 13: 1031-1048.

Chen S, Xu Y (2010). The influence of yeast strains on the volatile flavour compounds of Chinese rice wine. J Inst Brew. 116: 190-196.

Curioni PMG, Bosset JO (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. Int Dairy J. 12: 959-984.

Efiuvwevwere BJO, Akona O (1995). The microbiology of 'Kunun-zaki', a cereal beverage from Northern Nigeria, during the fermentation (production) process. World J Microbiol Biotechnol. 11: 491-493.

Halm M, Lillie A, Sorensen AK Jakobsen M (1993). Microbiological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. Int J Food Microbiol. 19: 135-143.

Hounhouigan DJ, Nout MJR, Nago CM, Houben JH, Rombouts FM (1993). Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of mawe, a fermented maize dough from Benin. Int J Food Microbiol. 18: 279-287.

Johansson ML, Sanni A, Lonner C, Molin G (1995). Phenotypically based taxonomy using API 50CH of lactobacilli from Nigerian ogi, and the occurrence of starch fermenting strains. Int J Food Microbiol. 25: 159-168.

Kunene NF, Geornaras I, von Holy A, Hastings JW (2000). Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented weaning food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. Appl Environ Microbiol. 66: 1084-1092.

Kutyauripo J, Parawira W, Tinofa S, Kudita I, Ndengu C (2009). Investigation of shelf-life extension of sorghum beer (Chibuku) by removing the second conversion of malt. Int J Food Microbiol. 129: 271-276.

Lennerz BS, Vafai SB, Delaney NF, Clish CB, Deik AA, et al. (2015). Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. Mol Gene Metabol. 114: 73-79.

Linton CJ, Wright SJL (1993). Volatile organic compounds: Microbiological aspects and some technological implications. J Appl Bacteriol. 75: 1-12.

Karovicova ZKJ, Kohajdova J (2007). Fermentation of cereals for specific purpose. J Food Nutr Res 46: 51-57.