"ASSESSMENT OF MICROBIOLOGICAL CHANGES DURING PRODUCTION OF MALTED AND FERMENTED FINGER MILLET FLOUR"

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ABSTRACT

Finger millet is widely consumed as porridge though not commercially grown in Rwanda. Traditional techniques of malting and fermentation are found to enhance bioavailability of nutrients. Hence the study aimed to assess microbiological changes among non-malted, malted and malted and fermented flour. Grains were purchased from local market and subjected to malting and milled. A portion of the malted milled flour was subjected to fermentation by mixing with water in the ratio of 2:1; and then allowed to auto ferment at 30°C for 48 hours. The fermented dough was mechanically dried and then milled into flour. All three samples of flours were microbiologically studied using Total Plate Count (TPC), Lactic Acid Bacteria (LAB), yeasts and moulds count. Data were analyzed using Microsoft Office Excel and results were presented as Logarithm of colony forming unit per gram (log cfu/g). Analysis showed that LAB greatly increased from 4.66 log cfu/g, 6 log Cfu/g to 6.24 log cfu/g while TPC greatly decreased from 5.69 log cfu/g, 5 log cfu/g and to 4.84 log cfu/g in non-malted, malted and malted and fermented flour respectively. Yeasts count also varied from non-malted, malted flour and to malted and fermented flour with results of 3.3 log cfu/g, 4.66 log cfu/g and 3.9 log cfu/g respectively. Moulds were absent in non-malted and malted flour while they were found to be low in malted and fermented finger millet flour.

Keywords: Finger millet, Lactic Acid Bacteria (LAB), yeasts, moulds, malting, fermentation

IINTRODUCTION

Finger millet scientifically known as *Eleusine coracana* is one of the varieties of millet found in the family of gramineae (Pragya & Raghuvanshi, 2012). Finger millet is a prominent drought resistant crop and is used as a staple prime food in India as well as in African countries. It serves as a good source of carbohydrate, protein, dietary fiber, amino acids and phytochemicals and it is nutritionally rich in total minerals at the level of 2.7% including calcium, magnesium, phosphorous and manganese (Subastri, et al., 2015). Finger millet has the highest calcium and potassium content among all cereals with an amount of 34 mg and 408 mg per 100 g respectively (Shobana *et al.*, 2013). Many products can be processed from finger millet including the malted and fermented flour and beverages. Malting process of finger millet is responsible for increasing the bio-accessibility of minerals such as manganese, calcium and iron at levels of 17%, 12% and 10.4% respectively. This is due to an increase in the activity of phytase enzyme leading to a decrease of phytate content during grains sprouting (Kalpana *et al.*, 2010). Fermentation plays an important role of allowing the growth of desired microorganisms such as bacteria, yeasts and moulds and it is widely used as food preservation method because it results in increasing food acidity and thus preventing growth of harmful microorganisms. It helps in providing a wide variety of flavors, and significantly improves the nutritional properties of the raw material (Kohajdová & Karovicová, 2007).

Malted and fermented flour is prepared from germinated finger millet and the flour is then subjected to natural fermentation process. This flour is largely used in preparation of weaning food, instant mixes and many other products such as porridge (Subastri *et al.*, 2015). The flour from malted finger millet increases amount of amino acids, phytochemicals and free radical scavenging activity (Barugahara *et al.,* 2015).

The change in microbiological load of the malted and fermented finger millet flour is very observable. It is dominated by Lactic Acid Bacteria (LAB), while yeast counts are found to be low with coliforms and moulds being absent (Usha & Chandra, 1997). However, the presence of yeasts in fermented compounds enables them to produce highly desirable aroma compounds. During malting and fermentation processes, the microbial changes include growth of LAB and yeasts which increase titratable acidity, decrease the pH and inhibit the development and multiplication of harmful microorganisms such as coliforms and enterobacteriaceae, thus extend cereal products shelf life (Muyanja & Namugumya, 2009). Microorganisms which are usually found in malted and fermented finger millet flour are *Lactobacillus fermentum*, *Lactobacillus salivarius, Pediococcus* and some species of *Leuconostoc* (Shankar & Usha, 2014).

Hence the study aimed to assess microbiological changes during malting of finger millet and fermentation of finger millet flour in comparison with the non-treated. Thus, this research will also contribute in extending the conservation time of finger millet flour which is often shortened by traditional processing method such as grinding and sieving. It might help also in diversifying the consumption of finger millet based products in Rwanda. Many researches have been published on microbiological changes during fermentation of cereal products but none has been found on microbiological changes during production of malted and fermented finger millet flour which was done in this research.

2. Materials and Methods

Samples of finger millet were purchased from local (Nyabugogo) market. They were transported in plastic sacks and stored in airtight containers to prevent contamination. Samples were analyzed within 48 hours.

2.1. Sample preparation

The samples passed through seven major unit operations that included sorting, washing, drying, grinding, sieving, weighing, malting and fermentation**.** The grains were initially subjected to manual cleaning by removal of dirty materials and dusts. Further cleaning and sorting was done to remove low quality and damaged grains that were separated from good quality grains by winnowing. Grains were washed using water in stainless steel container to remove soil materials which adhered to grain surfaces. Wet grains were dried in a mechanical drier (UNITEMP Drying cabinet, LTE Scientific Ltd., Greenfield, Odham, UK) and then used for analysis, this served as control, while a portion of the wet grains were used for malting. Malting was done by soaking moist grains for 48 hours, drained from excess water, wrapped in moist muslin cloth and allowed to germinate at ambient temperature. They were mechanically dried and the rootlets removed. The dried grains, non-malted and malted, were ground separately using a laboratory scale cutting mill (JISCO,model: J-NCM, Korea) and sieved using standard mesh sieve (BS No:30). Using an analytical balance (Accuris™ Precision Balance), appropriate weight of sample was portioned and used for analysis. A portion of the malted flour was subjected to fermentation by mixing water with malted flour in the ratio of 2:1 and then allowed to ferment at 30 °C for 48 hours (Usha & Chandra, 1997) and mechanically dried (UNITEMP Drying cabinet, LTE Scientific Ltd., Greenfield, Odham, UK) at 60 °C for 8 hours. All the three types of flour *viz.,* non-malted, malted and malted and fermented flour were subjected to the

microbiological analysis described below.

2.2. Sample analysis

Microbiological analysis was carried out on the above mentioned three types of samples and analyzed for Total Plate Count (TPC), Lactic Acid Bacteria (LAB) count, yeasts and molds count. TPC was analyzed by inoculating samples of appropriate dilutions into sterile Petri dishes containing well prepared plate count agar. The Petri dishes were then incubated at 35°C for 24 hours (FSSA, 2012). LAB was also analyzed by inoculating sample on MRS agar. Incubation was done at 30°C for 72 hours (FSSA, 2012). Yeasts analysis was conducted by inoculating sample dilutions on Petri dishes having Potato Dextrose Agar (PDA) with yeast supplement and chloramphenicol to suppress bacterial growth. They were then incubated at 30°C for 72 hours (FSSA, 2012). Colonies on the first plate with countable colonies between 30 and 1300 colonies (and its duplicate) were counted and their average recorded as colony forming unit per g (Cfu/g). Results were expressed as logarithm (Cfu/g).

2.3. Confirmatory tests

After the preliminary tests of counting of colonies in different culture media, they were confirmed by microscopic examination and differential staining using Gram staining technique and catalase test.

Microscopic examination: The morphology of the microorganisms was studied using a microscope to determine the genus. On a clean and sterile microscopic slide, 1 or 2 loops of a colony were placed in the center. A coverslip was placed over the sample avoiding air bubbles, using a thin film of Vaseline. The morphology of the microorganism was observed under the microscope.

Gram staining: The differential staining technique was used to differentiate the Gram negative bacteria from Gram positive bacteria. The difference in reaction is aconsequence of differences in the structure of the bacterial cell wall which make them to react separately that forms the key feature in their identification. Bacteria that retain the colour of crystal violet when treated with a differentiating agent are Gram positive while those that do not retain are stained in the contrasting colour of a counter stain that is usually pink / red and are hence termed as Gram negative.

Catalase test: This test is used as a confirmatory test. A small amount of bacterial colony was transferred to the surface of a clean, dry glass slide using a sterile loop in which a drop of 3 % H_2O_2 was placed. A positive result was characterized by a rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling; while a negative result was shown by the absence of bubbles or only by the appearance of a few scattered bubbles.

2.4. Statistical analysis

Results were expressed as means calculated using Microsoft Office Excel 2007. Tables and graphs have also been prepared using Microsoft Office Excel 2007. The significance of microbiological changes was determined using ANOVA single factor at 5% as level of significance.

3. Results and discussions

The study was conducted with the purpose of assessing microbiological changes during production of malted and malted and fermented finger millet flour. The acidic condition in the latter product reduced the growth of harmful bacteria due to the decrease of pH during fermentation.

3.1. Microbial changes in non-malted flour

The microbial changes in the non-malted flour that served as control indicated 3.34 log Cfu/g for yeasts, 5.69 log Cfu/g for TPC and 4.66 log Cfu/g for LAB. This is an indication of the presence of natural microflora as no metabolic changes occurred in microorganisms naturally present in the flour and its environment. The high amount of total bacterial counts may be attributed to the natural microflora in the raw materials or contamination during different handling steps. These results corroborated the findings of the study by Badau (2006) that found large contamination levels in non-malted pearl millet flour. Low amounts of LAB and yeasts may be due to the status of the sample because these parameters are usually increased with the level of fermentation process.

3.2. Microbial changes before and after malting

TPC decreased from 5.69 log Cfu/g in non-malted finger millet to 5 log Cfu/g in malted finger millet flour. This decrease may be due to the fact that raw grains may have been exposed to contamination during sprinkling of water, personnel and equipment used in polishing and milling which explains the higher amount of total bacterial counts but results showed no significant difference at $p>0.05$. However, malting decreased total microbial counts due to the effect of drying which reduce viable microorganisms which may have been present in raw flour (Livingstone, Sandhu, & Malleshi, 1992). LAB increased from 4.66 log Cfu/g to 6 log Cfu/g. This is probably due to the reason that most LAB are unable to utilize starch directly and extensive starch degradation by malting has the advantage of releasing soluble sugars, promoting lactic fermentation (Badau, 2006). These facts explain the increase of LAB from non-malted to malted flour and data showed a significant difference at $p<0.05$.

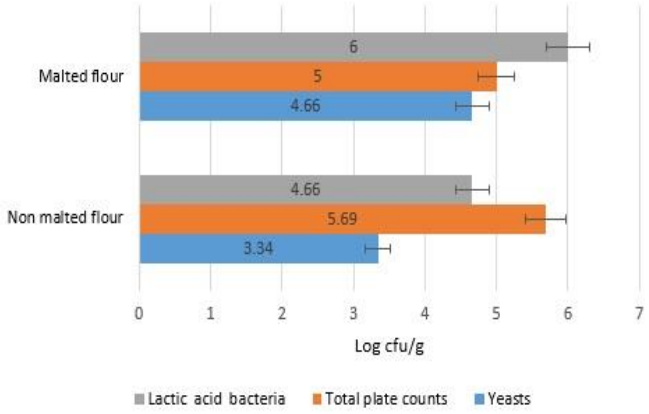

Figure 1: Comparison of microbial changes before and after malting

Yeasts amounts were found to be 3.34 log Cfu/g in non-malted and 4.66 log Cfu/g in malted flour (Fig 1). LAB is particularly important in fermentation because they produce desirable acids, flavor compounds, and peptides that inhibit the growth of undesirable microorganisms. The decrease of TPC and increase of LAB and yeasts after malting increased the amount of simple sugars.

3.3. Microbial changes before and after fermentation

Total plate counts decreased significantly at $p<0.05$ during fermentation from 5 log Cfu/g in malted flour to 4.84 log Cfu/g in malted and fermented finger millet flour. the results obtained corroborated with the findings obtained in the study on fermented finger millet which showed that total bacterial counts in millet decreased upon fermentation process (Usha & Chandra, 1997). Moreover, another study conducted by Shankar & Usha (2014) on fermented finger millet beverage confirmed the inhibition of pathogens during fermentation.

Contrarily, LAB increased significantly at $p<0.05$ from 6 log Cfu/g in malted to 6.24 log Cfu/g in malted and fermented finger millet flour. The increase in LAB count confirms the fact that they are the principal agents of natural fermentation of malted flour (Aydin *et al.,* 2009).

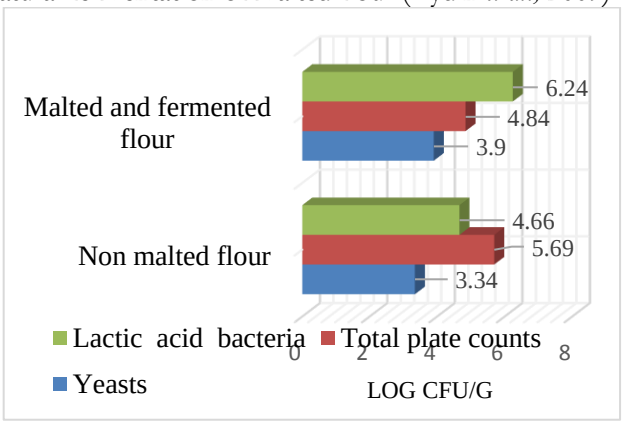

Figure 2: Comparison in microbial changes between non-malted (control) and malted and fermented finger millet

Yeasts decreased from 4.66 log Cfu/g in malted and 3.9 log Cfu/g in malted and fermented finger millet flour (Fig 2) significantly ($p<0.05$). These results were found to be similar to the studies conducted by Usha & Chandra (1997) which showed that yeasts decreased with the natural fermentation of finger millet.

3.4. Microbial changes during production of malted and fermented finger millet flour

Natural fermentations are usually carried out by yeast and lactic acid bacteria forming a complex microbiota that acts in collaboration. Results from this combination of malting and fermenting treatment revealed that TPC significantly decreased at $p<0.05$ from non-malted, malted and to malted and fermented flour with values of 5.69 log Cfu/g, 5 log Cfu/g and 4.84 log Cfu/g respectively (Fig 3). These findings were similar to the one obtained by Ilango & Usha (2013) which showed that pathogens were inhibited during the fermentation of millet beverage. LAB significantly increased at $p<0.05$ from 4.66 log Cfu/g in non-malted to 6 log Cfu/g in malted and to 6.24 log Cfu/g for malted and fermented finger millet flour. These results are in accordance with the observation that LAB is part of the microflora that dominate cereal fermentations (Chavan & Kadam, 1989). Bubbling was also observed during the fermentation indicating heterolactic fermentation (Usha & Chandra, 1997). LAB is particularly important in fermentation because they produce desirable acids, flavor compounds, and peptides that inhibit the growth of undesirable organisms.

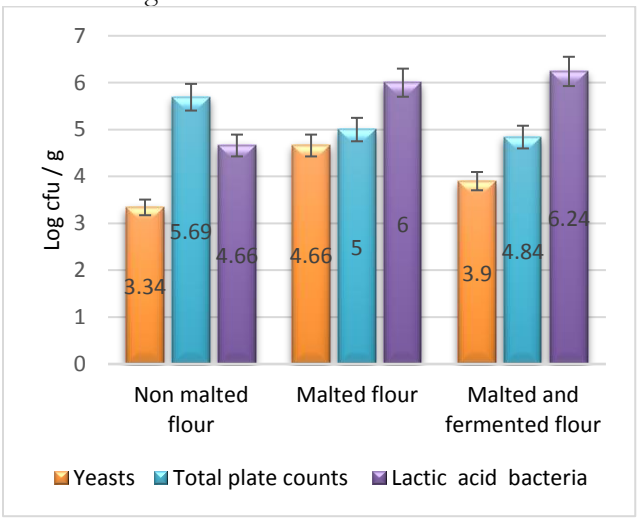

Figure 3: Comparison of microbial changes among non-malted, malted and fermented finger millet flour

Yeast counts decreased with fermentation process similarly to studies conducted by Usha & Chandra (1997) due to the reason that the fermentation is natural rather than being alcoholic one. However, the difference

showed by yeasts counts was not significantly different at $p>0.05$. Molds were absent in cultured samples except for malted and fermented finger millet flour. These results are similar to the ones obtained by Shankar & Usha (2014) which showed the presence of molds.

Table 1: Confirmatory tests

Microorganism	Microscopic examination	Catalase Test	Gram staining	Reference
LAB	Non-motile & rod shaped	Negative	Positive	Robert Kranz, *et al* (2006)
Yeasts	Hyphae formation	-	-	Linnea A. Qvirist *et al* (2016)
Molds	Hyphae formation	-	-	Linnea A. Qvirist *et al* (2016)

As revealed by the above stated confirmatory results (Table 1), LAB were characterized by their rod shape, catalase negative, gram positive, non sporing, and a strictly fermentative (Bennani *et al.,* 2017) characteristics; while yeasts and molds were present as microscopic filaments called hyphae. Since are not well structured on yeast cells, they are called pseudo-hyphae (Benson, 2001).

4. Conclusion

This study was conducted with the purpose of assessing the microbiological changes during production of malted and fermented finger millet flour. Total plate counts were marked by a significant decrease and LAB was characterized by a significant increase from non-malted to malted and fermented finger millet flour at $p<0.05$. Yeasts changes were shown by a non-significant increase at $p>0.05$ from non-malted to malted flour and significant decrease from malted to malted fermented finger millet flour at $p<0.05$. Molds were only found in malted and fermented finger millet flour. The research recommended nutritional evaluation and sensory analysis on malted and fermented finger millet flour

5. References

Aydin, A., Paulsen, P., & Smulders, F. (2009). The physico-chemical and microbiological properties of wheat. *Turkish Journal of Agriculture* , 445-454.

Badau, M. (2006). Microorganisms Associated with Pearl Millet Cultivars at Various Malting Stages. *Internet Journal of Food Safety* , 66-72.

Barugahara, E. I., Mbugua, S., Karuri, E., & Gakenia, M. (2015). Improving the nutritional value of traditional finger millet porridges for children aged 7-24 months in Bujenje County of Western Uganda. *African Journal of Food Science* , 426-436.

Bednarski, A. (2006). *Identifying Unknown Bacteria Using Biochemical and Molecular Methods.* St Louis: Washington University.

Bennani, S., Mchiouer, K., Rokni, Y., & Meziane, M. (2017). Characterisation and Identification of lactic acid bacteria isolated from Moroccan raw cow ' s milk. 2508, 4934-4944.

Benson, H. J. (2001). *The Fungi: Yeasts and Molds.* © The McGraw−Hill Companies.

Chavan, J. K., Kadam, S. S., & Beuchat, L. R. (1989). Nutritional improvement of cereals by fermentation. *Critical Reviews in Food Science and Nutrition* , 349-400.

FSSA . (2012). *Microbiological testing, Manual of methods of analysis of foods.* New Delhi: Ministry of Health and Family welfare/Government of India.

Kalpana, P., Sushima, W., & Krishnapura, S. (2010). Bio accessible Mineral Content of Malted Finger Millet (Eleusine coracana), Wheat (Triticum aestivum), and Barley (Hordeum vulgare). *Journal of Agriculture and Food Chemistry* , 58, 8100-8103.

Kohajdová, Z., & Karovicová, J. (2007). Fermentation of cereals for specific purpose. *Journal of Food and Nutrition Research* , 51-57.

Linnea, A. Q., Filippo, C. D., Strati, F., Stefanini, I., Sordo, M., Andlid, T., et al. (2016). Isolation, Identification and Characterization of Yeasts from Fermented Goat Milk of the Yaghnob Valley in Tajikistan . *Frontiers in Microbiology* , 1-17.

Livingstone, A. S., Sandhu, J., & Malleshi, N. (1992). Livingstone, A.S., Sandhu, J.S.,&Malleshi, N.G. Microbiological evaluation of malted wheat, chickpea, and weaning food based on them . *Journal of tropical pediatrics* , 7-74.

Muyanja, C., & Brenda S. Namugumya (2009). Traditional Processing, Microbiological, Physiochemical and Sensory Characteristics of Kwete, A Ugandan Fermented Maize Based Beverage. *African Journal of Food, Agriculture, Nutrition and Development* , 1046-1059.

Pragya, S., & Raghuvanshi, R. (2012). Finger millet for food and nutritional security. *African Journal of Food Science* , 77-78.

Shankar, I., & Usha, A. (2014). Assessment of the microbiological quality of koozh, a fermented millet beverage. *African Journal of Microbiology Research* , 308-312.

Shobana, S., Krishnaswamy, K., Anjana, R., Palaniappan, L., Sudha, V., Malleshi, N., et al. (2013). Finger Millet (Ragi, Eleusine coracana L.): A Review of Its Nutritional Properties, Processing, and Plausible Health Benefits. *Advances in Food and Nutrition Research* , 6-10.

Subastri, A., Ramamurthy, C., Suyavaran, A., Mareeswaran, R., Mandal, P., Rellegadla, S., et al. (2015). Nutrient profile of porridge made from Eleusine coracana (L.) grains: effect of germination and fermentation. *Journal of Food Science and Technology* , 6024-6030.

Usha, A., & Chandra, T. (1997). Microbial population biochemical change in fermenting finger millet (Eleusine coracana). *World journal of Microbiology and Biotechnology* , 13, 533-537.