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APPLICATION OF REFRIGERATED AND FROZEN SORGHUM MALT SLURRIES IN THE PRESERVATION OF STARTER CULTURES FOR OBUSHERA FROM UGANDA

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ABSTRACT

Industrial production of traditional fermented beverages is limited by lack of quality commercial starter cultures. *Saccharomyces cerevisiae* MNC21Y and *Lactobacillus plantarum* MNC21 can be used to ferment cereal beverages such as Obushera. These cultures are unavailable as commercial starters due to lack of appropriate propagating and distributing procedures. The purpose of this study was to evaluate the use of refrigerated and frozen sorghum slurries as carrier media for the starters. Starters were propagated in sorghum slurries (30°C for 24 h) and stored at 5°C and -18°C for 90 days. Viability of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* was determined by cell counts after surface plating and pour plating, respectively. Fermentation ability was determined by inoculating sorghum slurries with starters and monitoring pH, acidity and flavor development. Viability was higher for starters stored at 5°C (*S. cerevisiae*: 6 log cfu.g^{-1} and *Lb. plantarum*: 7-9 log cfu. g^{-1} during 90 days) than those at -18°C (*S. cerevisiae*: 2 cfu.g^{-1} and *Lb. plantarum*: 4 log cfu.g^{-1} after 30 days). Refrigerated starters acidified Obushera (pH ≤ 4.5) faster (10-20 h) than frozen ones (18-24 h). Refrigerated or frozen *S. cerevisiae* + *Lb. plantarum* starters in sorghum malt slurries can remain viable for at least one or three months, respectively and produce Obushera with characteristic flavors.

Keywords: *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, Sorghum, Starter cultures, Viability

INTRODUCTION

Globally, traditional fermented foods are important as dietary sources of nutrients such as carbohydrates, proteins, fiber, minerals and vitamins (Fusco, 2017). Fermentation of these foods contributes to enhancing nutritional value, increasing sensory diversity, prolonging product shelf life and ensuring food safety (Tamang, 2016; Fusco, 2017). Traditional fermented foods are also associated with health promoting effects which are be attributed to the probiotic effects of some of the starter cultures (Tamang, 2016; Fusco, 2017).

Obushera is a collective name for popular traditional fermented or non-fermented cereal beverages consumed in western, southwestern and central Uganda (Mukisa, 2012). The beverages are mainly produced from flour of either malted or un-malted sorghum or millet grain. Obushera is used as weaning food, thirst quencher and as a beverage on social functions such as wedding ceremonies (Mukisa, 2012). The production of Obushera has for long been largely carried out by local artisans. Industrial scale production of the product in Uganda only started in 2008 with Multiline International Limited introducing Obushera under the brand name 'Bessa'. Several companies currently produce packaged and branded Obushera. Despite these developments, the production of Obushera still relies on a spontaneous fermentation.

Spontaneous fermentations are initiated by the natural flora present on raw materials, utensils, processors or the environment. The fermentations result from the competitive

activity of the diverse flora with organisms better adapted to the substrate eventually dominating (Wirawati *et al.* 2019). Adopting spontaneous fermentations for industrial processes is challenging because initiation of fermentation takes a relatively longer time compared to when starter cultures are used and the fermentations may proceed in an unpredictable and uncontrollable manner (Wirawati *et al.* 2019). The processes are also associated with high risks of failure and inconsistencies in quality attributes and safety (Wirawati *et al.* 2019; Byakika *et al.* 2019). Developing starter cultures is one of the vital steps towards standardizing traditional fermentations for industrial commercial production (Soro-Yao *et al.* 2014). Through this approach, starters with desirable properties such as fast acidification and flavor production can be identified and applied thus ensuring reduction in processing time, consistent product quality and safety (Soro-Yao *et al*. 2014; Mukisa, 2012).

Starter culture combinations of *Saccharomyces cerevisiae* MNC21Y and *Lactobacillus plantarum* MNC21 can potentially be used for production of an acceptable product with a flavor profile similar to that of traditionally produced Obushera (Mukisa, 2012; Mukisa *et al*. 2017). Application of these starters also enables production of Obushera in 10 – 12 hours as opposed to 24 hours or more as is expected in traditional spontaneous fermentations. These starters are, however, not yet commercially available for use by Obushera processors since no starter carriers or starter culture delivery methods for these particular starters have been developed or evaluated to date.

Starter cultures may be preserved and distributed in liquid, spray-dried, frozen or lyophilized forms (Kringelum and Kragelund, 2010). The media used to carry starter cultures include milk derived carriers such as reconstituted skimmed milk (RSM) liquid nitrogen and nutritive media (Kringelum and Kragelund, 2010; Parente, Cogan and Powel, 2017). While starter cultures in these media exhibit maximum survival, the expense of the storage conditions limits the use of these carriers. Additionally, starter culture preparations usually contain other costly ingredients such as cryoprotectants (sodium glutamate, sucrose, lactose) and growth factors (Leroy and Vuyst, 2009).

Locally available plant materials such African locust bean, soy bean, starch can be used as cost effective means for propagation and distribution of starter cultures (Leroy and Vuyst, 2009; Aderibigbe, Visessanguan and Jureeporn, 2015). Slurries of millet or sorghum can potentially be used for the production, distribution and storage of these starters since Obushera produced from these slurries supports the growth of *S. cerevisiae* and *Lb. plantarum* (Mukisa, 2012). The use of millet or sorghum malt slurries, which are locally available and affordable raw materials, could potentially be a more economically feasible alternative for starter culture preservation and distribution. There is, however, need to establish the viability and activity of these starters under cold storage. Therefore, the purpose of this study was to investigate the potential of using refrigerated and frozen slurries of sorghum as carrier media for Obushera starter cultures.

MATERIALS AND METHODS

Sorghum malt preparation

SESO 3, a red seeded 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 then washed using pressurized water. Ten kilograms of grain were soaked in 15 liters of potable water containing 0.3% sodium hydroxide (Merck, Germany) and allowed to steep 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 water was drained and the grain transferred to germination beds at 25°C. Germination was halted after two days when the rootlets of the grain reached 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 room temperature (25-27°C) till further use.

Propagation of microbial strains

Lactobacillus plantarum MNC 21 and *Saccharomyces cerevisiae* MNC21Y isolated

from Obushera were used in the study. *Lb. plantarum* MNC21 was grown in 100 ml of MRS broth (CONDA, Madrid, Spain) at 30°C for 24 h. *S. cerevisiae* MNC21Y was grown in 100 ml of Yeast Mold Broth (CONDA, Madrid, Spain) at 30°C for 24 h. The cells were separately centrifuged at 7500 x g for 10 min (Centrofriger – BL II, JP Selecta, Barcelona, Spain) and the pellets washed thrice using sterile quarter strength Ringer's solution (Oxoid Limited, Basingstroke, Hampshire, England). The pellets were then separately suspended in 10 ml of sterile quarter strength Ringer's solution.

Preparation and inoculation of sorghum malt 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 sorghum malt was mixed with potable water to make a slurry of 12.5 % total solids. The slurry was heated with continuous stirring to 90°C and held at that temperature for 15 min. The hot slurry (200 ml) was then aseptically transferred to sterile 250 ml glass bottles and allowed to cool to 30°C. The porridge was inoculated with 6.0 log cfu.ml^{-1} of *Lb. plantarum* MNC 21 and *S. cerevisiae* MNC 21Y cultures and incubated at 30°C for 24 h. Samples were drawn at 0, 4, 8, 12 and 24 h to measure cell counts, pH and titratable acidity.

Storage of starter cultures in fermented sorghum malt slurries

Starter cultures can be stored in a refrigerator at 2 – 5°C or frozen with cryoprotectant at −20°C to −40°C, −80°C or −196°C (Parente, Cogan and Powell, 2017). Other authors have reported storing starters at −20°C to 10°C (Kringelum and Kragelund, 2010). In this study 5°C and −18°C were used because they are the common chiller and freezer cabinet temperatures, respectively observed for refrigerators in Uganda (Makumbi et al, 2015). Therefore, most processors of Obushera with access to refrigerators are likely to store the starters at either 5°C or −18°C. To store the starter cultures, 50 ml of the fermented sorghum malt slurries were distributed in sterile 100 ml plastic bottles. Some of the bottles containing the fermented sorghum malt slurries were stored in a refrigerator and the rest in a freezer.

Determining the fermentation ability of the stored starter cultures

Samples were drawn periodically to determine cell counts and fermentation ability of the stored cultures. Fermentation ability of the stored cultures was determined by inoculating 500 ml of freshly prepared and sterile sorghum malt slurries (12.5% total solids) with 1% (v/v) of the stored starter cultures. The inoculated slurries were incubated at 30°C for 24 h. Samples were drawn periodically to determine pH and titratable acidity (0, 4, 6, 12 and 24 h), and flavor development (24 h).

Acidity and pH Analysis

The pH was measured using a pH meter (Mettler-Toledo AG model, Mettler-Toledo Group, Schwerzenbach, Switzerland). Titratable acidity was determined by titrating 10 ml of the sample against a 0.1M solution of sodium hydroxide using phenolphthalein as the indicator (Horwitz, 2000).

Flavor development by the stored starter cultures

The starter culture combination of *Saccharomyces cerevisiae* MNC21Y and *Lactobacillus plantarum* MNC21 is known to produce acceptable sensory attributes (aroma, taste, texture and color) which are similar to those of the traditional product (Mukisa, 2012; Mukisa *et al.* 2017). Therefore, beside evaluating viability and acidification potential of the stored culture, this study assessed the production of the typical flavor of Obushera. Flavor development was determined by the researchers (n = 3) who were all familiar with Obushera. The products were sniffed to detect for the characteristic flavor of Obushera. Samples were scored on consensus as follows: +++ = strong flavor development; ++ = mild flavor development; + = weak flavor development; ND = not detected.

Microbiological analyses

The Serial dilutions were prepared using ¼ strength ringer's solution (Oxoid Limited,

Basingstroke, Hampshire, England). Enumeration of *Lactobacillus plantarum* MNC21 was carried out by pour plating selected serial dilutions in MRS agar (CONDA, Madrid, Spain) and incubating at 30°C for 48 h. Enumeration of *Saccharomyces cerevisiae* MNC21Y was carried out by surface plating selected serial dilutions of the culture in Potato Dextrose Agar (CONDA, Madrid, Spain) with chloramphenical supplement and incubating at 30°C for 72 h. Microbial counts were determined at days 0, 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 during storage of starters at 5°C and only up to day 30 for starters stored at -18°C.

Statistical analysis

Means were subjected to one way analysis of variance (ANOVA) to test for significant differences at a 5% level of significance. The least significant difference test (Fisher's LSD) was used to determine means that were significantly different from one another after the ANOVA test. All statistical analyses were performed by XLSTAT (2010, Addinsoft, Paris, France). Experiments were carried out in triplicate.

RESULT AND DISCUSSION

Changes in cell counts during storage

Figure 1 summarizes counts of the of *L. plantarum* MNC21 and *S. cerevisiae* MNC21Y mixed culture stored in sorghum malt slurries at -18°C and 5°C. *L. plantarum* MNC21 generally had higher ($p < 0.05$) counts than *S. cerevisiae* MNC 21Y throughout the storage period. There was a drastic decline in cell counts during storage at -18°C with both *L. plantarum* MNC21 and *S. cerevisiae* MNC21Y counts dropping below the desired concentration of 6.0 log cfu.ml^{-1} in ≤ 5 days. For cultures stored at 5°C, cell counts remained above the desirable level (6.0 log cfu.ml^{-1}) throughout storage for 90 days.

The drastic decline in cell 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 (Meneghel *et al.* 2017). In addition, the formation of extracellular ice in the suspension medium results in high solute

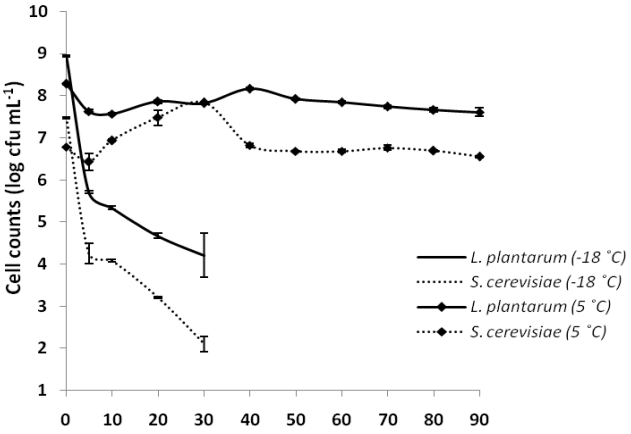

Figure 1. Counts of cultures stored at -18 ºC and 5 ºC. Error bars show standard deviations of three independent fermentations

concentration which results in osmotic stress (Meneghel *et al.* 2017). This may explain why the viability of the cells stored at refrigeration temperature (5°C) was higher ($p < 0.05$) than for those stored at freezing temperature (-18°C). Addition of cryo-protectants such as glycerol, glucose, sucrose or skim milk to cell suspensions prior to freezing has been reported to minimize the effects of cellular freeze damage (Tedeschi and De Paoli, 2011). In the current study the absence of a cryo-protectant in the Obushera most likely contributed to the observed sharp decline in cell viability of frozen cultures.

Fermentation ability of the stored starter cultures

Figure 2 and 3 show the fermentation ability (changes in pH and acidity) of the mixed *L. plantarum* MNC21 and *S. cerevisiae* MNC21Y culture stored at -18°C and 5°C. The fermentation ability varied with time of storage ($p < 0.05$) and was most efficient on day 0. However, unlike cultures stored at 5°C (Figure 3), the fermentable activity of cultures stored at -18°C (Figure 2) declined sharply in the subsequent days of storage. On day 0 it took approximately 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 -18 ºC this pH value was obtained between 15 – 24 h of fermentation while it took a shorter time (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, increase in titratable acidity 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 (Figure 3) is attributed to high cell counts (Figure 1). A drop in cell viability results in reduced cell metabolism which is observed as a reduction in the rate of fermentation thus leading to low acid production (Figure 2 and 3). Rapid acidification of Obushera is always desirable because it inhibits growth of pathogens which comprise majority of the microbial population at the start of fermentation (Mukisa, 2012). The pathogens are not only a food safety concern but may also produce off flavors when they grow in the product (Muyanja, 2001).

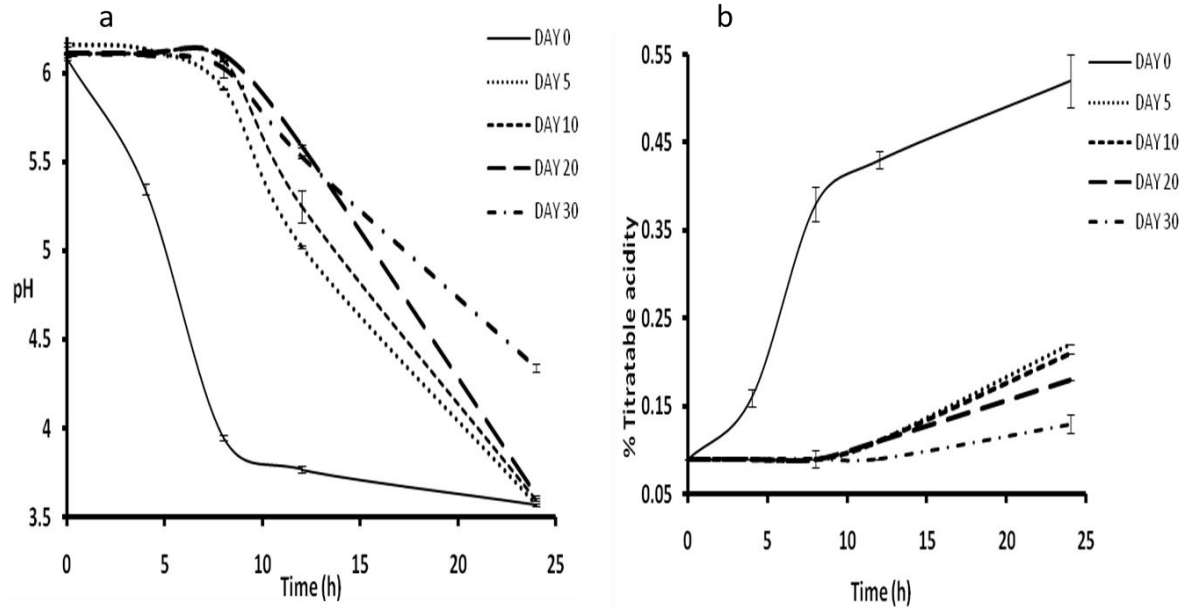

Figure 2. Changes in pH (a) and titratable acidity (b) of Obushera fermented using the *L. plantarum* MNC21 and *S. cerevisiae* MNC21Y mixed culture stored at -18 ºC. Error bars show standard deviations of three independent fermentations

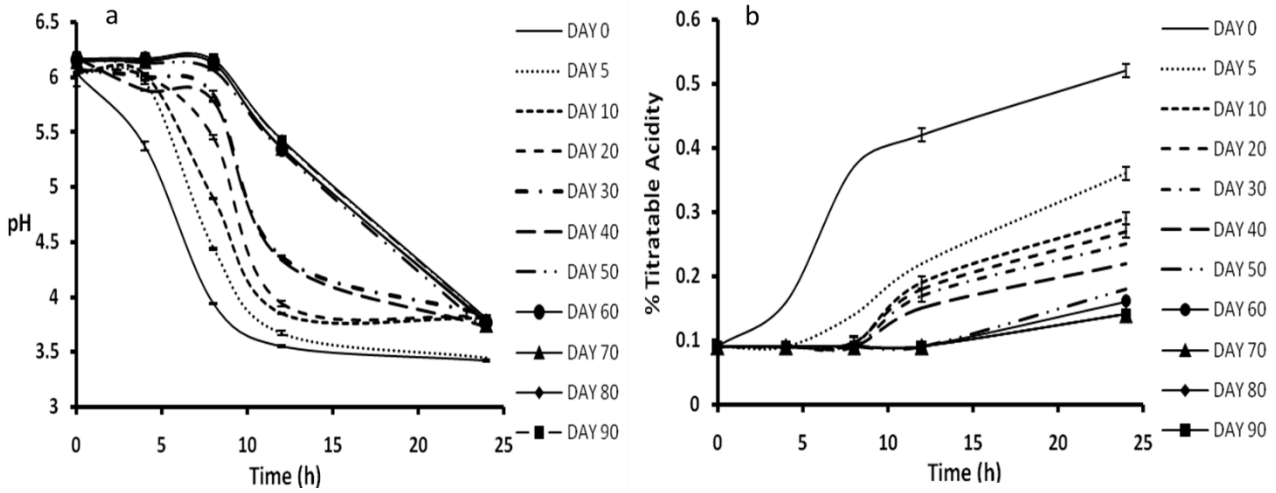

Figure 3. Changes in pH (a) and titratable acidity (b) of Obushera fermented using the *L. plantarum* MNC21 and *S. cerevisiae* MNC21Y mixed culture stored at 5 ºC. Error bars show standard deviations of three independent fermentations.

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 weak flavor development was noted for the rest of the days. For cultures stored at 5°C, a strong flavor characteristic of Obushera was observed up to the 40th day of storage after which there was mild flavor development.

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

Day of storage	**Intensity of characteristic flavor of Obushera**	
	Cultures at -18 ºC	**Cultures at 5 ºC**
0	+++	+++
5	+	+++
10	+	+++
20	+	+++
30	+	+++
40	ND	+++
50	ND	++
60	ND	++
70	ND	++
80	ND	++
90	ND	++

+++ = strong flavor development; ++ = mild flavor development; + = weak flavor development; ND not detected.

The strong flavor development observed on day 0 of storage (Table 1) was due to the high cell viability (Figure 1). *L. plantarum* and *S. cerevisiae* produce organic compounds and volatile acids which are responsible for the characteristic flavor of fermented Obushera (Mukisa, 2012). At high cell viability cells exhibit high metabolism which results in high production of flavor compounds. This explains why weak flavor development was noted at reduced cell viability on days 5 – 30 of storage for the frozen starter. In contrast, strong flavor development lasted longer due to the higher viable counts of the starter culture that was kept at refrigeration temperatures. Reduction in intensity of flavor for refrigerated cultures after day 40 could be due to reduction in cell metabolism probably due to cellular damage caused by prolonged refrigeration and or prolonged exposure of cells to lactic acid and other cellular toxins.

CONCLUSION

The purpose of this study was to investigate the potential of using refrigerated and frozen slurries of sorghum as carrier media for Obushera starter cultures. The study has shown that refrigerated (5°C) and frozen (-18°C) slurries of sorghum can be used to store the Obushera starters (*S. cerevisiae* MNC21Y and *Lb. plantarum* MNC21) for 90 days and 30 days, respectively. Therefore, refrigerated and frozen storage of lactic and yeast starters in cereal malt slurries can be adopted as an inexpensive technology for starter culture storage and distribution among small and medium scale processors of fermented foods and beverages. Further studies should evaluate the use of cryoprotectants in improving the shelf stability of the starter cultures.

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