Investigation of shelf-life extension of sorghum beer (Chibuku) by removing the second conversion of malt

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A B S T R A C T

The effect of removing the second step of malt conversion in the brewing of Chibuku beer was investigated with the intention of extending the shelf-life of the product. Chibuku was brewed in the laboratory scale fermenters using Delta Beverages' standard brewing procedure. A variation was made where the second malt conversion was not conducted on one brew. The effect of increasing pasteurisation time was also investigated. The extension of shelf-life was determined by following the physicochemical and the sensory profile of the products for a period of ten days under sub-tropical ambient conditions. Ethanol productions were similar between the control and test beers (without second conversion malt). A product with overall acceptability of 70% was made from the brew without the second malt conversion and with 15 min pasteurisation at 80 °C. The product was, however, low in bite and head retention, but had less bacterial load, decreased acid production, and improved keeping quality by at least two days. However, due to contamination of the pitching yeast with lactic acid bacteria (LAB), total acids rapidly increased after 168 h and caused unacceptable sourness. Increasing pasteurisation time to 20 min reduced bacterial load of the wort to figures as low as 2×10³ cfu/ml. General hygiene levels of the brewery were acceptable and no clostridia were detected in the product or contact surfaces along the production line. Bacterial contamination of the product mainly comes from the raw materials with pasteurisation greatly reducing this load. If improved, the procedure has the potential of extending the shelf-life of the beer to beyond 168 h.

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1. Introduction

Chibuku, an opaque beer, is one of the industrialised alcoholic beverages in Zimbabwe, and there are more than 20 opaque beer breweries that produce over 420 million litres of the beer each year (Parawira et al., 2005). The beer product is an opaque pinkish-brown liquid with a thin consistency due to its high content of suspended and dissolved solids (3.6% w/v), with an alcohol content of approximately 3–5%, pH around 3–4 and lactic acid levels around 0.5 g/l (Casey et al., 1984; Bvochora and Zvauya, 2001). Opaque beer, also known as doro, hwahwa, mhamba or utshwala, in different regions of Zimbabwe, is also a popular beverage in several countries in Africa (Gadaga et al., 1999). Different cities in Zimbabwe produce different brands of sorghum beer, namely, Ingwebu, Go Beer, Pungwe, Thabani, and Simba. It is considered a nutritious product because it contains a mixture of organic acids, alcohols, vitamins and other growth factors produced by lactic acid bacteria (LAB) and yeasts (Van Heerden, 1989; Holzapfel, 2002). The beer is marketed and consumed while still actively fermenting, and is effervescence and has a refreshing aroma.

Chibuku is made from straight run maize, sorghum malt, sorghum malt, barley malt, water, lactic acid and a top fermenting strain of the yeast, Saccharomyces cerevisiae. The opaque beer brewing process involves the blending of sorghum malt and meal, barley malt and straight run maize grits, the extraction and breakdown of carbohydrates from these raw materials to make a sugar solution, followed by its subsequent fermentation with yeast to produce ethanol and carbon dioxide (Fig. 1). Essentially, the process involves lactic acid fermentation as well as alcoholic fermentation.

The major biological changes occurring in the brewing process are catalysed by natural lactic acid bacteria and yeasts from barley and sorghum malt, and the S. cerevisiae yeasts introduced as a starter culture. Like other industrialised processes, saccharification plus lactic acid souring occur first, while alcoholic plus lactic acid fermentations occur last (the first acidification is due to added lactic acid while the second is due to inherent LAB in the raw material) (Wood, 1985). The beer is sold while microbiologically active, hence beneficial bacteria may end up spoiling the product. Opaque beer from Delta Beverages plants in Zimbabwe is made by the “double cook” method (pasteurisation) for utilization of starch in the malt and also pasteurising the wort.

Most traditional, African cereal-based fermented foods deteriorate rapidly and become unacceptable to consumers within one to four
The overall objective of this study was, therefore, to improve the shelf-life of Chibuku from the current 120 h to 168–240 h. This was to be achieved through investigating the effect of eliminating the second malt conversion (mashing) step in the brewing process so as to reduce the bacterial load, and hence reduce the rate and amount of acids formed. Removal of the second malt conversion step was combined with an extension of the pasteurisation time during preparation of the beer. The general hygiene levels along the production process (Fig. 1) were also investigated in order to determine external sources of contamination of the product.

2. Materials and methods

2.1. Identification of external sources of contamination

Critical stages in the preparation of Chibuku were identified for hygiene checks. The following sampling points were identified: (1) before and after cleaning in place (CIP) of multi-purpose vessels (MPVs), (2) before and after pasteurisation, (3) after pitching, (4) before and after cleaning containers, and (5) packaged Chibuku product. All liquid samples (100 ml) were collected in sterile McCartney bottles. Swabs were also obtained from contact surfaces on the multi-purpose vessels. Each sample was diluted appropriately in quarter strength Ringer’s solution. Coliforms and enteric pathogens were used as indicators of general hygiene of the brewing process. The aerobic spread plate count was done on all samples. All dilutions, inoculations, and incubations were done according to standard methods given in Marshall (1992).

Coliform presence was determined by spreading 1 ml of an appropriately diluted beer sample on violet red bile salt agar (OXOID) and incubated at 35 °C for 48 h. Growth of red colonies indicated the presence of coliforms. Positive plates were confirmed on brilliant green lactose bile broth. Colonies were suspended in Ringers solution and 1 ml inoculated into tubes containing brilliant green lactose bile broth (BGBL) (LAB M) with inverted Durham tubes. Accumulation of gas in the Durham tubes after incubation at 35 °C for 48 h indicated a positive coliform test.

Presence of Enteric pathogens was detected by spreading 1 ml of appropriately diluted beer sample on Deoxycholate citrate agar (OXOID) and incubated at 37 °C for 24 h. Growth of red colonies indicated the presence of Gram-negative bacteria that could be considered as potential pathogens.

Total bacterial counts as indicators of hygiene were determined by spreading appropriately diluted 1 ml samples onto plate count agar (BIOLAB) using the spreading technique. Colony counts were done on a Darkfield Quebec colony counter after incubation of the plates at 37 °C for 48 h. Lactic acid bacteria (LAB) were enumerated by pour plating in MRS agar (BIOLAB) after incubation for 72 h at 30 °C.

2.2. Effectiveness of cleaning

The effectiveness of reducing the total microbial load by cleaning was checked in the fermentation vessels and the standard 2 l packaging containers. Samples before and after cleaning were taken and tested for cleanliness using total bacterial load on plate count agar.

2.3. Preparation of the beers for determination of improvement in shelf life

The effect of removing the second malt conversion in the brewing of Chibuku beer was investigated by brewing opaque beer in 18 l laboratory-scale fermenters using the standard procedure outlined in Fig. 1. A control beer was made by brewing Chibuku without changing any one of the brewing parameters. A beer in which the second malt conversion was eliminated was also brewed. The experiments were repeated twice and the results presented are the average of the duplicated brews. Microbiological, organoleptic and physico-chemical profiles of the two types of beer were compared.
2.4. Chemical analyses

Chemical and organoleptic changes were followed in the beers stored under sub-tropical ambient conditions for a period of 10 days. Chemical tests performed during the fermentation period were total reducing sugars, pH, total acids (titratable acidity), total dissolved solids and alcohol.

The pH of the samples was measured immediately after sample collection using a CYBERSCAN 500 pH meter at room temperature. Titratable acidity was done using the method outlined in the DELTA BEVERAGES Chibuku Raw Materials and Analytical Methods manual (1998). Each beer sample (100 ml) was filtered through a No. 1 Whatman filter paper after which 10 ml of the filtrate were titrated against 0.1 N NaOH using phenolphthalein indicator until a permanent pink colour persisted for 30 s. The titration was done in triplicate for each sample. The total acidity was then calculated as follows:

\[
\text{% Total acidity} = \frac{\text{ml of sample} \times 0.09}{\text{Weight of SG bottle plus distillate} - \text{Weight of empty SG bottles}}
\]

where 0.09 is a conversion factor used to change total acids from grams to percentages.

Alcohol content was determined by specific gravity. A well mixed beer sample was measured into a 100 ml volumetric flask. The sample was transferred into a distillation flask into which 100 ml of tap water was also added. Distillation was done until 100 ml of distillate were collected. The sample was cooled to 20 °C before the relative density, measured as specific gravity, was determined by filling a standardised specific gravity (SG) bottle and weighing it. The amount of alcohol was then determined from the SG conversion tables as percentages by volume. Specific gravity was determined as:

\[
\text{SG} = \frac{\text{Weight of SG bottle plus distillate} - \text{Weight of empty SG bottles}}{\text{Weight of distilled water}}
\]

Total dissolved solids were measured as sucrose in centrifuged samples of beer using an Atago No 1 Brix sugar refractometer and expressed as grams of dissolved solids per 100 ml of liquid (°Bx/v). Dissolved sugars were used to assess progress of fermentation.

2.5. Physico-chemical analysis

Head, settling and viscosity parameters were determined using methods developed by Taylor and Doudi, (2006, Hand Book for SOBA course, unpublished). Beer was poured into a 1000 ml measuring cylinder and foam height was measured after standing for 30 min. Settling height was measured after standing for 5 min. Percentage foam was calculated by determining the volume of foam on top of the liquid as a percentage of total liquid excluding foam. Percentage settling was calculated by determining the volume of clear liquid on top of the solids as a percentage of total liquid volume. Viscosity was measured using a Brookfield Synchro-lectric viscometer as outlined in the DELTA BEVERAGES Chibuku Raw Materials and Analytical Methods manual, 1998).

2.6. Sensory evaluation

A group of 10 experienced tasters was used to evaluate the beers to ascertain any difference between the control and the variant beers and also to assess shelf-life. The products were scored against Delta Beverages specifications. Brand identity, bite, colour, odour and head retention were used to ascertain acceptability of the product. Acceptability was based on like or dislike of the product and overall acceptability scores were out of ten and were expressed as percentages. Average scores of the tasters were statistically analysed using ANOVA and Bonferroni’s multiple comparisons test.

2.7. Indices to measure improvement in shelf life

Time taken to reach 0.5% (v/v) total acids was taken as an index of shelf life. According to Delta Beverages standards, the product is deemed unacceptable (spoiled) when total acids reach 0.5% (v/v). The time taken for the product to become unacceptable in terms of bite, sourness and odour was also assessed through sensory evaluation.

2.8. Statistical analysis

The Pearson’s Product Moment correlation coefficient at 0.01 level of significance was used to ascertain the extent of the relationship between removal of the second malt conversion and the beer composition with respect to total acids, lactic acid, and alcohol. A matched pair T test was performed to determine the differences between the two samples.

3. Results and discussion

3.1. General hygiene of the production process

Total bacterial counts, Gram-negative enteric pathogens and coliforms (Section 2.1) were used as indicators of general hygiene of the production process and packaging material. No coliforms or potential enteric pathogens were detected at any of the sampling points, suggesting that good hygienic was practiced during the brewing process. Contamination from other external sources seems to be very minimal as shown by these results. The absence of coliforms does not, however, mean the absence of contaminating organism. It should be noted that small numbers of bacteria remaining after cleaning in addition to the load already in the raw material pose great spoilage risk. Mesophilic heterofermentative lactobacilli are largely responsible for the spoilage of sorghum beer (Taylor and Doudi, 2006, Certificate Course in Opaque Beer Brewing, Continuing Education University of Pretoria, unpublished). Chibuku is still being viewed by many, as an inferior product that does not require strict quality control measures. The absence of a HACCP (Hazard Analysis Critical Control Points) system makes it difficult to ascertain the real cause of spoilage, and such a system needs to be developed for Chibuku beer.

Total bacterial counts were also done for the product at selected stages of production up to the pitching stage. The total bacterial counts were 2.55 × 10^5 cfu/ml after straining, 3.35 × 10^6 cfu/ml after pasteurisation, 2.65 × 10^10 cfu/ml after the second malt conversion and 6.5 × 10^10 cfu/ml after pitching (the high count could probably include yeasts assuming that the plate count agar allowed for their growth). Total counts decreased after ‘pasteurisation’ and increased after the second conversion malt was added and even further after pitching. These results suggest that the source of contaminating microorganism could be the second step of malt conversion and pitching yeast. The bacteria were largely lactic acid bacteria which grew anaerobically on MRS. Ropy strains of these lactic acid bacteria were detected visually by touching colonies with a wire loop and observing stretchability.

3.2. Effects of removing second malt conversion on Chibuku beer

3.2.1. Microbial load changes

There was a marked decrease in total bacterial counts (TBC) after pasteurisation (second heating) in the brews without the second malt conversion at 15 min pasteurisation time giving counts as low as 5.9 × 10^4 cfu/ml as shown Table 1. The lactic acid bacteria load also showed a similar pattern to TBC after pasteurisation. Increasing the pasteurisation time as shown in Table 1 decreased the microbial load further (though with a lesser margin) to around 2 × 10^3 cfu/ml.

There was not much difference in bacterial load caused by increasing the pasteurisation time above 15 min. Pitching with yeast
resulted in a substantial increase in counts of lactic acid bacteria at were presumably thermophilic lactic acid bacteria.

The second step of malt conversion has been considered to be the main source of the contamination (Mashanda, 1997). The results in Table 1 show that, even after pasteurising the wort and before addition of second conversion malt, some bacteria still remain in the wort. The difference in between the bacterial load after pasteurisation and the load after adding the second conversion malt gives the bacterial load introduced by the second conversion malt. Even after removing second malt conversion, the beer still spoiled and this, most probably, was due to inefficiencies in pasteurisation and bacterial contamination coming from the yeast used as inoculum. Increasing pasteurisation time to 20 min and 30 min has an effect of further reducing bacterial load, although the reduction is smaller than that due to removal of the second conversion of malt.

3.2.2. Chemical changes

3.2.2.1. Total dissolved solids. A general decrease in dissolved solids was recorded in all samples. There was a rapid decrease in total dissolved solids during the first three days of fermentation which levelled off thereafter in both samples (Fig. 2). The control beer had the lowest content of 5.6% (w/v) dissolved solids while the beer produced without the second malt conversion had 6.1% (w/v) by day 4. This was probably due to high bacterial load in the control beer which meant fast utilization of available solids. The rate of sugar utilization was therefore, higher in the control than the variant. Not all dissolved solids were available for utilization by yeast as shown by the levelling off of the solids after 72 h in the products. The levelling off may be due to inhibition of metabolic activity by the increasing alcohol (Zvauya et al., 1996).

3.2.2.2. Changes in alcohol during fermentation. There was a gradual increase in alcohol content as fermentation progressed until a maximum of 4% (v/v) was obtained on the fourth day in both samples (Fig. 2). After 120 h, there was a slight decline in alcohol content in all samples reaching levels as low as 3.52% (v/v) by day 9 (Fig. 2). The amount of potential fermentable sugars available for yeast at the beginning of the fermentation was almost the same; hence their pattern of fermentation was similar in all samples (Fig. 2). The sugar conversion efficiency of the yeast did not change as seen in the level of alcohol produced. As a result, alcohol production is not affected as evidenced by the maximum amount of alcohol being produced by all the samples. Since second conversion malt is added to aid the colour, texture and viscosity of the product, it had and insignificant effect on fermentable sugars. The trend in alcohol production is similar to previous work by Mashanda (1997) and Bvorchora (2000).

3.2.2.3. pH. The pH values of the products generally decreased during storage due to lactic acid production by lactic acid bacteria, reaching final values of 3.18 to 3.31 by day 10 (Fig. 3). The control product had the lowest pH by the end of fermentation. There was a drastic drop in pH for the first 24 h in all samples. The drop in pH was higher in the control. Removal of second conversion malt reduced total bacterial load, thereby giving a decreased reduction in pH.

3.2.2.4. Total organic acids. Total acidity was used as an indicator of spoilage and acceptability of the product. A general increase in total acidity was recorded for both samples (Fig. 3) giving a trend similar to that of lactic acid. For the first three days of fermentation, the difference in total acids was not significant between both the samples. Thereafter, total acidity in the control beer rapidly increased until 240 h. Acidity in the other beer increased much slower, only rapidly rising after 168 h. The control beer accumulated 0.5%v/v total acids by 120 h but this level of acid was not produced until after 168 h in the beer without second malt conversion. A paired sample t test for total acids at $t_{0.025}$ revealed a significant difference in acidity for beers with and without the second malt conversion. It therefore follows that removing second step of malt conversion greatly reduces the production of organic acids, with the possibility of reducing undesirable souring. However, the temperature and other conditions of beer storage (temperature, yeast quality) need to be carefully controlled if meaningful shelf life is to be attained through removal of the second malt conversion.

As expected product bite and total acidity was reduced in the without the second step malt conversion. The rate of acid build up was however, higher in the control. The decrease was due to the expected reduction in inherent lactic acid bacteria as the second malt conversion was eliminated. However product bite improved with time most likely due to contribution from lactic acid bacteria in the yeast and those remaining after pasteurisation. Removing the second malt conversion delayed the time needed to reach the unacceptable total acid threshold of 0.5% (v/v). It took 168 h to reach this threshold value using this modified process unlike the 120 h achieved using the normal brewing process. The pattern of pH and total acidity recorded is consistent with that reported by other workers for various fermented beverages (Odunfa and Adeyele, 1995; Zvauya et al., 1997; Mugula et al., 2001; Muyanja et al., 2003).

Table 1

Changes in loads of total bacteria (TB) and lactic acid bacteria (LAB) in Chibuku beer after removing the second step of malt conversion, and pasteurising for different times

<table>
<thead>
<tr>
<th>Sample</th>
<th>TBC (cfu/ml) sd</th>
<th>LAB (cfu/ml) sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal beer with 15 min pasteurisation (0.5)</td>
<td>1.35×10⁵ (0.2)</td>
<td>3.55×10⁴ (0.5)</td>
</tr>
<tr>
<td>Beer without second conversion malt at 15 min pasteurisation</td>
<td>5.9×10⁵ (2)</td>
<td>4×10⁴ (0.5)</td>
</tr>
<tr>
<td>Beer without second conversion malt at 20 min pasteurisation (0)</td>
<td>2×10⁴ (0)</td>
<td>2×10³ (0)</td>
</tr>
<tr>
<td>Beer without second conversion malt at 30 min pasteurisation (0.5)</td>
<td>4×10³ (0.5)</td>
<td>1×10³ (0.5)</td>
</tr>
</tbody>
</table>

sd = standard deviation.
3.3. Physico-chemical analysis

Generally, the laboratory brewed beer resembled commercial Chibuku beer in appearance. All the beer samples had a thin consistency on pouring except for the control beer which became slimy by day 7. The control beer had a head of 4.5% compared to 3.9% for the beer without second conversion malt. The overall head, as measured within 5 min, was acceptable and almost uniform in all samples, with the control having a better head retention. The control beer had an average viscosity of 58 during the fermentation period while beer produced without the second malt conversion had lower average viscosities of 49–52. Standard viscosity values range from 50–60 in normal Chibuku products. By the seventh day, the control beer wasropy with white pellicles on the surface, while roppiness and off-odours were recorded from the ninth day in the test beer. The control beer had a settling of 5% while the beer without the second malt conversion had a settling of 2%. Settling increased from the seventh day in all samples.

Head retention and creaminess (foaming ability) values were lower in the test beer and decreased as the beer matured. This may be due to loss of dipeptides and other proteins that are responsible for foam stabilization (O’Rourke, 2002). Protein in beer may be precipitated to give a whitish curd-like suspension (pellicles). This is foam stabilization (O’Rourke, 2002). Protein in beer may be precipitated to give a whitish curd-like suspension (pellicles). This is foam stabilization (O’Rourke, 2002). Protein in beer may be precipitated to give a whitish curd-like suspension (pellicles).

3.4. Sensory evaluation of Chibuku

The control beer and the test beer were described as resembling the original Chibuku brand with good taste and no off-odours. The products were acceptable according to the rating by the panel of tasters based on the parameters evaluated (Table 2). No unacceptable sourness, odour or bite was recorded to warrant rejection of the new, test beer. Bite of the new product was greatly reduced although the panel of tasters considered it generally good. The new product had a 70% acceptability rating compared to 80% acceptability in the control (Table 2). Statistical analysis of overall acceptability of the beers showed no significant differences (P > 0.05) between the beers at 72 and 120 h, and Bonferroni’s multiple comparisons test showed that there was no significant variation between the two products (P > 0.05). Acceptability was encouraging since this was the first attempt to produce a product with such variation. Acceptability of the new product was within Delta Beverages’ specifications which require all new products to have 70% acceptability or better.

3.5. Extension of shelf life

Acceptability of the products decreased after 72 h in the control and after 168 h in the test beer. The control beer deteriorated faster than the variant, with the latter remaining reasonably acceptable at 168 h. Removal of the second step of malt conversion in the brewing of Chibuku increased the time taken to reach 0.5% (v/v) total acids from 120 h to about 168 h (Fig. 3). However, the reduction in head retention makes the product less appealing. Acceptability of the new product was much better at 168 h when compared to the control beer (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percentage acceptance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>72 h sd</td>
</tr>
<tr>
<td>Normal beer/control (1)</td>
<td>80 (0)</td>
</tr>
<tr>
<td>Beer without second conversion malt (2)</td>
<td>70 (10)</td>
</tr>
</tbody>
</table>

sd = standard deviation.

4. Conclusions

Removal of the second step of malt conversion reduces total bacterial loads to levels as low as 3.3 log cfu/ml, causing less production of total acids during fermentation. Consequently, this delayed the time taken to reach the unacceptable total acid threshold of 0.5% (v/v). It took 168 h to reach this threshold value using this modified process unlike the 120 h achieved using the normal brewing process. Spoilage of the normal product as evidenced by appearance of pellicles and roppiness was observed at 168 h while these were not found in the test beer product even at 240 h. If removing the second malt conversion is to be adopted as a way of brewing sorghum beer with extended shelf life, it would be necessary to carry out further studies to optimize the process and determine acceptability of the product in the market. It would also be advantageous to identify the types and load of bacteria in raw materials and that remaining in the worst after pasteurisation, so as to effect targeted preservation measures. A comprehensive HACCP system for Chibuku needs to be established so as to ensure quality control and improve storage life. This is the first report on the effect of removing second conversion malt in the brewing of Chibuku; hence extensive research is still needed in the optimization of the process including the storage conditions that favour extended shelf-life and effective pasteurisation.

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