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Survival and Acidification Potential of *Lactobacillus Plantarum* MNC 21 Stored in Air-Dried Sorghum Flours

Yusuf Byenkya Byaruhanga, Stellah Byakika, and Ivan Muzira Mukisa*

Department of Food Technology and Nutrition, School of Food Technology Nutrition and Bioengineering, College of Agricultural and Environmental Sciences, Makerere University, Uganda
*E-mail: ivanmukisa@gmail.com

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ABSTRACT

Increased commercialization of indigenous fermented foods requires availability of affordable starter cultures. The starters should also maintain functionality when stored at ambient conditions, especially where erratic power supply makes constant refrigeration unachievable. This study evaluated the survival of *Lactobacillus plantarum* MNC 21 starter culture air-dried (at 25 or 30°C) in sorghum flour and stored at 25°C for 30 days. Two sorghum varieties (malted and un-malted) were used. To determine their fermentation efficiency during storage, sterile sorghum malt slurries were inoculated with the dried culture and fermented at 30°C for 24 h. Acidification potential was determined at 5 days intervals by measuring microbial counts, pH and titratable acidity. Microbial concentrations dropped from 8-9 log cfu/g on day 0 to 1 log cfu/g on day 30. Sorghum variety and whether it was malted or un-malted did not affect culture survival. Culture dried at 25°C had better survival during the first 10 days (8-9 log cfu/g) than that dried at 30°C (8 log cfu/g) but survival between days 20-30 was similar (1-4 log cfu/g). The acidification potential (ability to reduce pH to ≤4.5) decreased with storage time: 4 h (day 0), 24 h (day 15), > 24 h (day 20) to no acidification (days 25-30). Air drying of starter cultures in sorghum flours coupled with storage at ambient temperatures could be adopted as a short-term preservation method. This low-cost technology is suitable for processors in developing countries where maintenance of a cold chain is hampered by unreliable electricity supply.

Keywords: Air-drying, *Lactobacillus plantarum*, preservation, sorghum, starter cultures

INTRODUCTION

Traditionally, indigenous fermented foods are made by spontaneous fermentation. Wild cultures on the raw materials, utensils, processors and the environment are relied on to initiate the fermentation (Mukisa et al., 2012). Consequently, the sensory attributes and microbiological safety of the products are inconsistent. To address this, researchers have isolated starter cultures for some indigenous fermented foods (Mukisa et al., 2012). This is very important since these foods are becoming popular across ethnic boundaries and consumer preferences, something that has attracted the interest of many entrepreneurs. Therefore, preservation of the starter cultures is required to facilitate

commercialization of indigenous fermented foods.

Among the different mechanisms available for starter culture preservation, drying is usually preferred because of easier transportation and better-quality control of the dried cultures (Tripathi and Giri, 2014). Common drying methods include hot-air drying, freeze drying, spray drying, and vacuum drying among others (Tripathi and Giri, 2014). However, some of these methods are associated with high heat, mechanical shearing, dehydration and osmotic pressure which lower cell viability (Tripathi and Giri, 2014). Consequently, protectants or carriers such as skim milk powder, groundnut shells, pea pericarp, safflower shells, cajanus pericarp and sunflower shells are employed to increase cell survival (Gosavi and Bagool, 2013).

Despite the effectiveness of the different drying methods mentioned earlier, the technologies for these methods are quite costly making it difficult for them to be fully adopted, especially in low-income countries. Additionally, the need for cold storage of the dried cultures is challenging, particularly in the rural areas where electricity supply is lacking or unreliable. Therefore, there is a need for a low-cost drying method for starter culture preservation that does not necessitate additional cold storage conditions. Therefore, this study examined the effect of low drying temperatures (25°C and 30°C) on the survival of *Lactobacillus* (*L.*) *plantarum* MNC 21 carried in sorghum flour and stored at 25°C. *L. plantarum* MNC 21 is a strong and fast acidifying culture that was isolated from *Obushera*, a fermented sorghum-millet beverage from Uganda (Mukisa, 2012). The *L. plantarum* MNC 21 preserved in the flour will be used as a starter culture for *Obushera* and related fermented cereal products.

MATERIALS AND METHODS

Microbial culture

L. plantarum MNC 21 isolated from *Obushera* by Mukisa (2012) was used. From the stock 0.1 mL was delivered into 100 mL of sterile MRS broth (CONDA, Madrid, Spain) and incubated at 30°C for 24 h. The LAB was sub-cultured thrice and recovered by centrifugation at 7,500 x g (5600 rpm in a Centrofriger BL-II centrifuge, JP Selecta SA, Barcelona, Spain) at 4°C for 10 min. The cell pellets were then suspended in 10 mL of sterile Ringer's solution. Culture purity was verified using a microscope (020-518.500 DM/LS I/98, Leica, Germany).

Sorghum varieties

Sorghum was used in this study because it is one of the common staple crops in Uganda and several parts of Africa where it is used to produce non-fermented and fermented foods and beverages (Mukisa, 2012). Two sorghum varieties namely: *Epuripur* (white grained) and *Eyera* (brown grained) were used. The grains were obtained from the National Semi-arid Resources Research Institute in Serere, Uganda. The two sorghum varieties (*Epuripur* and *Eyera*) were chosen for this study because they are among the common varieties of sorghum grown in Uganda. White, brown, high and low tannin varieties were chosen to capture the effects of tannin content on the growth and viability of *L. plantarum* MNC 21.

Preparation of sorghum flours

To malt the sorghum grains, they were first sorted to eliminate foreign matter then washed using pressurized water. Ten kilograms of grain were soaked in 15 L of potable water containing 0.3% NaOH and steeped for 6 h. Thereafter, the water was drained and the steep vessel refilled with fresh water. The grain was further steeped for 10 h after which the water was drained and the grain placed on trays and germinated at 25°C. Germination was halted when the rootlets were about 1cm long; this took three days for *Epuripur* and two days for *Eyera.*

The grain was spread out into a 2 cm thick layer in a drying chamber at 65˚C. The grain was milled using a Wonder Mill (110 Volt model, California, USA) and held in moisture proof containers at ambient temperature (25°C).

Sterilization, inoculation and drying of the sorghum flours

Prior to sterilization, the sorghum flours were first conditioned by sprinkling them with 2 parts of sterile potable water for each part of flour, increasing their moisture content from 11% to 65%. The mixture was then microwaved (Samsung ME731K, Johor, Malaysia) at 600 W for 3 min. The microwaving power and time was arrived at after several preliminary experiments (results not shown). Three minutes was the shortest time required to sterilize the flours when using 600 W. The Total Plate Count, total coliforms and yeasts and molds tests were used to check sterility of the flour. A 10 log cfu/mL suspension of *L. plantarum* MNC 21 was aseptically sprayed into the sterile flour in a ratio of 1:1 (culture suspension: flour). The flour was divided into 2 equal parts and spread out in thin layers of about 5 mm thick. One part was dried at 25°C for 24 h in a sterile class II biology safety cabinet (SterilGARD 403A-HE-INT, Sanford, US) while the other was dried at 30°C for 24 h in a sterile incubator (Binder 240, Tuttlingen, Germany). The dried flour-culture mixtures were separately stored in sterile screw-capped glass bottles at ambient temperature (25°C) away from light. Cell survival was monitored for 30 days using plate counts.

Acidification potential of dried *L. plantarum* MNC 21

Acidification potential of the stored *L. plantarum* MNC 21 was determined at 5 days intervals for 30 days. For this test, sorghum malt flour (*Eyera* variety) was mixed with potable water to make a 1 L slurry of 6.3% total solids. The slurry was heated with continuous stirring to 90°C and held at this temperature for 15 min. The hot gelatinized mixture (100 mL) was aseptically transferred into sterile 250 mL glass bottles and cooled to ambient temperature. It was then inoculated in duplicate with 1 g of sorghum flour (containing about 6 log cfu/mL dried culture) and incubated at 30°C for 24 h. Samples were drawn at 0, 2, 4, 6, 8, 10, 12 and 24 h to determine pH and titratable acidity.

Sample analysis

Enumeration of *L. plantarum* MNC 21 counts, Total Plate Count (TPC) and total coliforms was done by pour plating selected serial dilutions of the sample in MRS agar, Plate Count Agar (PCA) and Violet Red Bile Lactose Agar (VRBLA), respectively. MRS agar was incubated at 30°C for 48 h, PCA at 37°C for 24 h and VRBLA at 37°C for 24 h. To determine yeast and mold counts, selected serial dilutions of the sample were surface spread on sterile preset Potato Dextrose Agar and incubated at 30°C for 5 days. All media was supplied by Laboratorios, CONDA, Madrid, Spain. The pH was determined using a pH meter (AG model, Mettler-Toledo Group, Switzerland). Titratable acidity (TA) was determined by titrating 10 mL of the extract against 0.1N NaOH using phenolphthalein indicator. Moisture content was determined using the hot air-oven method (AOAC, 2000).

Statistical analysis

Results were presented as means ± standard deviations (Mean ± SD) of two independent experiments. Data were subjected to one-way analysis of variance (ANOVA) to test for significant differences at $\alpha = 0.05$. Mean comparisons were made using the Least Significant Difference (LSD) test. Analyses were done using Statistix software (Statistix, 2021).

RESULTS AND DISCUSSION

Microwave sterilization and drying of sorghum flours

Table 1 shows the microbial counts of malted and un-malted sorghum flours before and after microwave sterilization. Microwaving the flours at 600W for 3 min was sufficient to reduce the total plate counts, total coliforms, yeasts and molds to undetectable levels.

Table 2 shows the moisture content of sorghum flours after microwaving, addition of wet culture and drying. Microwaving reduced ($p<0.05$) the moisture content of the conditioned flours from 65% to 3.9-4.2%. Spraying of the flours with wet culture increased their moisture contents to 48.8-49.3%. Drying of the flours containing the culture then lowered their moisture content to 10.8-12.9%.

The results of microbial inactivation by microwave treatment observed in this study agree with those of Najdovski et al. (1991) who achieved vegetative cell destruction at 650W for ≤ 5 min. Microwave energy has the ability to rapidly heat, disinfect and dry materials efficiently (Silva et al., 2021). The principle of operation of microwaves is based on rapid oscillation of water and polar molecules in an alternating electric field leading to friction and thus dissipation of heat energy which is in turn responsible for microbial inactivation (Cebrián et al., 2017; Najdovski et al., 1991). Kormin *et al*. (2013) reported that the heating efficiency of microwaves is determined by the dielectric constant and dielectric loss factor of the heated material.

Dielectric constant is a measure of the ability of the material to store electromagnetic energy while dielectric loss factor relates to the ability of a material to dissipate electromagnetic energy into heat (Budnikov et al., 2020). High dielectric constant and dielectric loss factor result in high and rapid heating of a material by microwave energy. The dielectric constant of a material increases almost linearly with increase in water content due to the strong dipolar character of water molecules (Bhargava et al., 2013). Therefore, conditioning (up to 65% moisture content) increased the dielectric properties of the sorghum flour resulting in effective heating and sterilization.

Safe storage of flours, particularly at ambient temperatures, requires that the flours have moisture content not more than 14% (UNBS, 2017). Otherwise, high moisture content favors growth of unwanted microorganisms such as spoilage fungi and it also accelerates bacterial cell degradation reactions leading to death (Fu et al., 2011). Drying the flours at 25°C and 30°C reduced the moisture content from 48.8-49.3% to 10.8-12.9% (Table 2). This implies that flour tempered to about 50% moisture content can be effectively dried at 25°C within 24 h thus eliminating the unnecessary energy cost associated with air-drying at 30°C. More so since drying at 30°C rather than at 25°C is more likely to lower cell survival because of heat and dehydration stress.

The choice of drying and storage temperatures used was based on the optimal growth temperature (about 28 - 30°C) of *L. plantarum* to minimize cell death due to heat damage. Additionally, these storage temperatures were investigated because they are the common prevailing temperatures in most parts of Uganda. On average, temperatures typically fluctuate between 25 – 30°C. So minimal energy is needed to achieve and maintain them. This is contrast to refrigeration temperatures which require electricity. In developing countries like Uganda, electricity supply is limited and unreliable which is challenge for many of many *Obushera* processors for who the preserved culture is intended. The study aimed at producing a preserved culture that

could be safely stored at room temperature by the intended users.

Survival of dried *L. plantarum* MNC 21 during storage

Figure 1 shows the changes in *L. plantarum* MNC 21 counts during storage at 25°C in different sorghum flours. There was a general decrease in the counts throughout storage. *L. plantarum* MNC 21 cell concentrations dropped from 8-9 log cfu/g at day 0 to 1 log cfu/g at day 30. Sorghum flour type (malted vs non-malted) and variety did not affect the survival of *L. plantarum* MNC 21 (p>0.05). *L. plantarum* MNC 21 dried at 25°C had better survival during the first 10 days (8-9 log cfu/g) than that dried at 30°C (8 log cfu/g) but survival between days 20-30 was similar (1-4 log cfu/g). Between days 10-20, survival of cultures dried at 30°C was higher (p<0.05) than for those dried at 25°C. Cell levels dropped to <6 log cfu/g after 15-20 days.

The higher survival of *L. plantarum* MNC 21 dried at 30°C than at 25°C between day 10 and 20 of storage could be due to adaptation to low moisture content. *L. plantarum* MNC 21 dried at 30°C had a moisture content of 11% while that dried at 25°C had 13%. Low moisture content triggers specific transport systems in *L. plantarum* resulting in production of solutes such as carnitine which maintain cell membrane integrity and enhance survival (Kets and De Bont, 1997). High moisture content, as observed for *L. plantarum* MNC 21 stored at 25°C, lowered cell survival due to its acceleration of degradation reactions (Fu et al., 2011).

The survival of dried cultures is also affected by storage temperature. Cell viability greatly reduces with elevated storage temperature as a result of accelerated lipid oxidation and protein denaturation which lead to degradation of vital cell macromolecules (Fu et al., 2011). Nuylert *et al*., (2022) observed that dried cultures retained higher viability when stored at 4°C than at 30°C. Jofré *et al*. (2015) also observed higher survival of starter cultures stored at 4°C than 22°C. Therefore, the storage temperature used in this study (25°C) was also responsible for the decrease in culture survival over time. Nevertheless, storage at ambient temperatures such as 25°C can be adopted for short term storage of the starter culture considering that refrigerated storage cannot be guaranteed due to lack of power or its erratic supply.

The presence of oxygen in storage containers also lowers culture survival over time (Tripathi and Giri, 2014). Although cultures were kept in closed containers, they were not under complete oxygen-free conditions. Stobińska *et al*. (2017) observed improved viability for *Lactobacillus gasseri* stored in an anaerobic chamber than those stored in the presence of atmospheric oxygen. Otero *et al*. (2007) also observed higher cell viability of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* stored under nitrogen and vacuum compared to those exposed to oxygen. Oxygen favors oxidative reactions and accumulation of free radicals within the cell. Failure to metabolize these radicals or transport them out of the cell results in irreversible cell damage (Otero *et al*. 2007). Therefore, viability of the *L. plantarum* MNC 21 starters could be improved by using vacuum packaging.

Acidification potential of the dried *L. plantarum* MNC 21

Figures 2 and 3 show changes in pH and TA of slurries fermented by dried *L. plantarum* MNC 21 stored in different sorghum flours. The acidification potential (ability to reduce pH ≤4.5) of the cultures reduced with storage time. The ability to reduce pH was measured as the time taken to reduce pH to ≤4.5. This time to reduce pH to ≤4.5 increased with increasing storage time:

4 h (day 0), 24 h (day 15), > 24 h (day 20) to no acidification (days 25-30); meaning that the potential to acidify or reduce pH by *L. plantarum* MNC 21 decreased with storage time. Sorghum variety and malting did not (p>0.05) have an effect on the acidification potential of the dried cultures. The amount of acid produced by the stored cultures decreased with time of storage (Figure 3). The final average TA on day 0 day was 0.3% but dropped to 0.1% on day 20.

The activity of lactic acid bacteria starter cultures can be expressed by its rate of acid production, which is a useful parameter in defining effectiveness of the culture in technological processes. As such, acid production determined by pH and %TA is a highly reliable and reproducible method. The drop in pH of the sorghum malt slurries on day 0 of storage (Figure 2) is in agreement with Byakika et al. (2020) and Mukisa *et al*. (2017). *L. plantarum* metabolizes sugars to lactic acid resulting in a pH decline and a corresponding increase in %TA (Byakika et al. 2020). On day 0 of storage the rapid drop in pH was due to the higher cell numbers in the flours than those present in the subsequent days. The number of microbial cells positively corresponds with amount of sugar metabolized and eventually the amount of acid produced. Therefore, the decline in cell counts (Figure 1), explains the decline in the rate of acid production (Figures 2a and 2b). Failure to have any pH drop and increase in %TA on days 25 and 30 was probably because cell numbers were so low (1 log cfu/g) that 1 g of flour was insufficient to initiate fermentation within 24 h. Although cells dried at 30°C had higher survival than those dried at 25°C, between 10-20 days, their fermentation efficiencies were similar (p>0.05) suggesting that high survival does not always translate into high cellular activity.

It is vital that starter cultures rapidly drop pH to ≤4.5 to consequently inhibit pathogens. In this study, it took approximately 2 weeks for the cell concentration of dried *L. plantarum* MNC 21 cultures to drop below 6 log cfu/g (Figure 1) and for them for be able to reduce the pH to ≤4.5 in less than 24 h (Figure 2a). These observations suggest that *L. plantarum* MNC 21 in air-dried sorghum flours can be stored for 2 weeks at ambient temperature before losing its ability to rapidly acidify products.

CONCLUSION

L. plantarum MNC 21 starter culture can be preserved by convective air-drying at 25°C or 30°C in either malted or un-malted sorghum flour. The dried culture can be effective within two weeks of storage at ambient temperature. Therefore, LAB starters may be preserved by air drying in cereal flours and remain effective for at least two weeks without the need for cold storage. This approach is potentially useful for short term storage and distribution of starter cultures, especially in places where refrigeration might be difficult to achieve due to erratic supply or absence of electricity. Further studies should evaluate the possibilities of using vacuum packaging and other protective agents to extend the shelf stability of the stored cultures.

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Table 1. Effect of microwaving at 600 W for 3 minutes on microbial counts of sorghum flours

Sorghum flour	Counts before microwaving (log cfu/g)			Counts after microwaving (log cfu/g)		
	TPC	TC	YM	TPC	TC	YM
Malted *Eyera*	5.4±0.0	5.0±0.1	1.3±0.1	NG	NG	NG
Un-malted *Eyera*	5.6±0.0	5.1±0.1	1.2±0.1	NG	NG	NG
Malted *Epuripur*	5.6±0.0	5.4±0.0	1.0±0.0	NG	NG	NG
Un-malted *Epuripur*	5.4±0.0	5.3±0.0	1.4±0.0	NG	NG	NG

Values are means ± standard deviations, (n = 2). TPC=Total Plate Count, TC=Total Coliforms, YM=Yeasts and Molds, NG=No growth on the 10^{-1} dilution.

Table 2. Moisture content of sorghum flours

Sorghum flour	% Moisture content of flour after microwaving	% Moisture content of flour + LAB before drying	% Moisture content of flour + LAB dried at 25°C	% Moisture content of flour + LAB dried at 30°C
Malted *Eyera*	$(4.2\pm0.1)^a$	$(48.8\pm0.2)^a$	$(12.9\pm0.3)^a$	$(11.3\pm0.2)^a$
Un-malted *Eyera*	$(4.0\pm0.0)^a$	$(49.1\pm0.5)^a$	$(13.2\pm0.4)^a$	$(11.1\pm0.3)^a$
Malted *Epuripur*	$(3.9\pm0.0)^a$	$(49.3\pm0.3)^a$	$(13.1\pm0.1)^a$	$(11.4\pm0.1)^a$
Un-malted *Epuripur*	$(4.1\pm0.1)^a$	$(48.9\pm0.1)^a$	$(12.7\pm0.1)^a$	$(10.8\pm0.1)^a$

Values are means ± standard deviations. (n = 2 for MC). Values in the same column with same superscripts are not significantly different (p>0.05). Flour moisture before microwaving was 65%

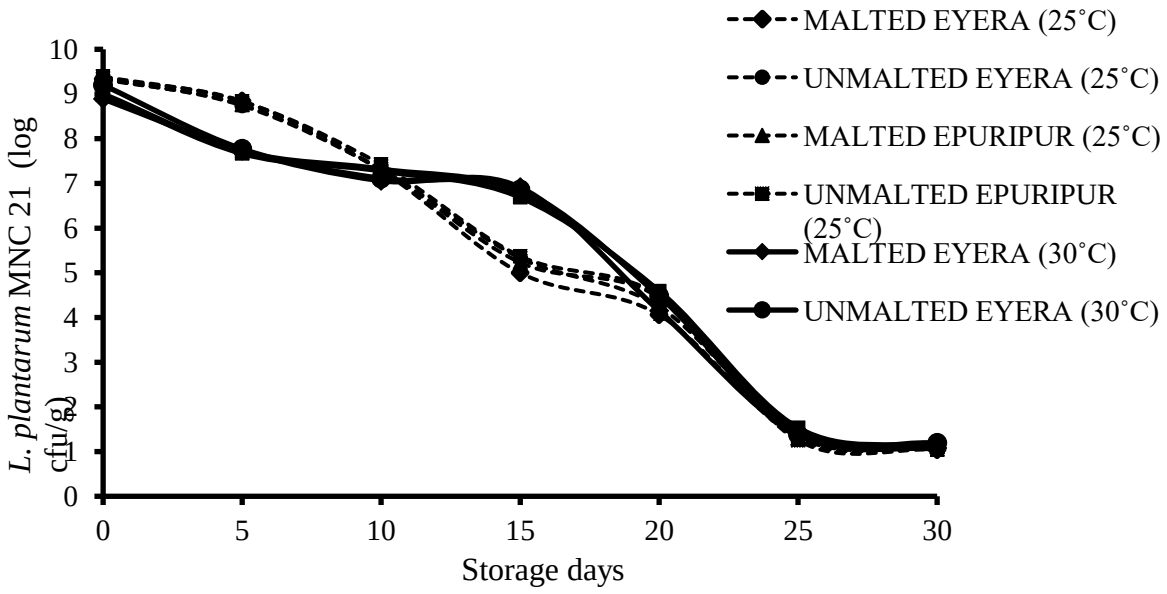

Figure 1. Counts of *L. plantarum* MNC 21dried at 25°C (dotted lines) and 30°C (solid lines) in malted and un-malted sorghum flours stored at 25°C.

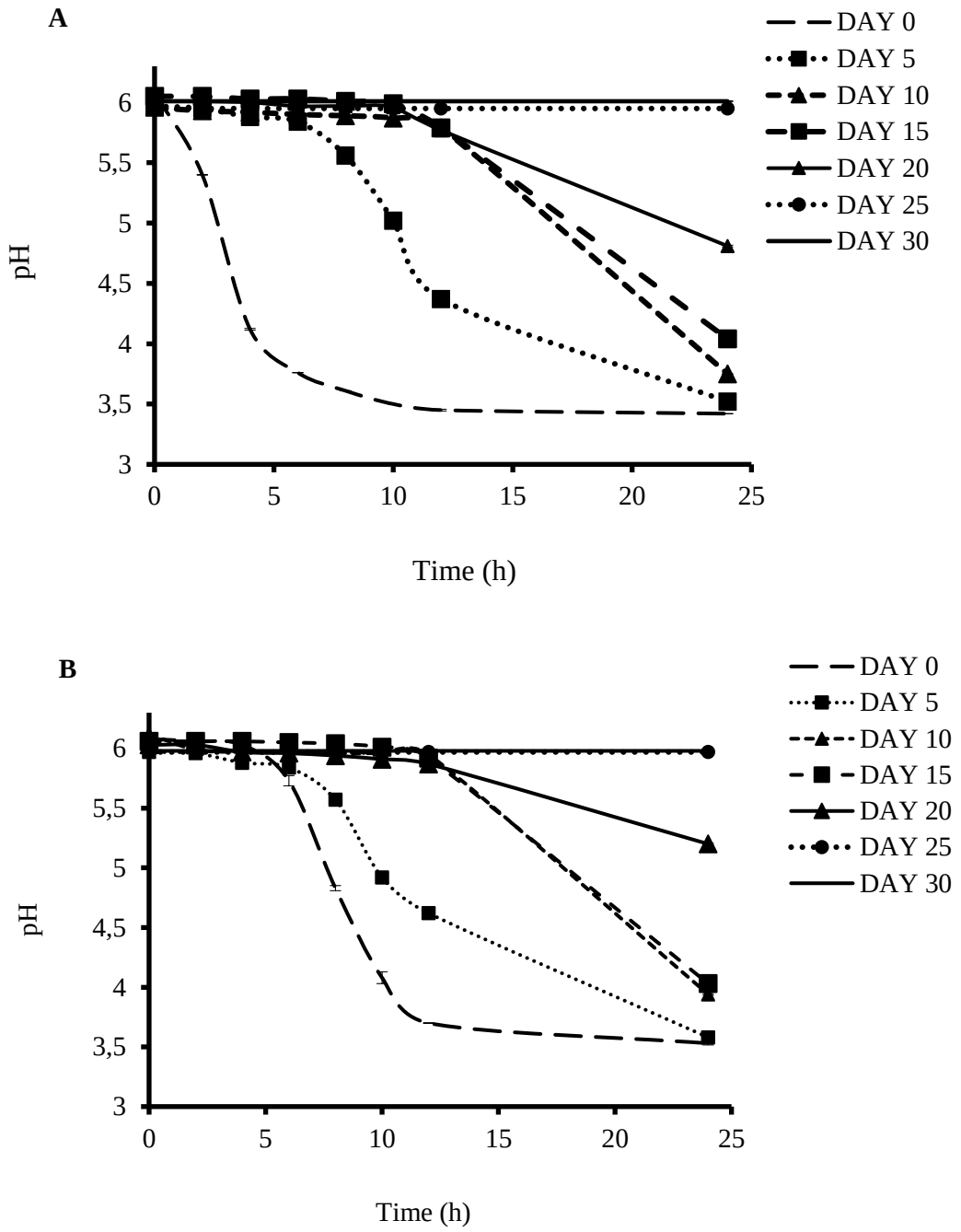

Figure 2. Changes in pH of *Obushera* fermented by *L. plantarum* MNC 21 dried in malted *Eyera* flour at 25°C (A) and 30°C (B), respectively.