Production and Nutritional Evaluation of Mahewu: A Non-Alcoholic Fermented Beaverage of South Africa

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ABSTRACT

Mahewu is a non-alcoholic sour beverage made from corn meal consumed in Africa and some Arabian gulf. A total of thirteen (13) lactic acid bacteria and ten (10) yeasts were isolated from fermenting maize meal during the traditional production of Mahewu. The microorganisms were screened for their ability to produce lactic acid, diacetyl, hydrogen peroxide and their growth at different pH with the aim of selecting starter cultures. Lactobacillus brevis and Saccharomyces cerevisae were identified and selected as starters based on the above parameters. Mahewu was produced using single and mixed starter cultures and its nutritional evaluation was investigated. The control was prepared using wheat flour as inoculum. The mahewu produced with combined starters of Lactobacillus brevis and Saccharomyces cerevisae was of better nutritional quality.

Keywords: Mahewu, bacteria, fermentation, lactic acid bacteria, single and mixed starter cultures

INTRODUCTION

Mahewu is a fermented maize beverage; it is non-alcoholic and popular among the indigenous people of South Africa (Schweigart and Fellingham, 1963). It is known by various names in South Africa. In Zulu, it is known as ‘Amahewu’, the Swazis call it ‘Emahewu’, the Pedis; ‘Metogo’, Sothos; ‘Machleu’ while the Vendas call it ‘Maphulo’ and the Xhosas; ‘Amarehwu’ (Coetzee, 1982). The most commonly used term is “Mahewu”. It is a refreshing drink largely consumed at schools, farms, mines and also acts as a weaning food for infants.

Mahewu is prepared by using 8-10% (w/w) maize flour as the major solid substrate in aqueous suspension. Wheat flour or maize bran is added to initiate the lactic acid fermentation (Byaruhanga et al., 1999, Holzapfel and Taljaard, 2004).

Studies done earlier on Mahewu have shown that the microorganisms responsible for its fermentation are lactic acid bacteria and yeast (Lactobacillus delbrueckii, Lactobacillus plantarum, Streptococcus thermophilus, Lactococcus lactis) (Odunfa and Adeyele, 1985; Holzapfel, 1989; Sanni, 1993 and Steinkraus, 1996).

An investigation on the survival of bacterial enteric pathogens in fermented Mahewu was conducted by Simango and Rukure, (1992) and it was concluded that fermented mahewu had bacteriostatic and bactericidal properties. Another study targeted the growth and survival of Bacillus cereus in fermented mahewu in which growth inhibition of the organism was observed (Byaruhanga et al., 1999).

Spontaneous fermentations have been used for the production of fermented foods based on the microflora present in the raw material (Vogel et al., 2002) but the quality of product and length of fermentation cannot be predicted (Togo et al., 2002), hence, the need for the use of a starter culture. A starter culture is a preparation containing a large number of variable microorganisms, which may be added to accelerate a fermentation process. It facilitates the predictability of its products (Holzapfel, 1997). Studies on the development of a starter culture for mahewu (Schweigart and Fellingham, 1963; Schweigart, 1971; Van Noort and Spence, 1976) led to the production of mahewu on a commercial scale (Edwards, 2003).

A survey on University of Zimbabwe students (age group 19-25years) found that over 33% of the students drink mahewu because they like the taste only, and 80% prefer to do so for its taste, in...
combination with other factors such as nutritional value. Studies have shown that fermentation improves the nutritional value of the food by enhancing the B-vitamin group in the products (Steinkraus, 2002).

Mahewu is known to offer some advantages over ogi in that the initial wild fermentation by fungi, etc is eliminated by boiling both the maize meal and water for steeping. Furthermore, it is pre-cooked and requires only mixing prior to consumption. Mahewu consists of coarse maize particles while ogi contains very fine pasty maize particles.

**METHODOLOGY**

**Sample Collection**

Western white maize (*Zea mays*) variety and wheat were purchased from Bodija Central Market in Ibadan, Oyo State. Sorting and removal of foreign material as well as broken seeds was done. The cleaned seeds were then taken in clean polyethylene bags for dry milling.

**Culture Media**

The culture media used in this study includes: De Mann Rogosa Sharpe (MRS) broth and Agar (for Lactic acid bacteria) and Malt Extract Broth and Agar which is used for isolating yeasts. The media were prepared according to the manufacturer’s instruction, homogenized in a water bath for 10 minutes and then sterilized in an autoclave at 121°C for 15 minutes and allowed to cool to 45°C before use.

**Enumeration and Isolation of Microorganisms from Steeping Maize Water**

Serial dilution of up to $10^{-10}$ was carried out on the steeping maize grain samples and plated out using MRS and Malt agar respectively. MRS plates were incubated in a carbon-dioxide enriched jar at 37°C for 48 hours while Malt extract plates were incubated aerobically at 30°C for 24-48 hours. Pure cultures were obtained by streaking out and colonial morphology and cellular characteristics of the colonies were studied. The pure cultures were sub cultured on slants at 4°C for subsequent use.

**Characterization of LAB Isolates**

The LAB isolates were characterized using macroscopic, microscopic, physiological and biochemical tests such as: Gram staining, Catalase test, Oxidase test, Motility test, Spore stain, Methyl Red test, Voges-Proskauer test, Hydrogen Sulphide test, Starch Hydrolysis, Production of Ammonia from Arginine, Casein Hydrolysis and the Carbohydrate fermentation pattern were monitored. Their growth at different temperatures and different pH, growth at 4% Nacl and homofermentative and heterofermentative tests was done.

**Characterization of Yeast Isolates**

The yeast isolates were characterized based on morphological, physiological and sugar fermentation pattern tests

Growth at different temperatures, Growth at 50% Glucose, urease test, nitrate assimilation, gelatin liquefaction, carbohydrate fermentation pattern for yeast Isolates

**Identification of Isolates**

The isolates were identified based on their macroscopic, microscopic and biochemical tests with reference to Bergey’s Manual of Systematic Bacteriology (Sneath, 1986) and Sanni and Lonner (1994).

**SCREENING OF ISOLATES FOR STARTER CULTURE SELECTION**

Quantification of Lactic acid produced by LAB isolates (A.O.A.C, 1990), quantification of diacetyl produced by LAB and yeast isolates (A.O.A.C, 1990), quantification of Hydrogen Peroxide produced by LAB isolates (Sanni et al., 1995)

**Preparation of Inoculum Size**

The starter cultures were selected on the basis of the results of tests carried out. The LAB species were transferred from slants to MRS plates and incubated anaerobically for 24hours. A colony was picked from each pure culture plate, grown successively in MRS broth before centrifugation at
4000rpm for 10minutes (Modified method of Kubuta, 2010). The pellets was washed in normal saline solution and centrifuged again. The colony forming units \((4.0 \times 10^8 \text{ cfu/ml})\) was determined by physically counting after growth of 1ml of bacterial suspension on MRS plates. The same method was used to obtain pure cultures of the yeast isolates. The cfu \((3.5 \times 10^8)\) was determined as described earlier (Mutula et al., 2003).

**Preparation of Mahewu using Starter Cultures**

After determining the inoculums size for each of the isolates, the starter cultures were then used as inoculum for the fermentation of the maize varieties. The Lactic acid bacteria used were *Lactobacillus brevis* and *Lactobacillus casei* and the yeasts used were *Saccharomyces cerevisae* and *Candida krusei*.

Mahewu was prepared as illustrated in Figure 1, adapted from methods by Haard et al. (1999). The mixture was incubated at 30°C and allowed to ferment for 24-36 hours. A similar procedure was used for controlled fermentation.

![Flow diagram for the traditional preparation of Mahewu](image)

**Physico-Chemical Changes during the Fermentation of Maize for Mahewu Production**

**pH**

Ten (10 ml) of the fermenting media was aseptically removed into sterile bottles and pH was taken with a Jenway pH meter equipped with a glass electrode.

**Total Titratable Acidity (TTA)**

Total titratable acidity was determined potentiometrically according to Nout et al. (1989) by titrating 10ml of fermenting samples against 0.1M NaOH using phenolphthalein (3drops) as indicator. The acidity was calculated as percent (w/v) lactic acid equivalent.

**Nutritional Analyses**

The moisture content, ash content, crude fat content, crude fiber content and crude protein content of mahewu samples was done using the A.O.A.C method of 1995.

**Determination of Mineral Contents**

The mineral analyzed were calcium, magnesium, potassium, sodium, iron and zinc. They were determined spectrophotometrically as described by A.O.A.C, 1995.

**Statistical Analysis**

The experimental data was analyzed using Analysis of Variance (ANOVA) to determine significant difference between the means and these were expressed as mean ± standard deviation (SD). The level of significance was set at \(P < 0.05\).The data were analyzed using SPSS version 17.0.

**RESULTS**

Tables 1 and 2 showed the morphological and biochemical characteristics of the isolates. All the LAB isolates were Gram positive rods/cocci with entire edges while the yeast isolates appeared shiny, whitish and greyish-white in colony.

Fig. 2 & 3 presents the percentage frequency occurrence of each LAB and yeast isolated from fermenting maize meal. *Lactobacillus brevis* had the highest frequency of occurrence (54%), followed by *Lactobacillus casei* (23%) and *L. plantarum* (15%) while *Lactococcus lactis* had the least frequency with 8%. For the yeast isolates, *S. cerevisiae* had the highest frequency of occurrence (70%) followed by *Candida krusei* with 30%.

**Table 2.** Biochemical and morphological characteristics of LAB isolated from fermenting maize meal during the production of “MAHEWU”

<table>
<thead>
<tr>
<th>Isolates code</th>
<th>Cellular morphology</th>
<th>Catalase</th>
<th>Grams reaction</th>
<th>Growth at pH5</th>
<th>Growth at Arginine</th>
<th>4% NaCl</th>
<th>Starch hydrolysis</th>
<th>Motility</th>
<th>Voges proshaner</th>
<th>Indole test</th>
<th>Oxidase test</th>
<th>H. S Production</th>
<th>Growth at 15°C</th>
<th>Lactose</th>
<th>Galactose</th>
<th>Mannitol</th>
<th>Malto</th>
<th>Raffinose</th>
<th>Arabinose</th>
<th>Adonitol</th>
<th>Sorbose</th>
<th>Glucose</th>
<th>Maltobiose</th>
<th>Sorbose</th>
<th>Xylose</th>
<th>Inositol</th>
<th>Fructose</th>
<th>Probable organism</th>
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**Table 3.** Biochemical and morphological characteristics of yeasts isolated from fermenting maize meal

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<tr>
<th>Isolates code</th>
<th>Catalase</th>
<th>Urease</th>
<th>Growth on 50% glucose</th>
<th>Growth at 30°C</th>
<th>Nitrate reduction</th>
<th>Glucitol lypofucation</th>
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<td><em>Candida krusei</em></td>
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<th>Sample code</th>
<th>Moisture (mg/100g)</th>
<th>Protein</th>
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<th>Ash</th>
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<td>12.04 ± 3.0g</td>
<td>3.65 ± 0.5g</td>
<td>1.37 ± 0.2g</td>
<td>1.40 ± 0.2g</td>
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<td>B(S.cerevisae)</td>
<td>92.78 ± 2.0g</td>
<td>14.78 ± 2.0g</td>
<td>3.57 ± 0.5g</td>
<td>1.34 ± 0.1g</td>
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<td>Control</td>
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<td>1.34 ± 0.2g</td>
<td>2.45 ± 0.5g</td>
<td>72.93 ± 5.0g</td>
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Table 4. Proximate analysis of “Mahewu” produced using different starter cultures

Values in the same column followed by the same letter are not significantly different according to Duncan’s multiple range test (p<0.05)

Table 5. Mineral composition of “Mahewu” produced using different starter cultures

Values in the same column followed by the same letter are not significantly different according to Duncan’s multiple range test (p<0.05)
Table 4 shows the results of the proximate composition of mahewu produced using different starter cultures. The result of the analyses revealed significant differences in the moisture content, protein content, ash content, crude fiber, fat content and carbohydrate content of mahewu produced using single starter and combined starters. The highest protein content of 14.78% was observed in mahewu produced using combined starters of L. brevis and S.cerevisae. The carbohydrate content was highest in mahewu produced using L.brevis singly (76.73%). Table 5 shows the result of the mineral composition of mahewu samples. Mahewu produced using the combined starters of L.brevis and S.cerevisae had the highest mineral contents compared to the one produced using single starter cultures.

DISCUSSION

*Lactobacillus casei, Lactococcus lactis and Lactobacillus plantarum, Saccharomyces cerevisae and Candida krusei* were isolated during the spontaneous fermentation of maize meal for mahewu production. This is in agreement with the work of SteinKraus et al., (1993) and Bvorchora et al., (1999) who reported the isolation of similar species during the production of Mahewu in South Africa. The fermentation of Mahewu using starter cultures in this study was characterized by a fall in pH and corresponding rise in TTA (lactic acid production) which was observed throughout the period of fermentation. These observations have been similarly reported in the spontaneous fermentation of maize and millet (Zvauya et al., 1997; Lei and Jakobsen, 2004 and Agarry et al., 2010). The decrease in pH as the fermentation progressed can be attributed to the increasing hydrogen ion content, due to the microbial activity on the carbohydrate and other food nutrients to produce organic acids (Adeyemi and Umar, 1994). The decrease in pH provides safety against bacterial pathogens. According to Gadaga et al. (2004), most pathogens are not able to survive under these conditions.

The increase in the titratable acidity was similar to the observations made by Mugula et al. (2003) and Annan et al. (2003) that high titrable acidity may have resulted from effect of fermentation and this has been reported to reduce incidences of diarrhea in people consuming them (Adeyemi and Beckley, 1986; Mensah et al., 1990).

The carbohydrate content decreased significantly in all the mahewu samples with mahewu produced using S.cerevisae singly having the lowest carbohydrate content of 67.46% and mahewu produced using L.brevis having the highest carbohydrate content of 76.73%. This decrease could be due to the selective utilization of carbohydrates as an energy source by the fermenting organisms (Dike, 2011). Also as reported by Chavan and Kadam (1989) and Haard et al. (1999) that generally, fermentation of cereals leads to a decrease in level carbohydrates.

There was a significant increase in the protein content of all the mahewu samples. High protein contents have been reported in fermented foods by Odunfa (1985) and SteinKraus (1985), they noted that fermentation process improves the nutritional quality of foods by increasing the protein contents. Fermentation significantly improves the percentage relative nutritive value (Protein quality) as well as the level of lysine in maize, millet and other cereals (Hamad and Field, 1979). The high protein content may also be due to the reduction in antinutrients such as phytate.

There was significant decrease in crude fibre and crude ash content of mahewu samples which can be attributed to the increase in protein content during fermentation (Wang and Fung, 1996; Omafuvbe et al., 2004). El-Tinay et al. (1979) reported that fibre tends to decrease during fermentation in dried milled flour samples than that of wet mill and mahewu is prepared using dried milled maize.

The fat content also decreased significantly in all samples. This occurrence may possibly be due to the utilization of oxidized lipids to generate energy for growth and cellular activities may have led to this increase in fat content (Ejiofor et al., 1987; Sanni and Ogbonna, 1991)

Mineral elements are important because they are essential for regulating and building the living and aids in fighting depression. From this study, mahewu produced using *L.brevis* and *S.cerevisae* had the highest mineral element composition compared to other samples. According to Obizoba and Atii (1994) and Fasasi (2009), mineral composition of cereals increase significantly during fermentation due to the activity of fermenting microorganisms.

CONCLUSION

It’s a fact from this research that *Lactobacillus brevis* and *Saccharomyces cerevisae* used as combined starters were better off in nutritional quality during the production of mahewu which may be recommended for industrial purposes.
REFERENCES


