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# Application of refrigerated and frozen sorghum malt slurries in preservation of starter cultures of traditional fermented cereal-based beverages - a case of Obushera from Uganda

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**Research note**

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## Abstract

# Objective

Industrial production of traditional fermented beverages in developing countries is limited by lack of commercial starter cultures. *Saccharomyces (S.) cerevisiae* MNC 21Y and *Lactobacillus (L.) plantarum* MNC 21 were identified as starter cultures for a Ugandan cereal beverage, *Obushera*. However, they are commercially unavailable due to lack of affordable appropriate propagating and preservation methods. In this study the starters were propagated in sorghum malt slurries (30 °C for 24 h) and stored at 5 °C and − 18 °C for 90 days. Viability and fermentation ability of the cultures was monitored.

# Results

Viability was higher for starters stored at 5 °C (*S. cerevisiae*: 6 log cfu/g and *L. plantarum*: 7–9 log cfu/g during 90 days) than those at -18 °C (*S. cerevisiae*: 2 cfu/g and *L. plantarum*: 4 log cfu/g after 30 days). Refrigerated starters acidified *Obushera* (pH ≤ 4.5) faster (10–20 h) than frozen ones (18–24 h). Refrigerating the starters in sorghum malt slurries preserves them for at least three months. This provides an affordable option for starter commercialization and industrial production of traditional fermented foods.


## 1 Introduction

Globally, traditional fermented foods are vital in the diet owing to their numerous benefits. Fermentation enhances the nutritional value, increases sensory diversity, prolongs shelf life and ensures safety of food (Nout 2009). *Obushera*, a traditional fermented sorghum/millet beverage from Uganda is a popular weaning food, thirst quencher and social drink (Mukisa 2012). Production has for long been by local artisans and industrial scale production only started in 2008 with Multiline International Limited introducing *Obushera* under the brand name '*Bessa.*' To-date there are many brands of *Obushera* on the market but all are spontaneously fermented.

Spontaneous fermentations are carried out by natural flora on raw materials, utensils, processors or the environment (Nout 2001). Adopting spontaneous fermentation for industrial production is challenging because the process takes long (24–36 h) and is unpredictable (Nout 2001). It is also associated with inconsistencies in product quality and safety (Holzapfel 2002). Developing starters is vital in standardizing traditional fermentations for industrial production (Holzapfel 2002). This way, starters with desirable properties like fast acidification and flavor production can be applied to reduce fermentation time and ensure consistent product quality and safety (Holzapfel 2002). Starter culture combinations (*Saccharomyces* (*S.*) *cerevisiae* + *Lactobacillus* (*L.*) *plantarum*) produce an acceptable product with a flavor profile similar to that of traditionally fermented *Obushera* (Mukisa 2012; Mukisa et al. 2017). Additionally, they produce *Obushera* in 10–12 h as opposed to ≥ 24 h as is with spontaneous

fermentations. These starters are, however, not yet commercially available because no affordable preservation methods were evaluated.

Starter cultures can be preserved in liquid, spray-dried, frozen or lyophilized forms using media like reconstituted skimmed milk, liquid nitrogen and nutritive media (Ananta et al. 2004; Carvalho et al. 2004; Peres et al. 2008). While cultures exhibit maximum survival in such media, ingredients e.g. cryoprotectants and the expense of the technology involved limit their use (Tartè 2009). Locally available plant materials like coco yam, rice, cassava, yam and *Garri* are reported as good starter carriers (Ofuya and Nnajiofor 1989; Okafor et al. 1999). Since *Obushera* supports *S. cerevisiae* and *L. plantarum* growth (Mukisa 2012), millet or sorghum slurries may be economically feasible alternatives for their preservation. This study evaluated refrigerated and frozen sorghum slurries as carriers of *Obushera* starters.

## 2 Methods

# 2.1 Material and methods

# 2.1.1 Microbial cultures

*L. plantarum* MNC 21 and *S. cerevisiae* MNC 21Y isolated from *Obushera* (Mukisa 2012) were used. *L. plantarum* MNC 21 and *S. cerevisiae* MNC 21Y were separately grown in MRS broth and Yeast Mold Broth, respectively. The broths were supplied by CONDA, Madrid, Spain. Cultures were incubated at 30 °C for 24 h. The cells were centrifuged at 7500xg for 10 min and the pellets washed thrice using sterile quarter strength Ringer' solution (Oxoid Limited, Hampshire, England). The pellets were suspended in sterile quarter strength Ringer's solution.

# 2.1.2 Sorghum malt

*SESO* 3, a red sorghum variety obtained from the National Semi-arid Resources Research Center in Serere, Uganda was used to prepare sorghum malt. The sorghum grain was placed on a suspended wire mesh and sorted to remove chaff. The grain was washed using pressurized water. Ten kilograms of grain were soaked in 15 L of potable water containing 0.3% NaOH then steeped for 6 h. The water was drained and the steep vessel refilled with fresh water. The grains were steeped for a further 10 h after which the grain was transferred to germination beds at 25 °C. After two days when the rootlets were 1 cm long, the grain was spread out in a 2 cm thick layer in a drying chamber at 65 °C. The grain was considered dry if it broke 'cleanly.' The dried sorghum malt was stored in moisture proof bags at ambient temperature till further use.

# 2.1.3 Preparation and inoculation of slurries

Dried sorghum malt was milled using a Wondermill (Grote Molen Inc., Pocatello, USA) at the bread texture control setting. At this setting, 99% of the resulting flour passed through a 1000 mm mesh and 85% through a 500 mm mesh. The flour was mixed with potable water to make slurry (12.5% total solids)

which was then heated with continuous stirring to 90 °C and held at that temperature for 15 min. The hot slurry was aseptically transferred to a sterile glass bottle and cooled to 30 °C. It was then inoculated with 6 log cfu/mL of *L. plantarum* MNC 21 + *S. cerevisiae* MNC 21Y mixture and incubated at 30 °C for 24 h. Samples were drawn at 0, 4, 8, 12 and 24 h to determine cell counts, pH and titratable acidity. Experiment was done in triplicate.

# 2.1.4 Storage of starter cultures

The fermented slurries were distributed in sterile bottles and some stored at -18 °C while others at 5 °C. Samples were drawn periodically to determine cell counts and fermentation ability. Fermentation ability was determined by inoculating 100 mL of sterile freshly prepared sorghum malt slurries (12.5% total solids) with 1% (v/v) of cultures and incubating at 30 °C for 24 h. Samples were drawn periodically to determine pH, titratable acidity and flavor development.

# 2.1.5 Analyses

### Acidity and pH

The pH was measured using a pH meter (Mettler-Toledo AG model, Schwerzenbach, Switzerland). Titratable acidity was determined by titrating 10 mL of *Obushera* against a 0.1M solution of NaOH using phenolphthalein as the indicator (Horwitz, 2000).

### Flavor development

Flavor development was determined by sniffing the products to detect the characteristic flavor of *Obushera.* The intensity of flavor was scored as follows: +++ (strong), ++ (mild) and + (weak).

### Microbiological analyses

*L. plantarum* MNC 21 was enumerated by pour plating selected serial dilutions of the culture in MRS agar then incubated at 30 °C for 48 h. The yeast was enumerated by surface plating selected serial dilutions of the culture on Potato Dextrose Agar supplemented with chloramphenicol then incubated at 30 °C for 72 h. Counts were determined on days; 0, 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 for cultures stored at 5 °C and 0, 5, 10, 20 and 30 for those kept at -18 °C.

### Statistical analyses

Means were subjected to one way analysis of variance to test for significant differences at $\alpha = 0.05$. The Fisher's LSD was used to separate the means. Analyses were done using XLSTAT software version 2010 (Addinsoft, Paris, France).

## 3 Results And Discussion

# 3.1 Microbial counts

Figure 1 summarizes counts of the culture mixture in sorghum malt slurries stored at -18 °C and 5 °C. Throughout storage, *L. plantarum* MNC 21 were generally higher ($p < 0.05$) than *S. cerevisiae* MNC 21Y. There was a drastic decline in counts during storage at -18 °C. Counts of both microorganisms dropped below the desired concentration (6 log cfu/mL) in ≤ 5 days. For cultures stored at 5 °C, counts remained above 6 log cfu/mL throughout 90-day storage period.

The drastic decline in counts of the cultures stored at -18 °C is associated with cellular freeze damage. Cellular damage is majorly due to intracellular ice crystal formation which damages the cellular structures (Fonseca et al. 2000). In addition, the formation of extracellular ice in the suspension medium results in high solute concentration which results in osmotic stress (Fonseca et al. 2000). This therefore, explains why the viability of the cells stored at 5 °C was higher ($p < 0.05$) than that of those stored at -18 °C. Addition of cryoprotectants such as glycerol to cell suspensions prior to freezing is reported to minimize the effects of cellular freeze damage by lowering the freezing point (Hubalek 2003). In the current study the absence of a cryoprotectant in the slurries possibly resulted in the observed sharp decline in viability of frozen cultures.

# 3.2 Fermentation ability

Figures 2 summarizes the fermentation ability (changes in pH and acidity) of the mixed *L. plantarum* MNC21 + *S. cerevisiae* MNC 21Y culture stored at -18 °C (Figs. 2a and 2b) and 5 °C (Figs. 2c and 2d). Fermentation ability was most ($p < 0.05$) efficient on day 0 of storage. However, unlike cultures stored at 5 °C (Figs. 2c and 2d), the fermentable activity of cultures stored at -18 °C (Figs. 2a and 2b) declined sharply in the subsequent days of storage. On day 0 it took about 6 h only for cultures stored at either temperature to drop the pH of the *Obushera* below the desirable value (pH ≤ 4.5). Thereafter for cultures stored at -18ºC this value was obtained between 15–24 h of fermentation whereas it took 6–18 h for cultures stored at 5 °C. Titratable acidity increased to 0.43% on day 0 but the increase was much lower (0.1 – 0.04%) on days 5–30 for cultures stored at -18 °C. For cultures stored at 5 °C, the increase was 0.43 – 0.13% between days 0–40 and 0.09 – 0.05% between days 50–90.

The trend in fermentation ability of the cultures is directly related to cell viability. The high fermentation ability of cells stored at 5 °C (Figs. 2c and 2d) is attributed to high cell counts (Fig. 1). A drop in cell viability results in reduced cell metabolism, observed as a reduction in the rate of fermentation thus leading to low acid production (Figs. 2a-2d). Rapid acidification of *Obushera* is always desirable because it inhibits growth of pathogens which are the majority at the start of fermentation (Muyanja 2001). The pathogens are not only a food safety concern but may also produce off flavors in the product (Muyanja 2001).

# 3.3 Flavor development

Table 1 summarizes the intensity of flavor development in *Obushera* at 24 h of fermentation. Strong flavor development for cultures stored at -18 °C was observed only on day 0 after which it weakened throughout the rest of the storage time. For cultures stored at 5 °C, a strong flavor characteristic of *Obushera* was observed up to the 40th day of storage after which it became mild.

Table 1
Intensity of flavor development in *Obushera* at 24 h of fermentation

| Day of storage | Cultures at -18 ºC | Cultures at 5 ºC |
|---|---|---|
| 0 | +++ | +++ |
| 5 | + | +++ |
| 10 | + | +++ |
| 20 | + | +++ |
| 30 | + | +++ |
| 40 | | +++ |
| 50 | | ++ |
| 60 | | ++ |
| 70 | | ++ |
| 80 | | ++ |
| 90 | | ++ |
| +++ = strong flavor development; ++ = mild flavor development; + = weak flavor development | | |

The strong flavor development observed on day 0 of storage (Table 1) was due to the high cell viability (Fig. 1). *L. plantarum* and *S. cerevisiae* produce organic compounds and volatile acids responsible for the characteristic flavor of fermented *Obushera* (Mukisa 2012). At high cell viability cells exhibit high metabolism resulting in high production of flavor compounds. This explains why weak flavor development was noted at reduced cell viability on days 5–30 of frozen storage. In contrast, strong flavor development lasted longer due to higher counts for the culture stored at 5 °C. The subsequent reduction in flavor intensity could be due to cellular damage caused by prolonged exposure to cold conditions and/or lactic acid and other cellular toxins.

# 4 Conclusions

*Obushera* starters (*S. cerevisiae* + *L. plantarum*) in refrigerated sorghum malt slurries had better survival and fermentation ability than those stored in frozen slurries. Therefore, storage of lactic and yeast starters in cereal malt slurries under refrigeration can be adopted as an inexpensive preservation technology. This technology is suitable for distribution of starter culture to processors of fermented foods in various parts of the country. Adopting this technology by developing a simple starter culture kit will ensure consistent product quality and safety.

# 5 Limitations

The limitation of this work is that the fermentation ability of the cultures was determined at laboratory scale. Whereas this gave a good indication of the cell viability, findings need to be further validated at pilot/large scale.

# Abbreviations

**cfu**: colony forming units, ***L***.: *Lactobacillus*, **MRS**: de Man Rogosa Sharpe, **PDA**: Potato Dextrose Agar, *S*.: *Saccharomyces*

# Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and material

Datasets from which conclusions of the manuscript have been drawn are presented in the paper.

### Competing interests

Authors declare no competing interests.

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### Authors' contributions

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# Figures

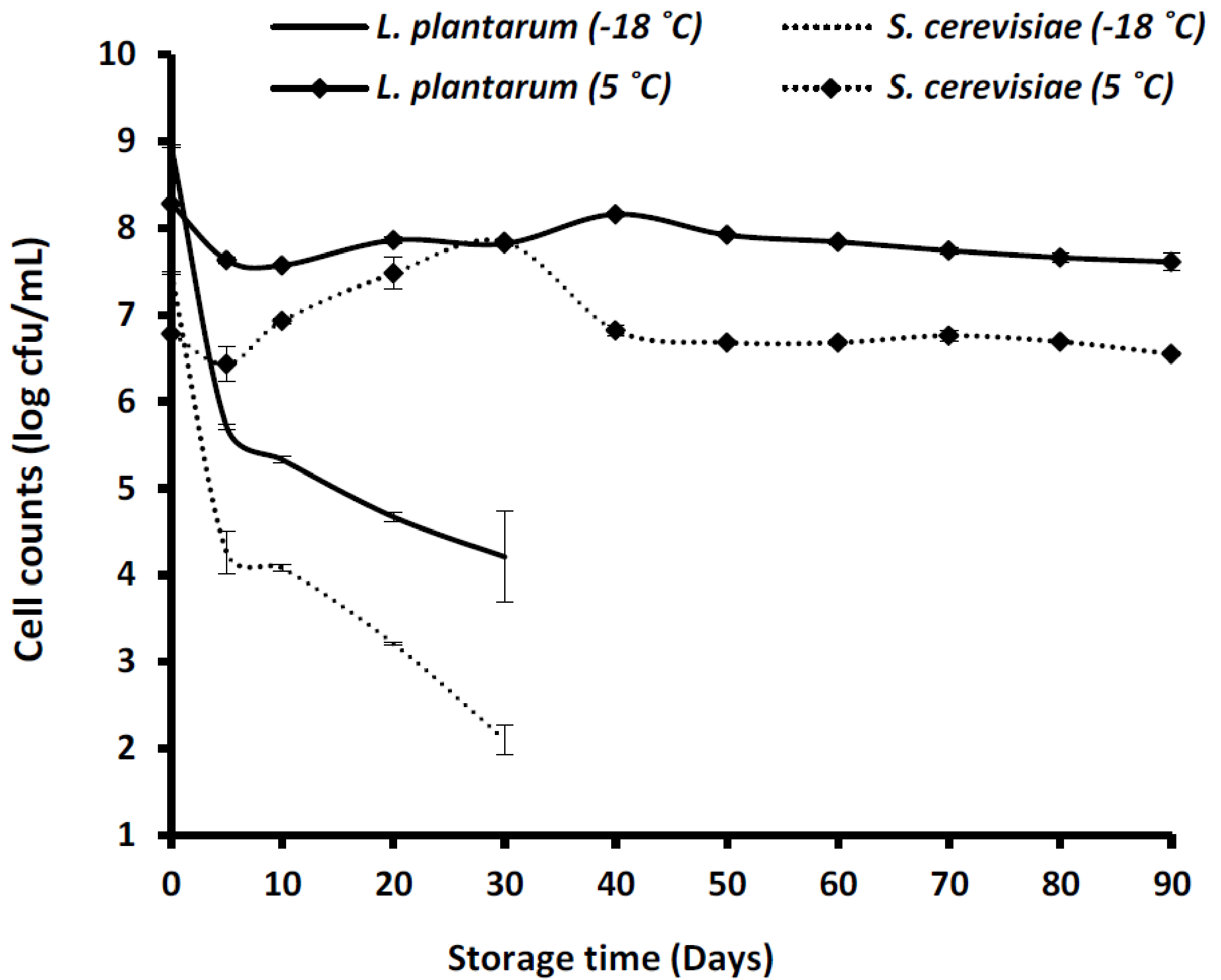


Figure 1

Counts of cultures stored at -18 ºC and 5 ºC. Error bars show standard deviations of three independent fermentations

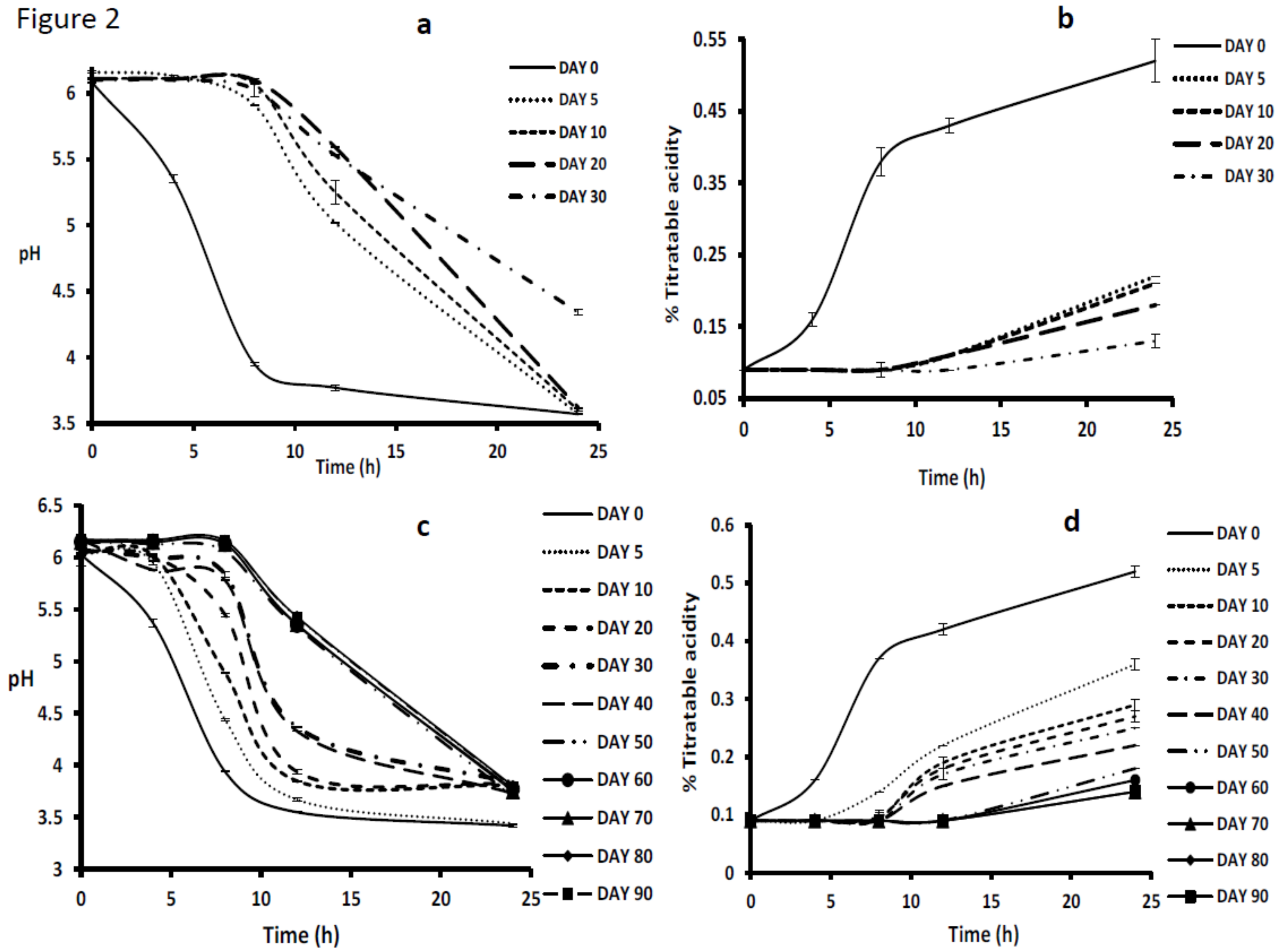


**Figure 2**

Changes in pH and titratable acidity of Obushera fermented by L. plantarum MNC 21 and S. cerevisiae MNC 21Y mixed culture stored at -18ºC (a and b) and 5ºC (c and d). Error bars show standard deviations of three independent fermentations