

**TRADITIONAL PROCESSING, MICROBIAL AND PHYSICOCHEMICAL
CHANGES DURING FERMENTATION OF MALWA****Muyanja C*¹, Birungi S¹, Ahimbisibwe M¹, Semanda J¹ and BS Namugumya¹****Charles Muyanja**

*Corresponding author email: ckmuyanja@yahoo.co.uk / ckmuyanja@agric.mak.ac.ug

¹Makerere University, Faculty of Agriculture, Department of Food Science and Technology, P.O Box 7062 Kampala, Uganda

ABSTRACT

A survey was conducted to characterise production methods of *malwa*; a Ugandan traditional fermented millet beverage, in four divisions of Kampala district using a questionnaire. *Lactobacillus* and *Lactococcus* spp and coliforms were enumerated in the raw materials and during fermentation using standard microbiological methods. Changes in chemical parameters were determined using standard methods. Similarities in production methods were observed among the *malwa* producers. All producers germinated millet grains (2-3 days) to make green malt. The germinated grains were sun-dried for 2-3 days. Moistened millet flour was subjected to solid state pit fermentation for one week to produce acidified fermented dough. The acidified fermented dough was roasted over an open fire to produce roasted acidified dough. The duration of fermentation of *malwa* varied between 2 and 4 days. Only 5% of the producers practiced back slopping. Producers (90%) reported that consumers preferred sour *malwa*. *Lactobacillus* and *Lactococcus* spp numbers in the sour dough, roasted sour dough and green malt varied between 3.48 and 5.38, 2.02 and 2.60, and 4.45 and 6.25 log cfug⁻¹ respectively. Coliforms in sour dough, roasted sour dough and green malt varied between 1.36 and 5.53 log cfug⁻¹. *Lactobacillus* spp increased from 2.73 to 6.60 log cfu mL⁻¹ whereas *Lactococcus* spp increased from 2.67 to 6.22 log cfu mL⁻¹ during 72 h of fermentation. The greatest increase in numbers was observed during the first 24 h. Coliforms decreased from 2.80 to 1.19 log cfu mL⁻¹ after 24 h with a slight increase to 1.26 log cfu mL⁻¹ after 48 h due to further addition of green malt. Coliforms were still detectable after 72 h. The pH decreased from 4.3 to 3.65 as titratable acidity increased from 0.69 to 1.47% lactic acid after 72 h of fermentation. Total soluble solids decreased from 17.7 to 7.7 °Brix during 72 h fermentation. Ethanol increased from 1.07 to 12% v/v. Carbohydrates and tannins decreased during germination and fermentation. Apparent increase in protein content was observed. The high numbers of *Lactobacillus* and *Lactococcus* spp and coliforms in the sour dough suggest their involvement in the solid state pit fermentation of millet flour. Higher numbers of *Lactobacillus* and *Lactococcus* spp in the green malt indicates that these organisms play a big role in the fermentation process of *malwa*.

Key words: *Lactococcus*, *Lactobacillus*, Millet, Fermentation, *Malwa*

INTRODUCTION

Cereals account for as much as 77 % of total caloric consumption in African countries [1] and contribute substantially to dietary protein intake in a number of these countries [2]. Millets are important food crops in arid and semi arid regions of the world [3, 4]. Finger millet (*Eleusine coracana L*) is one of the economically important cultivated millets. Finger millet production is concentrated in eastern and southern Africa, with Uganda and Tanzania, being the leading producers [5]. Finger millet is also known as African millet, koracan, ragi (India), wimbi (Tanzania), bulo (Uganda) and telebun (the Sudan). The major nutritional setback of millets with respect to nutrient bioavailability is the large amounts of polyphenols and tannins [1, 6]. These substances reduce the nutritional value of cereals by interfering with mineral bioavailability and digestibility of proteins and carbohydrates through complexing with minerals and inhibiting enzymes. Fermentation is suggested as the simple and economical means to improve the quality and utilization of cereals [1] as it reduces antinutritional factors especially tannins and the phytates [7].

In Uganda, finger millet is used in the production of fermented beverages like *malwa* (ajon) and *bushera* [3, 8]. *Malwa* is a beverage that is valued for its taste, flavour and aroma and is mainly produced at the household level in eastern and north eastern region of Uganda mainly by the Iteso tribe. *Malwa* deteriorates rapidly and has a short shelf life of about 2 days [9].

There is little information available on the production technologies for *malwa* as well as the microorganisms involved in fermentation. The present study reports on the production methods of *malwa*, the preparation of ingredients and the changes in the physicochemical parameters (pH, total soluble solids, titratable acidity and ethanol) and microbial numbers during fermentation. The effects of germination and fermentation on the carbohydrates, proteins, and tannins levels are also reported.

MATERIAL AND METHODS

Methodology of the Survey

A survey was conducted using a questionnaire in Wandegeya, Nsambya, Kikoni, and Naguru divisions of Kampala district where *malwa* is mostly produced. The questionnaire focused on the preparation of raw materials used, the fermentation process and its duration, utilization and storage of *malwa*. Eighty (80) randomly selected *malwa* producers were interviewed.

Sample collection

Samples of the raw materials at different stages, namely, germination, pit fermentation, roasting, and mash fermentation were obtained from different producers using sterile 500 mL plastic screw-capped glass bottles. Five samples from each stage were obtained and transported on ice in cooling boxes to the Department of Food Science and Technology, Makerere University, Kampala, Uganda, for analysis. Fermentation was monitored and samples were drawn at 0, 24, 48 and 72 h intervals

for microbial counts, pH, titratable acidity (TA), Total soluble solids (TSS), ethanol content, protein, carbohydrates and tannins determination.

Enumeration of Lactic acid Bacteria (LAB) and coliforms during *malwa* fermentation

A-10 g sample was transferred aseptically into 90 mL sterile 0.1% peptone (64271 Darmstadt, Germany) and homogenized for 30 seconds. The homogenate was serially diluted and aliquots from appropriate dilutions were used in duplicate on respective agar plates. Enumeration of Lactic acid bacteria (LAB) was carried out on MRS agar (Conda laboratories, Madrid, Spain) and M17 agar (Merck, Darmstadt, Germany). MRS and M17 agar were used to enumerate *Lactobacillus* and *Lactococcus* spp respectively. Plates were incubated at 30°C for 48 h. Coliforms were enumerated by pour plating on Violet Red Bile agar (VRBA) (Oxoid). After solidification, the VRBA plates were incubated aerobically at 37°C for 24 h. Colonies were counted by using a bench magnifier (Stuart, Switzerland). Colony forming units (cfu) were calculated according to IDF standard 153:1991, Method No: 9.3 [10].

Chemical analyses

The pH was determined using a digital pH-meter (CKI Digi-sense Model NO 607) with a combined electrode (pH C 2005-8, Radiometer analytical, Copenhagen, Denmark). The pH meter was calibrated using commercial buffers (Merck, pH 4 and 7). Total soluble solids (TSS) (°Brix) of *malwa* were determined at 20°C using an Abbe refractometer (Model IT, Atago, Japan) according to the method by Joslyn [11]. The titratable acidity was determined by titration using 0.1N Sodium Hydroxide, using 0.3mL of phenolphthalein indicator. The ethanol content of the samples was determined according to AOAC method No. 982.10 [12]. The protein content was determined using the Kjeldahl method according to AOAC method No. 992.23 [12]. The carbohydrate content was determined using the phenol Sulphuric acid method [13]. Tannin content determination was carried out using the modified vanillin-HCl method [14].

Data analysis

Data obtained was subjected to analysis of variance (ANOVA), (SPSS version 12) and Microsoft Office Excel, 2007. The mean values and standard deviations for microbial numbers of raw materials (sour dough, roasted sour dough and green malt) and during fermentation were computed. Means values and standard deviations for pH, titratable acidity, Total Soluble Solids, ethanol, carbohydrate, protein and tannins were also computed. The means were subjected to analysis of variance (ANOVA) to test for significant differences ($P < 0.05$). Fisher's Least Significant Difference was used to determine which means were significantly different.

RESULTS

Preparation of malwa at household level

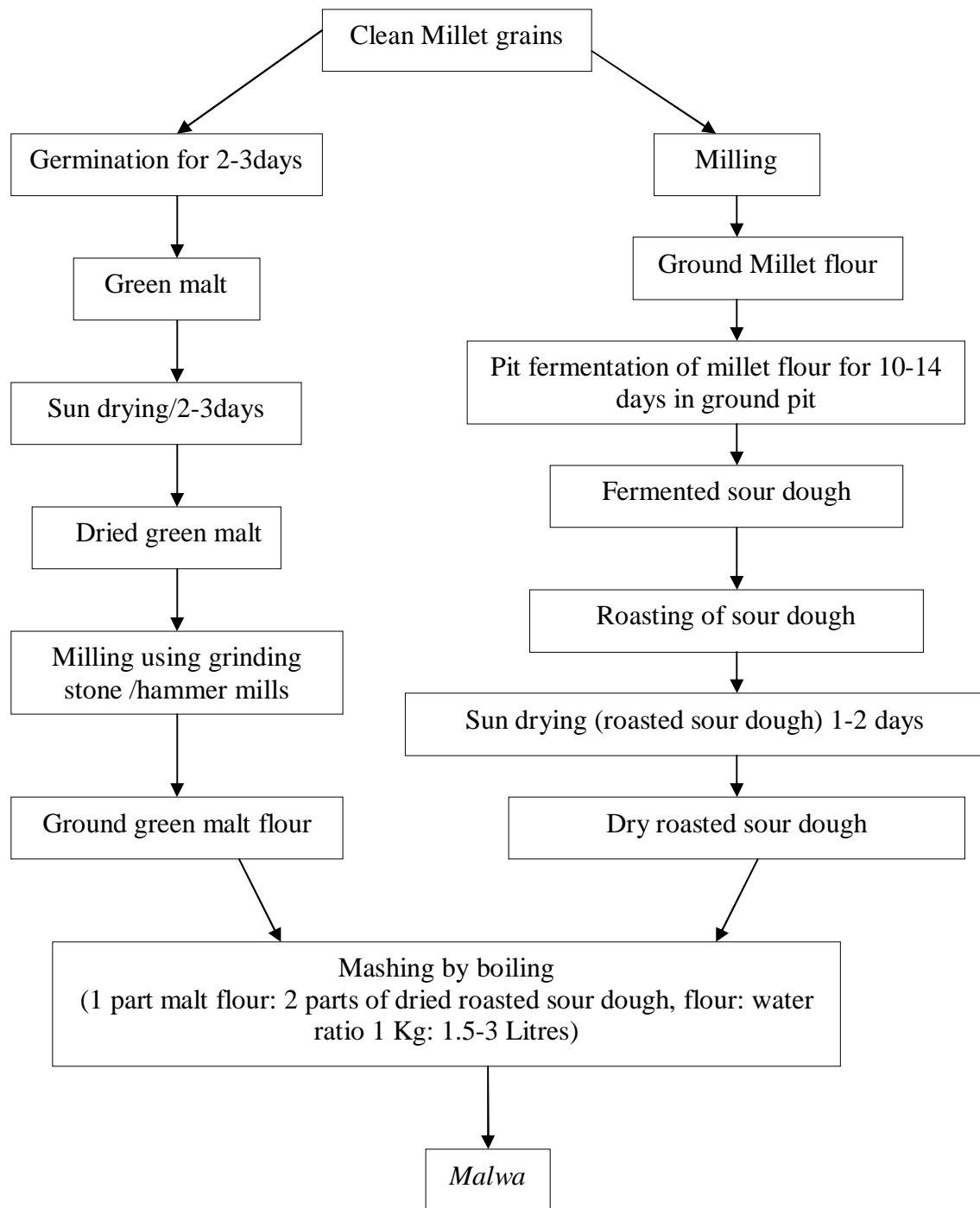


Figure.1: Flow chart for the traditional preparation of malwa

Microbial levels in raw materials

Table 1 shows the microbial numbers in raw materials used for production of *malwa*. The microbial numbers were determined in the raw materials obtained from two *malwa* producers for comparison. Higher numbers of microorganisms were observed in pit fermented dough than the roasted acidified dough. The green malt contained the highest microbial numbers. Roasting resulted in reduction in microbial numbers. The LAB in sour dough, roasted sour dough and green malt ranged from 2.02 ± 0.75 to 6.25 ± 0.10 log cfu/g. Coliforms in the raw materials varied between 1.36 ± 0.9 and 5.71 log cfu/g. There was no significant difference ($p=0.142$) in the microbial numbers in the raw materials for the two producers.

Changes in microbial numbers during fermentation

Table 2 shows the changes in microbial numbers during fermentation. Coliforms decreased from 2.80 ± 0.09 to 1.19 ± 0.33 log cfu/g during the first 24 h. After 48 h the coliforms slightly increased from 1.19 to 1.26 ± 0.01 log cfu/g due to addition of more green malt but decreased later. The *Lactobacillus* and *Lactococcus* increased from 2.73 ± 0.10 to 6.60 ± 0.10 log cfu/g and from 2.67 ± 0.03 to 6.22 ± 0.13 log cfu/g after 48 h of fermentation, respectively. The largest increase in microbial number was noted during the first 24 h and thereafter a slight decrease was observed.

Changes in pH, TA, TSS and ethanol during fermentation of *malwa*

Table 3 shows the changes in physiochemical parameters during fermentation. The pH decreased from 4.32 ± 0.28 to 3.65 ± 0.04 after 72 h. This corresponded with an increase in the titratable acidity from 0.69 ± 0.02 to 0.47 ± 0.02 % lactic acid. The most rapid production of lactic acid was observed between 24 and 48 h. Total soluble solids decreased from 17.7 ± 1.27 to 7.70 ± 0.14 °brix. The ethanol content increased from 1.07 ± 0.09 to 12.0 ± 0.18 % v/v corresponding with the decrease in TSS.

Changes in carbohydrates, protein and tannins content during germination and fermentation

Germination (4 days) led to a decrease of carbohydrate from 80.15 ± 0.73 to 75.56 ± 1.17 % representing a 5 % decrease. There was an apparent increase in protein content from 7.76 ± 0.27 to 7.93 ± 0.36 % during germination. Tannins decreased by 63% (Table 4). Fermentation caused carbohydrate to decrease by 12.5% whereas, proteins apparently increased by 8.2%. There was a rapid decrease in carbohydrate content towards the end of fermentation (Table 5). Tannins decreased from 0.07% in raw millet flour to 0.002 % in *malwa* (Table 5) giving a 97% decrease.

DISCUSSION

Preparation of *malwa* at household level

Malwa is made from clean dry finger millet grains. Typically, millet grain is sprinkled with water and put in a gunny bags and soaked overnight (12 h). The soaking of the grain for 12 h allows the grains to imbibe water to facilitate germination. The watering of the grains during germination is purposely done to provide additional water for sprouting. Grain sprouting is indicated by appearance of

the radicals and swelling of the grain on second day. During the first three days of sprouting, grain is turned twice daily and watered. Germination duration ranged between 3 and 4 days. After germination, the grains are sun dried for 1-2 days. The dried grains are either hand or hammer milled to produce ground green malt. Another portion of ungerminated millet grain is ground into flour and mixed with a small amount of water to produce stiff dough. The stiff dough is then placed in plastic sheets and buried in the soil to undergo solid pit fermentation for 10-14 days. The stiff dough is subjected to solid state pit fermentation to impart desirable changes in the dough. During pit fermentation, the prevailing conditions support the proliferation of various microorganisms including lactic acid bacteria. LAB leads to the production of organic acids during pit fermentation imparting acid taste to the dough hence referred to as sour dough. The purpose of souring is to achieve basic biochemical changes [15]. The microorganisms which are involved in pit solid state fermentation synthesise enzymes that degrade polymeric substances into smaller and more easily digestible compounds. The extracellular enzymes produced hydrolyze protein and starch into amino acids and sugars, respectively. These amino acids and sugars are the substrate for lactic-acid bacteria and yeasts, which produce the characteristic flavours of *malwa*. The sour dough is roasted at around 72°C on cut metallic metal drums under open fire with continuous stirring to avoid undesirable burnt flavour. The roasted sour dough (deep dark brown) is sun dried for 1-2 days. Roasting imparts desirable flavour and colour in *malwa*, and also gelatinises the starch in the dough rendering it easy for assimilation by malt diastatic enzymes during the mashing stage. Ungelatinised starch is slowly attacked by the malt enzymes [16].

For production of *malwa*, two parts of the roasted sour dough is mixed with 1 part of the green malt. Water is added to the mixture at a ratio of 1 Kg of grist to 1.5-3 Litres and mashed by boiling. The mixture is left to cool at ambient temperature before green malt is added. The mixture is fermented in plastic and metallic drums (100-200 Litres) at ambient temperature for 2-4 days. During the second day more green malt is added on to accelerate the fermentation process. The green malt added during the progression of fermentation is to contribute additional fermentable sugars and amylase enzymes which break down starch to support secondary fermentation. The endogenous amylolytic enzymes (and amylases) hydrolyze the cooked starch to fermentable sugars for the metabolism of microorganisms. *Malwa* producers (5%) used previously fermented portion (back slopping) to initiate the fermentation but indicated that it should be done carefully as it can spoil the whole batch. The producers reported that *malwa* was ready for consumption when bubbling ceases and a bit sour. The *malwa* is served in clay pot and consumed while being diluted with hot water using locally made straws. The *malwa* has a short shelf life and deteriorates rapidly depending on the brewing recipe and experience of the producer. Consumers preferred sour *malwa* to the sweet one (1-2 days old). However, consumers reported that sweet *malwa* causes stomach upsets. This can be explained by the presence of high numbers of coliforms in *malwa* at the low acid levels. Similar findings were reported for *bushera* (2-day old) [4]. Coliforms are usually active in the early stages of fermentation of fermented cereal-based beverages [17].

The lower numbers of coliforms in sour dough and roasted sour dough than in green malt can be attributed to acidity which inhibits the growth of coliforms whereas the reduced number in roasted sour dough are due to heat destruction [3, 8]. Unlike other fermented plant materials where the coliforms first increase and then followed by a decrease, in *malwa*, coliforms decreased throughout the fermentation time. A slight increase in coliforms was noted when green malt was added as fermentation progressed. The contradiction of our results may be explained by the fact that the materials used for preparation of *malwa* first undergo pit fermentation which contributes to a low initial pH and high acidity during the early stages of fermentation hence decreasing coliforms numbers.

Although yeasts were not enumerated in this study, the increase in ethanol content with fermentation can be attributed to an increase in yeasts. The presence of yeasts from the green malt at mashing and during fermentation has been reported [9].

The increase in LAB numbers has been reported [4] with a slight decrease as fermentation progressed [9]. Most LAB grow best at a pH range of 4.0-4.5 [18]. The pH continued to fall below this range as fermentation of *malwa* progressed hence the slight decrease in LAB numbers observed. These results are in agreement with other studies on fermented African foods [19, 20].

The acidification was reported when the raw millet flour was buried in the ground for a week [9]. The author further reported that green malt added during fermentation contributes acidic bacteria, particularly the *Lactobacillus spp*, which lead to further drop in pH. The increased acidity and simultaneous drop in pH have been documented in other African fermented beverages [4, 8, 19]. A decrease in TSS was observed and is an important potential parameter, which can be used to monitor the rates of fermentation and alcohol production [21]. The increase in ethanol content and the decrease in TSS can also be attributed to the alcoholic fermentation carried out by the yeasts [9]. Similar results were obtained during 'Busaa' fermentation [22].

The decrease in the carbohydrate was attributed to a decrease in starch content during germination and natural fermentation [1, 23]. Germination was reported to increase total soluble sugars, reducing and non-reducing sugar content of pearl millet with a parallel decrease in its starch content [2]. Starch and soluble sugars (maltose, glucose and fructose) are principal substrates for fermenting organisms [7]. The utilization of carbohydrates by the germinating grains with concomitant production of carbon dioxide may account for the lower carbohydrate content of germinated grains. The decrease in carbohydrate content during fermentation can be attributed to the vast numbers of different microorganisms present at relatively low acidity (high pH) which utilize the carbohydrate before the limiting factor of high acidity (low pH) sets in to inhibit enzyme action [9]. The decrease in pH with fermenting time is one of the limiting factors for the fermenting organisms and only acid tolerant microorganisms will dominate towards the end of fermentation as reported by others [2, 18, 24].

The results obtained in this study suggest that there is an increase in protein content of *malwa* as a result of fermentation. Protein content of *orubisi/amarwa* was reported to increase from 2.0 to 2.7% during 120 h fermentation [25]. Protein content was also noted to increase in pearl millet with fermentation time [26]. However, other researchers reported that protein content of the millet was unaltered during fermentation [27]. Fermentation was reported to have a slight effect on the crude protein content of *mawe* (maize sour dough) [20]. The increases in protein content were attributed to synthesis of proteins by microorganisms [28]. During fermentation, some proteins are hydrolysed [29]. However, the hydrolysis and synthesis of proteins by microorganisms does not change the total amino acid content or the amount of nitrogen (as measured by Kjeldahl analysis). This means that synthesis of new proteins from amino acids resulting from proteolytic activity does not increase the total protein content unless an external nitrogen source is introduced during fermentation. The apparent increase in protein content observed in our study may reflect a loss in carbohydrates and volatile compounds rather than an actual increase in protein [7].

The results demonstrated the combined effect of germination and fermentation in reducing the tannin levels. Germination was reported to decrease the tannin content in sorghum and finger millet [28]. Reduction of tannin content during soaking and fermentation of grains has also been reported [26, 28]. Similar results were also reported during sorghum fermentation [1]. The decrease in tannin content was attributed to the presence of metabolic systems in the microflora responsible for fermentation that produce polyphenolases which break down tannins [1].

CONCLUSION

The production techniques of *malwa* were similar among the producers. The study has revealed that LAB dominate the production of *malwa*. The suppression of coliform growth can be attributed to the increase in titratable acidity and alcohol as fermentation progressed. The study has shown that fermentation and germination have a reducing effect on the carbohydrate content as a result of bio-conversion of carbohydrates to organic acids and other compounds such as alcohol. The results suggest that germination and fermentation may be used to improve the nutritive value of cereals as tannin decrease during both processing steps. The increasing titratable acidity, ethanol, and decreasing pH and TSS can be used to monitor the fermentation process of *malwa*. Further studies need to be conducted to investigate microbial strains that are involved in the fermentation of *malwa* for potential starter cultures development.

ACKNOWLEDGEMENTS

We thank the African Institute for Capacity Development (AICAD), Juja, Nairobi, Kenya for the financial support. The authors extend their gratitude to the respondents for their active participation during the conduct of the survey.

Table1: Microbial numbers (log cfu/g) in raw material for *malwa* production

Microorganisms	Prepared Raw Material		
	Sour dough	Roasted sour dough	Green malt
Producer 1			
Coliforms	4.30±0.15	1.36±0.9	5.26±0.13
Lactobacillus.spp	3.48±0.00	2.02±0.75	4.54±0.13
Lactococcus.spp	5.33±0.08	2.06±0.77	6.25±0.01
Producers 2			
Coliforms	4.40±0.10	1.36±0.10	5.53±0.10
Lactobacillus.spp	3.48±0.10	2.56±0.10	4.45±0.10
Lactococcus.spp	5.38±0.10	2.60±0.10	6.25±0.10

Values are means ± standard deviations of three independent determinations

Table 2: Changes in microbial numbers during fermentation of *malwa*

Fermentation Time (h)	Microbial number (log cfu/mL)		
	Coliforms	Lactobacillus. spp	Lactococcus. spp
0	2.80±0.09	2.73±0.10	2.67±0.03
24	1.19±0.33	6.27±0.21	5.46±0.08
48	1.26±0.01	6.60±0.10	6.22±0.13
72	1.10±0.21	6.27±0.04	4.24±0.09

Values are means ± standard deviations of three independent determinations

Table 3: Changes in selected physiochemical parameters during *malwa* fermentation

Time (h)	pH	Titratable acidity (%)	Total soluble solids	Ethanol (%)
0	4.32±0.28	0.69±0.02	17.7±1.27	1.07±0.09
24	3.93±0.07	0.79±0.05	13.0±1.41	7.66±1.71
48	3.76±0.28	1.32±0.04	8.20±0.28	11.9±0.06
72	3.65±0.04	1.47±0.02	7.70±0.14	12.0±0.18

Values are means ± standard deviations of three independent determinations

Table 4: Changes in Carbohydrates, Proteins and Tannin content (%) of finger millet with germination time

	Time (days)	Carbohydrates	Protein	Tannin
Raw finger millet	0	80.15±0.73	7.76±0.27	0.07±0.00
Germinated finger millet	4	75.56±1.17	7.93±0.36	0.026±0.00

Values are means ± standard deviations of three independent determinations

Table 5: Changes in Carbohydrate, Proteins, and Tannins content (%) during fermentation of *malwa*

	Time (days)	Carbohydrate	Protein	Tannins
Raw finger millet	0	80.15±0.73	7.76 ±0.27	0.07±000
During fermentation	1	79.85±0.17	8.24±0.04	0.006±0.00
	2	76.78±0.21	8.42±0.01	0.005±0.00
	3	74.03±0.06	8.47±0.01	0.003±0.00
	4	70.36±0.35	8.40±0.01	0.002±0.00

Values are means ± standard deviations of three independent determinations

REFERENCES

1. **Chavan JK and SS Kadam** Nutritional improvement of cereals by fermentation. *Crit. Rev. Food Sci. Nutr.* 1989; **28**:351-400.
2. **Khetarpaul N and BM Chauhan A** Research note on 'the effect of germination and fermentation on the *in vitro* starch and protein digestibility of pearl millet. *J. Food Sci.* 1990; **55 (3)**: 883-884.
3. **Steinkraus KH** Handbook of Indigenous Fermented Foods, 2nd Ed, Marcel Dekker, Inc, New York, 1996.
4. **Muyanja CMB, Narvhus JA, Treimo J and T Langsrud** Isolation, characterisation and identification of lactic acid bacteria from *bushera*: Ugandan traditional fermented beverages. *Int. J. Food Microbiol.* 2003; **20**:201-210.
5. **FAO.** Production Yearbook, Food and Agriculture Organization, Rome, (2000).
6. **Salunkhe DK, Jadhav SJ, Kadam SS and JK Chavan** Chemical, biochemical and biological significance of polyphenols in cereals and legumes. *Crit. Rev. Food Sci. Nutr.* 1990; **17**:277.
7. **Muyanja CMBK, Narvhus JA and T Langsrud** The use of starter cultures in the fermentation of *bushera*, a Ugandan traditional fermented sorghum beverages. *Uganda. J Agric. Sci.* 2004; **9**: 606-610.
8. **Namugumya B and CMBK Muyanja** Traditional processing, microbiology, physiochemical and sensory characteristics of kwete, A Ugandan fermented maize based beverage. *African. J. Food Agric. Nutr.. Develop.* 2009; **9 (4)** 1047-1059.
9. **O'Rourke T** Brewing up the Past. The Brewer International, 2000; **2 (8)**.
10. International Dairy Federation (IDF), Butter fermented milks and fresh cheese- Enumeration of contaminating microorganisms- Colony counting technique at 30⁰C, IDF standard 153:1991.
11. **Joslyn AM** Methods in food analysis, 2nd Ed, New York, Academic Press, 1970.
12. **AOAC.** Official Methods of Analysis. 9th Ed., Association of Official Analytical Chemists, Washington D.C, 1999.
13. **Dubois M, Gilles K, Hamilton J, Rebers P and F Smith** Colorimetric method based on phenol sulfuric acid, *Anal. Chem.* 1962; **28**:356.

14. **Price ML, van Scoyod S and LG Butler** Critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* 1978; **26**:1214–1218.
15. **Abegaz K, Fekadu B, Langsrud T and JA Narvhus** Indigenous processing methods and raw materials of *Borde*, an Ethiopian traditional fermented beverage. *J. Food Technol.* 2002; **7**(2): 59-64.
16. **Serna-Saldivar S and LW Rooney** Structure and Chemistry of Millet, In: Dendy DAV(Ed), Sorghum and Millets Chemistry and Technology. American Association of Cereal Chemists, Minnesota, 1995.
17. **Mbugua SK** The nutritional and fermentation characteristics of uji produced from dry milled maize flour (unga baridi) and whole wet milled maize. *Chem. Mikrobiol. Technol. Lebensm.* 1987; **10**:154–161.
18. **Frazier C W and DC Westhoff** Food Microbiology, 4th Ed, McGraw Hill, 1988.
19. **Mugula JK, Nnko SAM, Narvhus JA and T Sorhaug** Microbiological and fermentation characteristics of *togwa*, a Tanzanian fermented food. *Int. J. Food Microbiol.* 2003; **80**: 187–199.
20. **Hounhouigan DJ, Nout MJR, Nago CM, Houben JH and FM Rombouts** Changes in physio-chemical properties of maize during natural fermentation of *mawe*. *J. Cereal Sci.* 1993; **17**:291 – 300.
21. **Tiisekwa AB, Mosha TCE, Laswai HS and EE Towo** Traditional alcoholic beverages of Tanzania: production, quality and changes in quality attributes during storage. *Int. J. Food Sci. Nutr.* 2000; **51**:135-143.
22. **Nout MJR** Microbiological aspects of the traditional manufacture of *busaa*, a Kenyan opaque maize beer. *Chem. Mikrobiol. Technol. Lebensm.* 1980; **6**: 137–142.
23. **Asiedu M, Nilsen R, Lie Ø and E Lied** Effect of Processing (Sprouting and/or Fermentation) on Sorghum and Maize. I: Proximate Composition, Mineral and Fatty Acids. *Food. Chem.* 1993; **46**: 351-353.
24. **Kunene NF, Geornaras I, von Holy A and JW Hastings** Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. *Appl. Environ. Microbiol.* 2000; **66**: 1084–1092.
25. **Shayo NB, Kamala A, Gidamis AB and SAM Nnko** Aspects of manufacture, composition and safety of *orubisi*: a traditional alcoholic beverage in the North-western regions of Tanzania. *Int. J. Food Sci. Nutr.* 2000; **51**: 395-402.

26. **Usha A, Sripriya G and TS Chandra** Effect of fermentation on the primary nutrients in finger millet (*Eleusine coracana*). *J. Agric. Food Chem.* 1996; **44**: 2616-2618.
27. **Abdelmoneim OE and AH El-Tinay** Effect of fermentation on protein fractions and tannin content of low and high-tannin cultivars of sorghum. *Food Chem.* 1994; **49**: 265-269.
28. **Obizoba IK and JV Atti** Evaluation of effect of processing techniques on the nutrient and antinutrients content of pearl millet (*Pennisetum glaucum*). *Plant Foods Hum. Nutr.* 1994; **45**: 23-34.
29. **FAO.** United Nations Food and Agriculture Organization. Sorghum and Millets in Human Nutrition. FAO, Food and Nutrition Series, No. 27, Rome, 1995.