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Impact of Storage Temperature on Microbial Diversity and Probiotic Effect of Liquid Brewers' Yeast

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Abstract

Using probiotics as animal feed additives instead of antibiotics is gaining momentum to avert adverse negative effects on human health. Liquid brewers' yeast (LBY) is an industrial by-product containing probiotic microorganisms and is also used as a protein supplement for dairy animals. Nevertheless, value chain actors' lack of appropriate handling practices compromises the by-products' quality and safety. This study aimed to determine the effect of variation in temperature on microbial diversity and probiotic effects during the storage time of LBY sampled from distributors and farmers from Githunguri sub-county of Kenya. The samples were stored at 20˚C, 25˚C and 30˚C, then tested on 0, 5, 10, 15 and 20 days. The study's parameters involved determining the pH levels, lactic acid bacteria (LAB), total coliform count (TCC), mould, and yeast in LBY. The rate (k) of the reaction kinetics model was used to extrapolate the expected probiotic shelf life. The LAB and yeast populations were reduced in a first-order reaction at all storage temperatures. The rate of reduction in the numbers of LAB reduced with an increase in temperature (k = −0.019 and −0.023) at 20˚C and 30˚C, respectively. Yeast's highest rate of growth reduction was 25˚C (k = −0.009) and least at 30˚C (k = −0.043). The minimum effective concentration for probiotics of 10^6 CFU/mL needed to observe the beneficial physiological impact on farm animals was achieved between 34.9 and 35.5 days at the tested storage temperatures. The study provides insight into the unexploited low-cost probiotic potential of LBY in dairy production. Conversely, handling practices and environmental microbial contamination along the value chain can compromise product quality and safety. There is a need to advocate its use in dairy for improved

productivity and sensitize farmers to appropriate hygienic measures along the LBY value chain.

Keywords

Liquid Brewers' Yeast, Microbial Diversity, Probiotics, Shelf Life

1. Introduction

The dairy industry supports smallholder farmers' livelihoods and economies across Africa [1]. The increased human population, urbanization, disposable income, and greater diversity to meet nutritional needs have increased the demand for dairy products [2]. The desire to raise animals in numbers is not feasible due to the subdivision of land and the negative effect of climate change, hence the need to increase productivity per animal. The traditional forage fed to animals cannot provide adequate nutrients to exploit the genetic potential of ruminant animals without supplementation [3] [4]. The deficit has led to using feed additives such as antibiotics, ionophores, and probiotics in animal feeds. However, the growing consumer concern about the antibiotic levels in animal feeds has led to immense scrutiny of the quality and safety of food products in the market [4], as some antibiotic residues can persist through milk processing until the final dairy product is consumed thereby causing severe health complication [5]. Accumulation of antibiotic residues in the body can impair functions of the beneficial gut microbiota with a negative impact on human health [6]. Successful treatment of diseases considered typical under normal circumstances is impeded by developed acquired antimicrobial resistance (AMR) by microbes due to misuse of antibiotics in animal feeds. The effect has led to high morbidity and mortalities and increased financial implications [7]. Moreover, antibiotic residues in milk can alter the activities of probiotic microorganisms used in the dairy industry to initiate fermentation, leading to defects in fermented dairy products [8] and affecting global trade and consumer preference.

Smallholder farmers from developing countries face the challenge of inadequate and low-quality feeds due to insufficient mitigation measures towards the adverse effects of climate change. The consequences in the livestock sector cut across the entire food value chain, from farm production to human consumption [9]. Commercial concentrates that are protein sources, such as soybean meal and fish meal, could have been a solution, but the high cost and access to such concentrates have hampered this option [10]. Moreover, the feeding approach and actual feeds to animals have an impact on the environment, such as the magnitude of greenhouse (GHG) gas emissions [11]. Strategies towards adaptation and mitigation to limit the adverse effects of climate change are inevitable [12]. As a cheap alternative, smallholder farmers around the factories often used waste and by-products from local food industries [13] [14]. Liquid brewers' yeast is a protein-rich material produced by breweries worldwide. It is treated as waste by

most small and mid-sized breweries, meaning this material has the potential for upcycling [15]. Liquid brewers' yeast is usually obtained at the end of beer fermentation by a process known as flocculation. Due to LBY having a high chemical oxygen demand (COD) value of 0.53 kg/hL, it cannot be disposed of into wastewater streams without prior treatment, as it would severely affect the environment. The by-product is mainly utilized in animal feed formulations as a low-cost source of protein [16]. However, the handling and storage conditions of LBY along the supply chain from the producer to the farmer influence its microbial diversity and quality. Minimal information on contamination levels of LBY as a result of handling is available. Equally, the feasibility of its application in commercial animal feed ingredients as a low-cost probiotic and protein source is lacking.

According to Baker *et al.* [17], using LBY as a cheap protein supplementation has positively affected feed quality and utilization. In addition to a nutritional benefit, LBY in ruminant animals has increased milk production and improved digestion [17] [18]. It has also been shown that yeast present in the LBY are probiotic, which improves the gut microflora of ruminant animals, especially in situations where sub-therapeutic antibiotic treatment is frequently used [17] [19] [20]. The primary effect of yeast appears to be stimulating cellulolytic bacteria growth in the rumen and enhancing fibre digestion. Additionally, yeast increases the proportion of lactate-utilizing bacteria, reducing the risk of dysbiosis and producing vitamins and enzymes [17] [21]. Alteration of the ruminal environment and population of microorganisms in the host animal enables the shift of fermentation towards efficient feed utilization for increased productivity and a decrease in negative environmental effects such as greenhouse gas emissions [22]. Also, a high level of α- and β-acids in the yeast in the presence of hops used in the beer fermentation act as antimicrobials, particularly inhibiting hyper ammonia-producing bacteria (HAB). Hyper ammonia-producing bacteria are known to cause significant amino acid degradation, leading to a loss of valuable proteins. Still, the hop acids in the LBY protect these proteins from degradation by HAB [16]. The antimicrobials produced by yeast act against pathogenic microorganisms and spoilage ones, such as mycotoxin-producing mould, thereby preserving LBY and ensuring safety [23].

Feeding animals on fresh LBY could avoid the cost of preservation, such as drying, and ensure quality and safe feed [24]. Value chain actors' lack of storage and conservation facilities, especially in the developing world, is the leading cause of LBY spoilage [25]. It has been proposed that LBY be transported from the brewery to the farms within the shortest period possible to minimize spoilage [26]. According to Alaru [2], LBY value chain actors in this area do not keep the by-product under refrigeration; therefore, storage temperatures are subject to prevailing weather conditions. Liquid brewers' yeast's high polysaccharides, proteins, and moisture make it more prone to microbial development and deterioration [24]. So, preservation should be embraced. Terefe [24] states that environmental conditions, higher moisture, and fermentable sugar in LBY accele-

rate quality deterioration during storage. Conversely, improper handling of LBY hastens mould growth and mycotoxin production, high dry matter losses, unpleasant odour, lowered nutritional value, and reduced feed palatability. Information on the microbial population in LBY is beneficial because it could guide measures to minimize health risks.

This study aimed to predict shelf life based on storage conditions and the viable load of contaminants (coliforms and mould) and potential probiotics (yeast and LAB) in LBY used by dairy farmers from Githunguri sub-county, Kenya. It was hypothesized that the findings of this study would inform appropriate conditions that LBY supply chain actors can use to handle the by-product without interfering with its potential probiotics effect and safety upon supplementation to dairy cows and avert any negative impact on human health on consumption of the derived dairy products.

2. Materials and Methods

2.1. Sampling and Sample Collection

This study was conducted in Githunguri sub-county within Kiambu County, Central Kenya. Githunguri sub-county was selected due to its proximity to East Africa Breweries Limited (EABL). The samples of LBY were purposively taken from selected seven different sources: three distributors (D1, D2, and D3) and four farmers (F1, F2, F3, and F4). The distributors and farmers were systematically selected; each distributor supplied a farmer with LBY. Sampling at the distributors' level was conducted immediately after the supplier delivered the by-product and at the farmer's point on the day of purchase. Sampling was done in 250 mL containers, then immediately cooled to below 10˚C and transported to the laboratory for analysis.

2.2. Sample Storage and Prediction of Shelf Life

Samples obtained from each source were stored at three different temperatures: 20˚C, 25˚C, and 30˚C for 20 days. Besides the initial analysis, the microbial load and pH of the samples were analyzed during the storage period on days 5, 10, 15, and 20. Regression plots between potential probiotics (Yeast and LAB) count and temperature during the storage were used to determine the rate of chemical reactions. It was established from the regression analysis that the growth of yeast and LAB in LBY during storage followed a first-order reaction. From the reactions kinetics equation obtained from the regression plots, the rate constant (k) was then used to extrapolate the expected shelf life of LBY under each storage temperature as follows:

$$\text{Predicted shelf life} = \frac{I_o - 6.00}{Exp(Lnk)}$$

where: I_o = Mean initial yeast/LAB count in LBY, 6.00 = A concentration of viable probiotic cells of at least 10^6 CFU/mL needed to observe a beneficial physio-

logical effect on farm animals [27], and *Lnk* = slope of the first order regression model.

2.3. Microbial Analysis

According to Alaru [2], the microbial load was determined, where 1 mL of each LBY sample was aseptically obtained and introduced in 9 mL of sterile buffered peptone water (Oxoid, UK). The LBY sample was then serially diluted seven-fold. Exactly 1 mL of each dilution was obtained and inoculated on a petri dish in duplicates by pour plating. Microorganisms were cultured as follows:

a) Total Coliform Count (TCC) were obtained by inoculating samples with MacConkey agar (Oxoid, UK). The Petri dishes were left to cool at room temperature, followed by incubation at 37˚C for 48 hours in an inverted manner.

b) Yeast and mould were obtained by inoculating samples with Potato Dextrose Agar (PDA) supplemented with 0.01% chloramphenicol (Oxoid, UK) and incubated at 25˚C for five days.

c) Lactic acid bacteria were enumerated on pour plates of de Man, Rogosa, and Sharpe (MRS) agar (Oxoid Ltd, Basingstoke, Hampshire, England), incubated at 37˚C for 24 hours anaerobically using the Anaerocult A pack (Merck, Darmstadt, Germany).

3. Results

Analysis of variance for the effect of source, storage temperature, and period on LBY microbial load and pH is shown in **Table 1**. The storage period (days) of LBY significantly affected TCC, LAB, yeast, and mould growth, as well as pH ($p \leq 0.001$), whereas the source showed a significant effect on the growth of mould ($p \leq 0.05$) alone. Whereas temperature variations significantly affected mould ($p \leq 0.05$) growth levels, the impact in TCC was observed in the temperature levels, days, and their interactions. The pH was not significantly affected by any interaction of tested factors. Interaction between storage temperature and days significantly affected microbial growth in LBY. In comparison among the factors and their interactions, storage period recorded the highest mean square values for microbial growth and pH. Notably, CV for TCC was the highest at 108.584, followed by mould at 73.718.

The initial microbial load in LBY from different sources is shown in **Table 2**. It was established that LBY from various sources had variations of TCC at $\log_{10}$ 5.42 ± 0.05 to 8.05 ± 0.08 CFU/mL, LAB at $\log_{10}$ 7.21 ± 0.23 to 8.30 ± 0.17 CFU/mL, yeast at $\log_{10}$ 5.57 ± 0.28 to 8.45 ± 0.27 CFU/mL and mould at $\log_{10}$ 0.00 to 8.00 ± 0.07 CFU/mL. A comparison of distributors shows a significant difference in TCC and yeast count from the three sources, with no mould count for D3. A wider variation was exhibited in samples from farmers, but notably, no mould count was recorded on samples from farmer 4. Overall, there was no significant difference in the mean microbial load in LBY samples from distributors and farmers.

Table 1. Mean square values for the effect of source, storage period, and temperature on microbial load and development of pH in liquid brewers' yeast.

SOV	DF	TCC	LAB	Yeast	Mould	pH
Source	1	0.048ns	0.017ns	0.108ns	8.118*	1.156ns
Day (period)	4	89.678***	4.223***	6.992***	45.785***	1.402***
Temp	2	17.207***	0.858ns	0.314ns	11.742*	0.053ns
Source*days	4	0.027ns	0.071ns	0.106ns	5.120ns	0.250ns
Days*temp	6	17.206***	1.268**	1.514***	15.761**	0.003ns
Source*temp	2	1.288ns	0.166ns	0.196ns	2.688ns	0.033ns
Source*days*temp	6	1.208ns	0.159ns	0.148ns	10.191*	0.017ns
Error	65	1.387	0.304	0.240	3.744	0.110
R^2	-	0.849	0.585	0.716	0.620	0.494
CV	-	108.584	8.065	7.227	73.718	7.810

Key: CV = coefficient of variation; DF = degree of freedom; LAB = lactic acid bacteria; R^2 = coefficient of determination; SOV = source of variations; Temp = temperature; TCC = total coliform count; ns = not significant; significant *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

Table 2. Initial microbial load in liquid brewers' yeast from different sources.

		Microbial count in $\log_{10}$ CFU/mL			
Source of LBY		TCC	LAB	Yeast	Mould
Distributors (D)	D1	5.42 ± 0.05^{bc}	$7.71 \pm 0.22b^{c}$	6.44 ± 0.25^{c}	5.00 ± 0.71^{c}
	D2	7.51 ± 0.07^{b}	7.83 ± 0.16^{b}	7.60 ± 0.16^{b}	4.56 ± 0.02^{c}
	D3	8.05 ± 0.06^{a}	7.81 ± 0.21^{b}	8.05 ± 0.18^{a}	0.00 ± 0.00^{d}
Farms (F)	F1	6.50 ± 0.05^{c}	7.84 ± 0.09^{b}	6.37 ± 0.22^{a}	6.23 ± 0.17^{b}
	F2	8.05 ± 0.08^{a}	$7.68 \pm 0.21b^{c}$	8.05 ± 0.22^{a}	8.00 ± 0.07^{a}
	F3	5.99 ± 0.06^{bc}	7.21 ± 0.23^{c}	5.57 ± 0.28^{d}	6.52 ± 0.04^{b}
	F4	8.04 ± 0.04^{a}	8.30 ± 0.17^{a}	8.45 ± 0.27^{a}	0.00 ± 0.00^{d}
Mean	Distributors	6.99 ± 0.80^{a}	7.78 ± 0.04^{a}	$7.36 \pm 0.48a$	3.19 ± 1.60^{a}
	Farmers	7.14 ± 0.53^{a}	7.69 ± 0.18^{a}	7.01 ± 0.62^{a}	5.19 ± 1.77^{a}

Means with different superscripts in the same column indicate statistically significant differences ($p < 0.05$); CFU = colony forming units; LAB = lactic acid bacteria; TCC = total coliform count.

Microbial growth levels and pH development in LBY during the storage period are shown in **Figure 1**. It was found that the microbial load of TCC, LAB, yeast, and mould in LBY decreased during the storage period. There was a drastic decline in TCC load from $\log_{10}$ 7.16 CFU/mL on day 0 to $\log_{10}$ 2.34 CFU/mL on day 5, followed by a further drop to $\log_{10}$ 0.00 CFU/mL on day 10 and a drop

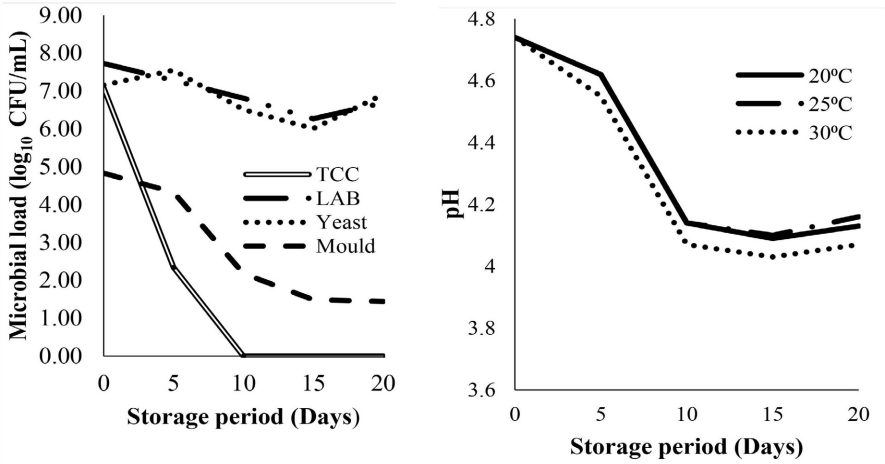

Figure 1. Microbial load in $\log_{10}$ colony forming units per millilitre of liquid brewers' yeast and pH levels at different temperatures during the storage period. LAB = lactic acid bacteria; TCC = total coliform count.

in mould count from initial 4.83 CFU/mL to 4.33 CFU/mL in day five followed by a sharp drop to 2.18 CFU/mL in day ten and a gradual decline to 1.49 CFU/mL and 1.44 CFU/mL were observed in day 15 and 20 respectively. An increase in yeast count from the initial $\log_{10}$ 7.16 CFU/mL to 7.55 CFU/mL on day 5, followed by a gradual drop up to 6.51 to 6.01 CFU/mL on day 10 and 15, respectively, and a final increase to 6.91 CFU/mL in day 20 was recorded. Lactic acid bacteria gradually declined from an initial 7.73 CFU/mL to 6.27 CFU/mL on day 15 and increased to 6.91 CFU/mL on day 20. A slight drop in pH of LBY stored at different temperatures up to day 5, followed by a drastic decrease to day 10, and a minimal increase towards days 15 and 20 was observed.

The microbial load of TCC, LAB, yeast, and mould of LBY at different temperatures during storage is shown in Figure 2. It was observed that TCC in LBY stored at 20˚C drastically dropped from an initial population of 7.08 CFU/mL to $\log_{10}$ 0.00 CFU/mL by day 5, while for LBY stored at 25˚C and 30˚C dropped to $\log_{10}$ 0.00 CFU/mL by day 10. For mould, the count dropped to $\log_{10}$ 0.00 CFU/mL by day 20 for LBY stored at 25˚C and 30˚C, but for LBY stored at 20˚C, mould count dropped to 1.80 CFU/mL on day 20. No significant effect of tested temperature on LAB and yeast was observed.

The regression plot of the probiotic microbial growth rate in LBY at different storage temperatures is shown in Figure 3. As the storage period progressed for LAB, the reduction rate in numbers reduced with an increase in temperature from 20˚C (k = −0.019) to 30˚C (k = −0.023). But for yeast, the highest rate of growth reduction was observed at 25˚C (k = −0.009) and the least at 30˚C (k = −0.043).

The influence of storage temperature on the shelf life of LBY is shown in Figure 4. It was established that the three storage temperatures did not vary significantly on the predicted number of days for LBY to remain with a potential probiotic population of 10^6 CFU/mL. Based on the LAB population, an increase in storage temperature from 20˚C to 30˚C slightly increased the expected time to reach the recommended minimum effective concentration for probiotics from

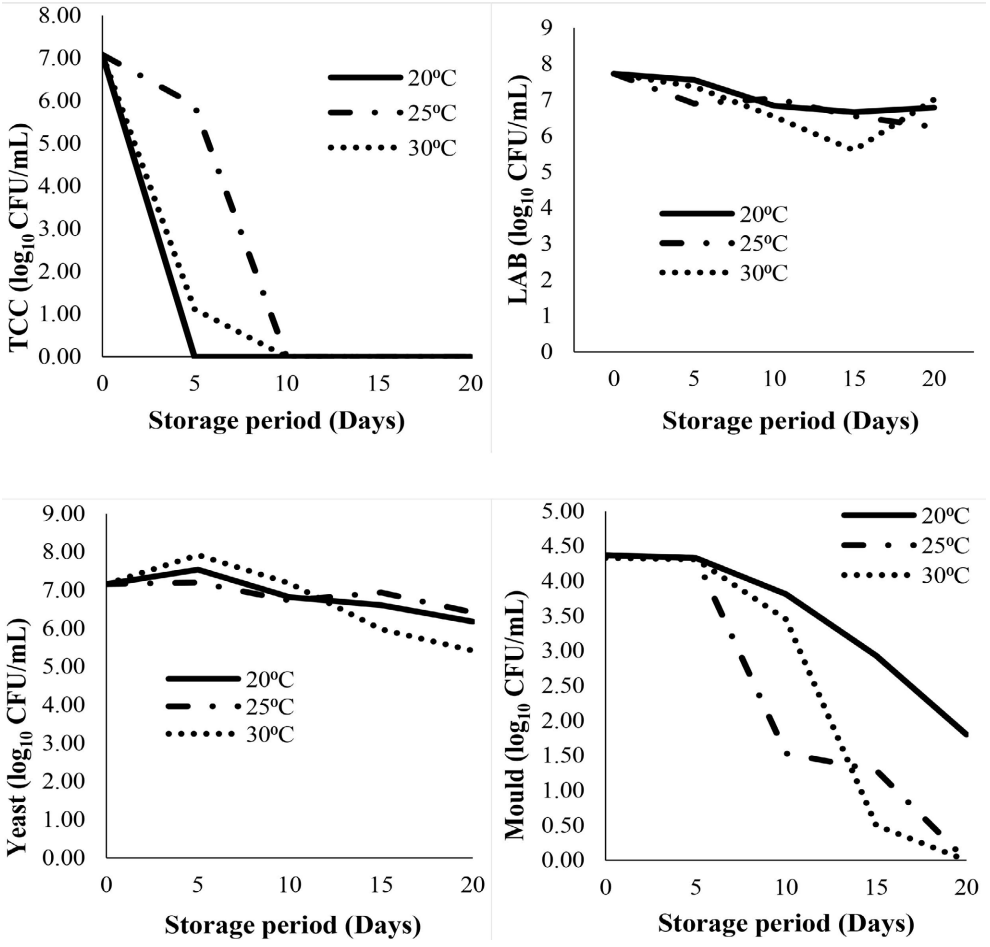

Figure 2. The microbial load of the total coliform count, lactic acid bacteria, yeast, and mould in log_{10} colony forming units per millilitre of liquid brewers' yeast stored at different temperatures. LAB = lactic acid bacteria; TCC = total coliform count.

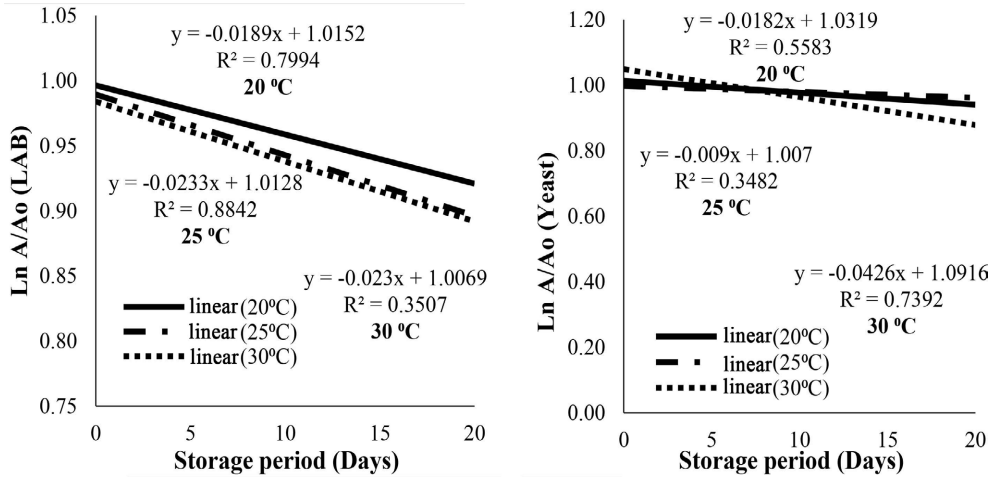

Figure 3. First-order regression plot of lactic acid bacteria and yeast; the potential probiotic microorganisms in liquid brewers' yeast at different storage temperatures. A° = initial concentration; A = concentration after a given duration; LAB = lactic acid bacteria; Ln = natural logarithm; R^2 = coefficient of determination.

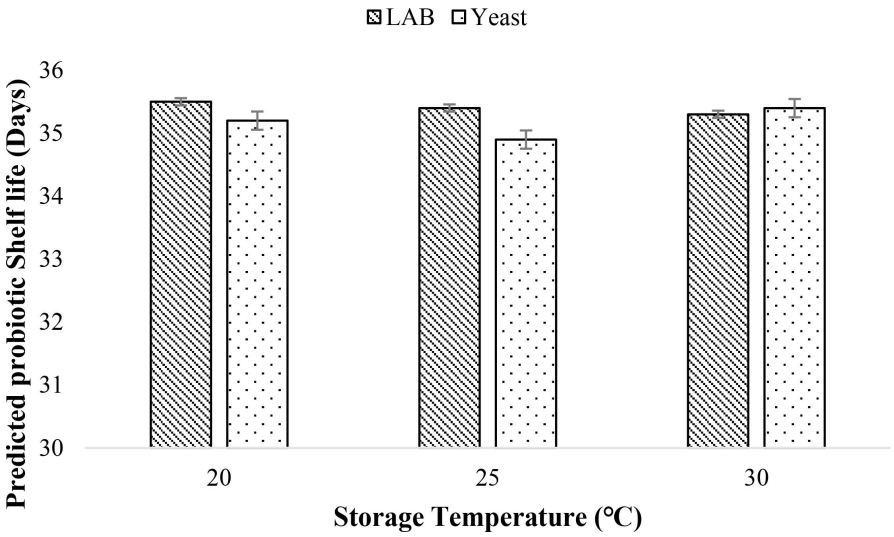

Figure 4. Influence of storage temperature on time required for yeast and lactic acid bacteria population to reach recommended minimum effective concentration for probiotics. LAB = lactic acid bacteria.

35.3 to 35.5 days. But based on yeast population, LBY stored at 25˚C showed an expected time to be 34.9 days, while at 30˚C exhibited an expected time to be 35.4 days.

4. Discussion

The microbial load and pH of LBY were significantly influenced by the days of sample storage ($p \leq 0.001$), as shown in **Table 1**. The level of microbial load shown in **Table 2** indicates variations among the sources where LBY was obtained, which could be attributed to the corresponding degree of contamination along the value chain. Liquid brewers' yeast usually undergo autolysis at 80˚C for 45 sec to 1 minute to destroy viable yeast cells, followed by a viability test at breweries. The autolysis process is repeated if the viability test turns positive [28]. Thus, any viable cell in the by-product points to contamination. The high numbers of TCC, which are hygiene indicators with a coefficient of variation of 108.58%, show very high variability of hygiene standards among the handlers of LBY. The finding ascertains the assumption of contamination happening at the source. A very high coefficient of variation (72.48%) on the presence of mould, potential spoilage microbial for LBY from all sources except D3 and F4, indicates probable environmental contamination. The production of off-flavours and slimy texture by mould in LBY impairs animal intake. Subsequently, there is a risk of contamination of milk with aflatoxin [29]. Thus, the information on the microbial population in LBY is essential as a health precautionary guide on minimizing food and feed safety risk factors [24] [30]. Probable routes of contaminants at the source could be attributed to the handling practices by actors along the value chain, such as equipment used, personnel hygiene, and mixing of the previous stock of LBY and the new one [29]. A prior study by Alaru [2] showed

that storage containers at farms and distribution points typically range from 20 to 2000 and even 10,000-litre plastic containers/tanks rather than stainless-steel tanks. The unhygienic handling of the by-product was verified at some farms where open feeding troughs were used as LBY storage facilities. Cleaning is usually performed by splashing water with or without detergents on the equipment. The procedure is inadequate for cleaning the tanks, especially the plastic ones, which contain 'dead spaces' that cannot be easily accessed during cleaning. Yet, scratches and dents during cleaning act as hideouts for microbes [31]. Again, improper hand washing or failure to use protective gear by the personnel handling LBY are practices that act as risk factors for contamination of the by-product at the farm. Hence, handling and the environment contributed to the variation in microbial load in the sampled sources. Appropriate measures must be in place to prevent any negative effects on quality and safe use. Liquid brewers' yeast contains low dry matter (8% - 10%) and a high crude protein (CP) level (50%), whereas soya bean meal has dry matter (DM) of 85% - 90% and 40% - 50% CP. Cognisant of these facts, a deliberate effort must be made to utilize it as a low-cost protein to replace soya bean meal in feed formulation [28], simultaneously providing probiotic benefits. However, the high moisture content and nutritional composition, especially the proteins and polysaccharides, promote the development of microorganisms and accelerate the deterioration of the by-product [24]. The feed companies can establish a mechanism for utilization in liquid form within one week from the delivery date [2]. To save on transport costs, such companies can be within a 60 Km radius of the breweries [28].

During storage, as indicated in **Figure 1**, coliforms were reduced to zero. In contrast, mould was decreased by about three-fold with a corresponding reduction in pH, while LAB and yeast reduction were insignificant. The findings indicate the possibility of LAB and yeast-producing metabolites (acidity and antimicrobial compounds) that significantly alter the environment and become unfavourable to coliforms and mould, which are probable pathogens and spoilage microorganisms in LBY [32]. The LAB can produce organic acids like acetic, formic, propionic, and lactic acids, which are suggested for use in the food preservation approach [33]. The antagonistic and symbiotic ability exhibited by fungi that produce fungal secondary metabolites (FSM) enable stimulation of survival and reproduction and deter the growth of microorganisms that compete with them for nutrients under natural conditions [34]. Also, the findings signify a probable mutual synergistic relationship between yeast and LAB that can be beneficial as probiotics in animal nutrition. The pH declined, stabilized, and increased slightly towards day 20. The findings correspond with those of other researchers who attributed such a shift in pH to the consumption of organic acids (lactic and acetic) by yeast present in the microbiota of such by-products [27]. The pH is an essential parameter in LBY preservation. Its range was between 4.03 and 4.74, which is high enough to inhibit the growth of most spoilage microorganisms. Still, it is crucial to note that reduced pH levels produce an undesirable effect on crude protein and the metabolizable energy of the by-product

[35]. Conversely, the higher mean square values for storage time on microbial count and pH than any other factors demonstrate that time is the most critical parameter for measuring both spoilage and probiotic shelf life prediction of LBY.

However, as indicated in **Figure 2**, storage temperature did not affect LAB and yeast but varied in effect on TCC and mould. The result could imply that storage temperature influences the rate of destruction of pathogenic and spoilage microorganisms in LBY. The effect could be attributed to the rate at which yeast and LAB produce antimicrobial compounds in LBY that make the environment unfavourable for survival and the multiplication of other microorganisms. Sun *et al.* [23] indicated that fermented feed such as LBY limits the growth of pathogens such as *E. coli* due to LAB production of organic acids and other compounds. Hence, the presence of more counts of LAB and yeast in LBY prequalifies it as a potential probiotic feed that could offer different benefits to the animal besides protein supplementation when used [15] [16] [17] [36].

Reaction kinetics analysis showed that the population decline of LAB and yeast in the LBY during storage is in a first-order reaction, as shown in **Figure 3**. For LAB, the higher the storage temperature, the slower the population decline rate as storage time progressed. On the contrary, LBY stored at 25˚C showed the highest rate of population decline for yeast (k = −0.009), while 30˚C had the least (k = 0.043). The lag phase growth of yeast is preceded by exponential growth, use of the available oxygen, and anaerobic conditions is developed. If the utilization of fermentable wort sugars and assimilable nutrients is enhanced, the availability of carbon and nutrients will be limited, and ethanol concentration will increase [37]. This observation showed that the rate of population decline reduces when the by-product is stored at near optimum growth temperature. The optimum growth temperature for LAB is 37˚C. For yeast, it is 25˚C, indicating that the by-product could easily be handled at favourable growth temperatures for yeast in diverse agroecological zones of Kenya. However, these different rates of population decline did not significantly differ in the predicted number of days for preserving LBY, as indicated in **Figure 4**. Irrespective of using either yeast count or LAB, the effective probiotic shelf life was predicted to be between 34.9 and 35.5 days. A study by Kamphayae *et al.* [14] demonstrated that a preservation period of LBY of up to four weeks could assure superior fermentation quality. As much as these predicted days guarantee that probiotic microbes will be above the effective concentration of 10^6 CFU/mL, the challenge could be the presence of mould that survived for a long time, as shown in **Figure 2**. These could contribute to mycotoxin formation during storage and spoilage of LBY by forming a slimy texture [29]. A strategic effort must be in place by all actors along the supply chain to reduce any forms of environmental contamination of the by-product as much as practically possible.

5. Conclusion and Recommendation

The study established that the minimum effective concentration for probiotics of

10^6 CFU/mL needed to observe beneficial physiological effects on farm animals is between 34.9 and 35.5 days at the tested storage temperatures. The findings further prove that LBY can be used as a source of low-cost probiotics in dairy production. Nonetheless, inappropriate handling and environmental microbial contamination along the value chain can compromise product quality and safety. There is a need to advocate for LBY use in dairy production as a source of probiotics and protein supplements and sensitize farmers on appropriate hygienic measures along the LBY value chain for improved product quality and safety.

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Conflicts of Interest

The authors declare no conflicts of interest whatsoever regarding the publication of this paper.

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