



## **The Effect of Using Biological Treatment on Microbial Growth during the Malting of Sorghum**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JAMB/2021/v21i1130405

#### Editor(s):

(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

#### Reviewers:

(1) Mohamed Helal Elsayed, Northern Border University, Saudi Arabia.

(2) Wedean Ghassan Ali Al Hadban, University of Baghdad, Iraq.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/75855>

**Original Research Article**

**Received 13 August 2021**  
**Accepted 23 October 2021**  
**Published 12 November 2021**

### **ABSTRACT**

Malting is an important industrial product with a huge market outlet. Sorghum grain carries a numerous and variable, microbial population that mainly consists of bacteria, yeasts, and filamentous fungi. Sorghum malt is heavily reliant on chemical control of moulds and coliforms. This research aimed at investigating ways of improving malt quality and safety, using starter cultures of lactic acid bacteria and yeast, during the steeping stage of malting. All the steep treatments contained a sizeable population of moulds, greater than 4logcfu/mL, at 0hrs of steeping. A 3Log decrease was recorded in the steep treatment containing only single culture of *Lactobacillus plantarum*. All the steeping treatments achieved varying levels of anti-nutrient reduction. The *Lactobacillus plantarum* CLB8 steep reduced the phytate level by as much as 47% when compared to the phytate level in sorghum grain. The combined cultures of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1 reduced the phytate content by as much as 40% when compared to the sorghum grain without treatment. When compared to the control steep, the *Lactobacillus plantarum* CLB8 steep improved the anti-nutrient degradation by 31%. The combined cultures of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1 reduced the phytate content by as much as 23% when compared with the control steep. The polyphenol content was reduced

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by about 46% in the *Lactobacillus plantarum* CLB8 steep and 29% in the combined cultures of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1 steep when compared to the polyphenol content in the whole sorghum grain. Only the *Lactobacillus plantarum* CLB8 steep had better polyphenol reduction than the control with a 9.6% reduction more than the control. It was concluded that lactic acid bacteria can be apply as a biological control organism in malting of grains.

**Keywords:** Sorghum malting; mould; lactic acid bacteria; yeast; biocontrol.

## 1. INTRODUCTION

Sorghum malt is used extensively in many African countries to produce local 'opaque' beer, although it is also used in modern breweries as adjuncts to produce 'Lager' beer. It's generally known that naked cereal grains such as sorghum and millet used in beer production are not protected by the presence of husks and are prone to mycotoxins contamination due to the presence of toxinogenic moulds [1]. Most producers of the local sorghum opaque beer are artisans who rely on the traditional floor malting technique to produce sorghum malt. Such a process stimulates the proliferation of microorganisms especially coliforms and mould. Such proliferation of microorganisms can affect the quality and safety of the malt for consumers. The proliferation of fungi is of greater concern because of their ability to produce mycotoxins [2]. Some grain colonizing microbes have positive effects during malting, in the sense that they can improve seed germination and hence the process ability of the malt, but the presence of others during malting is rather disadvantageous (production of mycotoxins, reduction of the filtration rate, premature yeast flocculation, etc. [3]. Hence the need to make a choice among the members of the microbial ecosystem of the grain. Naturally occurring moulds grow easily on sorghum grains during malting or high moisture storage conditions which are the main stage of African opaque beer process production. The growth of moulds such as *Aspergillus flavus*, *Penicillium parasiticus*, *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium roseum* and *Fusarium moniliforme* on grains or during malting are known to elaborate aflatoxins, trichothecenes, fumonisins, ochratoxin A and zearalenones, among other mycotoxins [4]. Most traditional malting process does not have any mechanism for controlling the microbial load during malting. Although in the industrial brewing sector a variety of chemical treatments are used. The use of these chemicals could lead to the accumulation of chemical residues on the products, greatly affecting the consumer's health

and the environment [5]. For the traditional producers, accumulation of toxins would negatively impact on the health of its users, its use as a weaning food and for local beer brewing [2]. The potential for using microorganisms to detoxify mycotoxins has been reported to be promising [6]. The continued use of chemical compounds for treatment has also been documented to select for resistance in bacteria and fungi [7]. Malt is a food product and the addition of chemical additives to enhance germination should be discouraged. Microorganisms indigenous to sorghum may be exploited and isolated as starter cultures to augment the hydrolysis process, thus giving us an attractive alternative to the use of chemicals. Hence, because of the high microbial load associated with the traditional malting of sorghum, a safe method of biological control needs to be developed. This safe method can also be used as an alternative to the use of chemicals in the industrial malting of sorghum. The maltster has several ways to combat undesirable microorganisms; carefully selected microbial cultures can be used as starters during soaking to modify the malting microbial ecosystem dynamic [8].

*Lactobacillus* species are known to be fastidious organisms and have been applied to improve the microbiological stability, quality and safety of silage and sorghum used in malting [9]. *Saccharomyces* spp. and *P. pentosaceus* cultures have also been tested by Lefyedi [2] while Bwanganga et al. [10] used *Bacillus subtilis* successfully for biocontrol treatment. Success with Biological procedure to reduce the amount of mould and coliform in malting will lead to production of high quality malt with reduced consumer's exposure to chemicals and mycotoxins as well as better economic accrual to the producer. substitute that is capable of reducing the moulds and detoxifying their metabolites.

This research aimed at investigating ways of improving malt quality and safety, using starter

cultures of lactic acid bacteria and *Saccharomyces cerevisiae*, during the steeping stage of malting.

## 2. METHODOLOGY

### 2.1 Collection of Microorganisms

Cultures of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1 were obtained from microbiological laboratory, University of Ibadan, Oyo State.

### 2.2 Confirmation of Isolates

Lactic acid bacteria were subcultured into a selective medium, de man, Rogosa and Shape (MRS) agar. The isolates were confirmed base on their morphology and cultural characteristics. Further confirmation was made using catalase test.

The yeasts were subcultured in Sabouraud Dextrose Agar (SDA) supplemented with 40 mg/L Chloramphenicol for selective confirmation of yeast after which Microscopic examination of the isolate will be carried out using wet mount method according to Thais and Danilo [11].

### 2.3 Laboratory Scale Malting Process

Laboratory scale malting process was carried out according to a modified method of Lefyedi [12]. Samples (150 g) of the two sorghum cultivars were rinsed and then steeped at 25°C for 48hr in 300 ml of sterile distilled water. The test cultures, LAB and Yeast cells excluding cells and spent media were, added into the 300 mL steeping water to make a final concentration of cultures of about  $10^7$ – $10^8$  cfu/mL. Mixed cultures were also investigated with equal proportions of the LAB and Yeast cultures prepared in the ratio 50:50, to make up the  $10^7$ – $10^8$  cfu/mL of the mixed cultures in the steep water. The un-inoculated grain was similarly steeped at 25°C for 48 hr. After steeping, the grain was rinsed and then germinated.

### 2.4 Physico-Chemical and Nutritional Analyses of the Steeped Sorghum pH Determination

The pH of the fermenting substrates was measured daily with the electrode of a pH metre standardized with the appropriate buffer.

### 2.4.1 Total titratable acidity determination

The amounts of the lactic acid produced in the steep water were determined at set intervals by the standard titration procedure for total titratable acidity (TTA) according to Association of Official Analytical Collaboration (A.O.A.C) [13]. Lactic acid content determination was done by titrating 25mL of the supernatant fluid of the substrates on addition of 3drops phenolphthalein as indicator, 0.1M Sodium hydroxide (NaOH) was slowly added from a burette into the samples until a pink colour appeared. Each ml of 0.1M NaOH is equivalent to 90.08mg of lactic acid.

Determination of tannins, phytate were done according to Markkar et al. [14,15]. While determination of moisture content, ash content, Estimation of crude protein, Carbohydrate determination and total extracts (crude fat) determination were done according to A.O.A.C. [13].

### 2.5 Statistical Analysis

All analyses were conducted in triplicates. Mean scores of some of the results and their standard deviation were reported. Data were subjected to analysis of variances, and Duncan multiple range tests was used to separate the Means using SPSS version 20.

## 3. RESULTS AND DISCUSSION

Overall there was significant increase in lactic acid count in all sorghum treatments within 24 hours (Fig. 1). This agrees with the report of O'Sullivan [16]. The most significant microbiological change that occurs during the malting process is the increase in the lactic acid bacteria count in comparison to the changes that occur to the microbial counts of *Pseudomonads*, coliforms and fungi [17].

The enteric bacteria population of only the steep treatments initially increased within the first 8hours by about 2logcfu/mL (Fig. 2). This can be attributed to an increase in moisture content of the bacteria environment in the steep. The increase in moisture content coupled with the release of nutrients from the grain made the environment conducive for bacteria proliferation [2]. A decline in enteric bacteria population was noticed after 8hours of steeping. This was more noticeable in the steep treatments containing only LAB culture and the treatment containing both LAB and Yeast in combination. The

reduction in bacterial population can be attributed to antagonistic action of LAB. The production of antimicrobial substances like lactic acid leads to a reduction in the pH of the steep water which makes the medium less conducive for microbial growth. LAB are well known to produce antimicrobial substances like H<sub>2</sub>O<sub>2</sub>, diacetyl, lactic acid and organic acids which are inhibitory to the growth of some pathogens.

Of all the biocontrol treatments tested in this study, only the *Lactobacillus plantarum* steep reduced the total mould count by about 3Log CFU/mL, from an initial 4.88 Log CFU/mL at 0hr to below 1.5 Log CFU/mL (Fig. 3). This is below the limit of 3 Log CFU g/L recommended by the Association Française de Normalisation [18].

Titrateable acidity was determined analytically by titration and is not the same as total acidity. Titrateable acidity is a measure of the hydrogen ions required to obtain a specific end point and is always lower than total acidity [19]. There is no direct relationship between titrateable acidity and pH due to variations in buffer capacity, however higher acid levels are usually associated with lower pH values and vice-versa [20]. In this study, an increase in total titrateable acidity was followed by a corresponding decrease in pH. (Fig. 5 and Fig. 6) LAB are generally known to produce lactic acid during fermentation. They are even classified as heterofermentative or homofermentative based on their lactic acid production pattern. This explains the drop in pH in the steep treatments. The treatment with LAB starter had a faster drop in pH than those without LAB as a starter.

There was a general increase in total aerobic count in all steep treatments over 48 hours. This is as a result of increased moisture level in the grain and the mobilization of endogenous grain nutrients (Table 1). The moisture level and the availability of nutrients from the grain can lead to proliferation of microbes [21]. The high microbial load and the possible presence of mycotoxins in sorghum malt implies that sorghum malt is not a very safe product to be used for beer brewing and for the preparation of weaning foods without adequate treatment.

All the steep treatments contained a sizeable population of moulds, greater than 4logcfu/mL, at 0hrs of steeping. A 3log decrease was recorded in the steep treatment containing only single culture of *Lactobacillus plantarum*. Laitila et al.

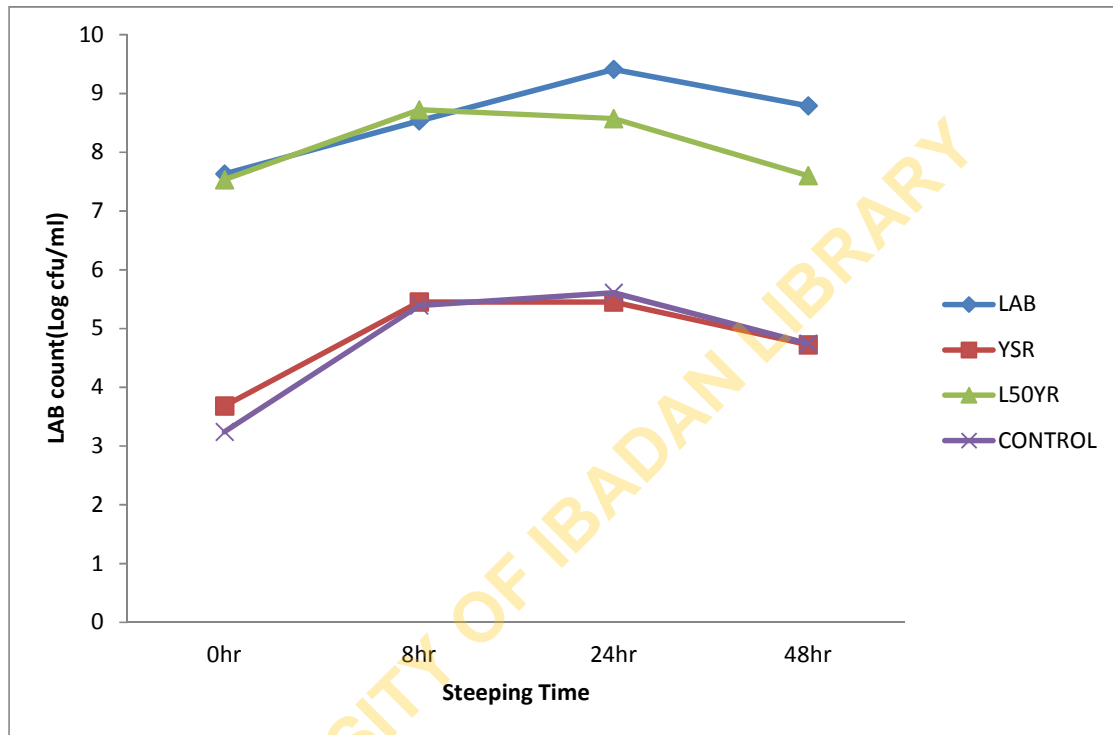
[22] and Lavermicocca et al. [23] suggested that the antifungal activity of *L. plantarum* could be the results of many organic acids such as lactic, acetic and phenyllactic acids. *L. plantarum* and *L. sanfrancisco* produce special organic acids (3-phenyl-L-lactic acid and caproic acid, respectively) that have antifungal properties [24, 25,26]. Yeasts have also been reported to produce organic acids that have inhibitory effect on some microorganisms. This could be responsible for the marked decrease in mould population between 24h and 48 h with the treatment containing *Saccharomyces cerevisiae* alone. Strains of *S. cerevisiae* that have been isolated from indigenous fermented foods and beverages and thereafter been successfully used as starter cultures are Ghanaian fermented maize dough for 'kenkey' and 'koko' production [27], Ghanaian fermented sorghum beer, Zambian 'munkoyo' maize beverage and Nigerian 'ogi' based on maize [28]. Masoud and Kalltoft [29] reported *in-vitro* inhibition of Ochratoxin A production from *Aspergillus ochraceus* by three yeasts (*Pichia anomala*, *P. kluyveri* and *Hanseniaspora uvarum*). Fungal strains of *Trichoderma* have also been demonstrated to control pathogenic fungi through mechanisms such as competition for nutrients and space, fungistasis, antibiosis, rhizosphere modification, and myco-parasitism [30].

All the steeping treatments achieved varying levels of anti-nutrient reduction (Table: 2). The *Lactobacillus plantarum* CLB8 steep reduced the phytate level by as much as 47% when compared to the phytate level in sorghum grain. The combined cultures of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1 reduced the phytate content by as much as 40% when compared to the sorghum grain without treatment. When compared to the control steep, the *Lactobacillus plantarum*CLB8 steep improved the anti-nutrient degradation by 31%. The combined cultures of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1 reduced the phytate content by as much as 23% when compared with the control steep. Lactic fermentation has also been shown to reduce the phytate content of sorghum and some non-tannin containing cereals [31]. The polyphenol content was reduced by about 46% in the *Lactobacillus plantarum* CLB8 steep and 29% in the combined cultures of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1 steep when compared to the polyphenol content in the whole sorghum grain. Only the *Lactobacillus plantarum* CLB8 steep had better

polyphenol reduction than the control with a 9.6% reduction more than the control.

These antinutritional factors, coupled with the lysine, tryptophan and methionine deficiencies in cereal proteins contribute to malnutrition in developing countries [32]. Fermentation may serve to improve the nutritional value of cereal

staples through the reduction of antinutritive factors [33]. Fermentation also provides optimum pH conditions for enzymatic degradation of phytate which is present in cereals in the form of complexes with polyvalent cations such as iron, zinc, calcium, magnesium and proteins. Such a reduction in phytate may increase the amount of soluble iron, zinc and calcium several fold [34].



**Fig. 1. Profile of LAB Count during steeping of experimental and control sorghum in water for 48h**

Key: Control = Sorghum steep without microbial culture

LSR = Sorghum steep with *Lactobacillus plantarum* CLB8

L50YR = Sorghum steep with equal portions of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1

YSR = Sorghum steep with *Saccharomyces cerevisiae* CYT1

**Table 1. Proximate composition of treated Sorghum subjected to steeping for malt production**

	Moisture	Crude fat	Crude fibre	Crude fibre	Ash	CHO
Control	38.56±0.05 <sup>b</sup>	12.23±0.05 <sup>d</sup>	3.59±0.02 <sup>d</sup>	1.03±0.01 <sup>a</sup>	0.91±0.02 <sup>a</sup>	75.39 ±0.11 <sup>a</sup>
YSR	37.29±0.04 <sup>a</sup>	10.99±0.08 <sup>b</sup>	3.29±0.03 <sup>c</sup>	1.14±0.03 <sup>b</sup>	1.01±0.02 <sup>b</sup>	76.34±0.10 <sup>b</sup>
LSR	38.87±0.02 <sup>c</sup>	11.41±0.05 <sup>c</sup>	2.89±0.04 <sup>a</sup>	1.24±0.02 <sup>c</sup>	1.18±0.01 <sup>c</sup>	77.07±0.08 <sup>c</sup>
L50YR	39.30±0.03 <sup>d</sup>	11.35±0.05 <sup>c</sup>	3.26±0.03 <sup>c</sup>	1.74±0.05 <sup>d</sup>	1.28±0.02 <sup>d</sup>	75.4 ±0.01 <sup>a</sup>
CFW	44.41±0.05 <sup>e</sup>	10.80±0.13 <sup>a</sup>	2.99±0.02 <sup>d</sup>	1.78±0.03 <sup>d</sup>	2.70±0.04 <sup>e</sup>	77.19±0.13 <sup>c</sup>

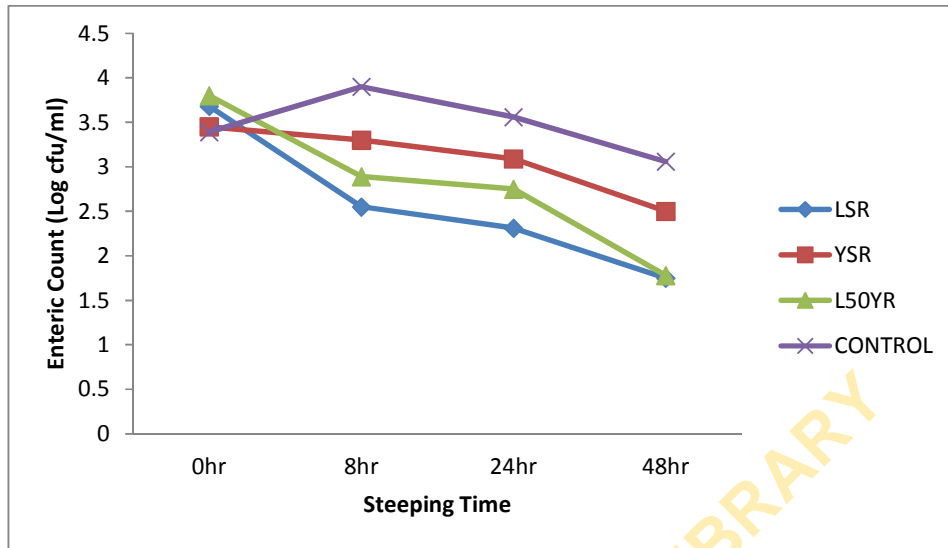
Mean values in the same column followed with different lower case letters are statistically significantly different at  $p < 0.05$

Key: Control = Sorghum steep without microbial culture

LSR = Sorghum steep with *Lactobacillus plantarum* CLB8

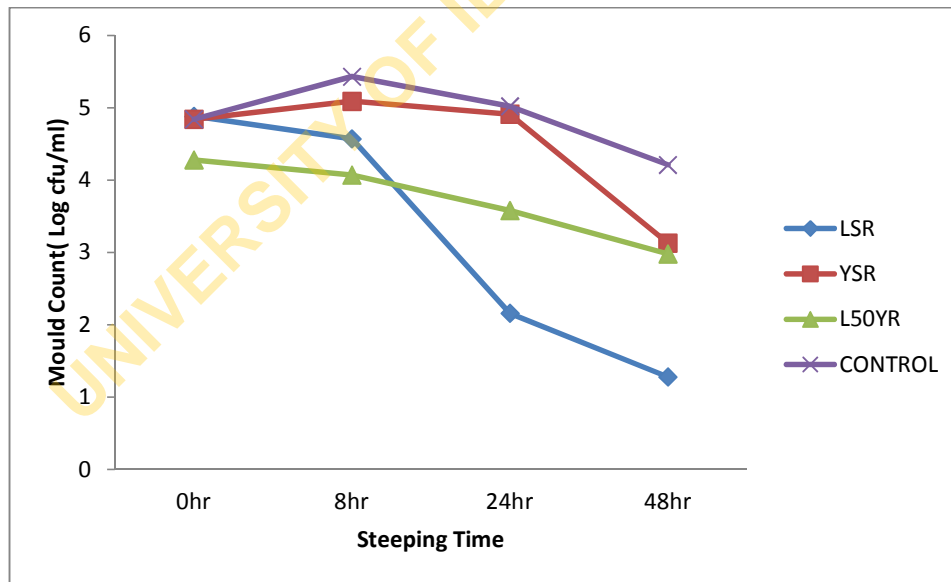
L50YR = Sorghum steep with equal portions of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1

YSR = Sorghum steep with *Saccharomyces cerevisiae* CYT1



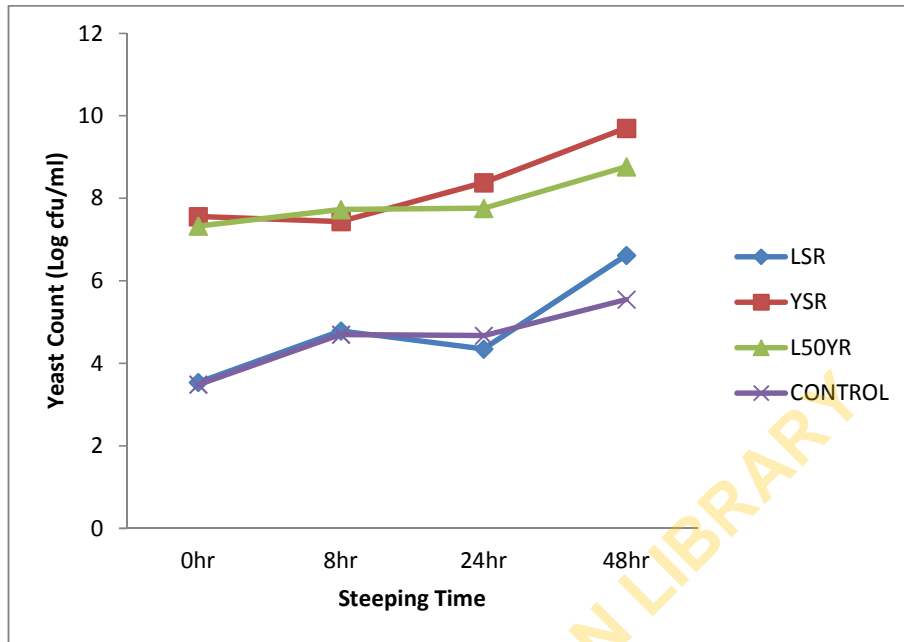
**Fig. 2. Profile of Enteric Bacteria count during steeping of experimental and control sorghum in water for 48h**

Key: Control = Sorghum steep without microbial culture  
 LSR = Sorghum steep with *Lactobacillus plantarum* CLB8  
 L50YR = Sorghum steep with equal portions of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1  
 YSR = Sorghum steep with *Saccharomyces cerevisiae* CYT1



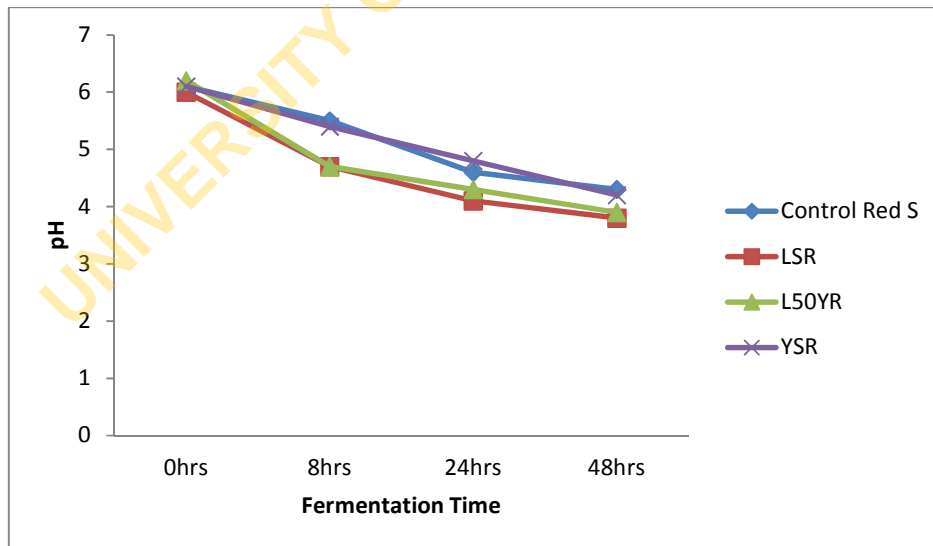
**Fig. 3. Profile of Mould Count during steeping of experimental and control sorghum in water for 48hrs**

Key: Control = Sorghum steep without microbial culture  
 LSR = Sorghum steep with *Lactobacillus plantarum* CLB8  
 L50YR = Sorghum steep with equal portions of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1  
 YSR = Sorghum steep with *Saccharomyces cerevisiae* CYT1



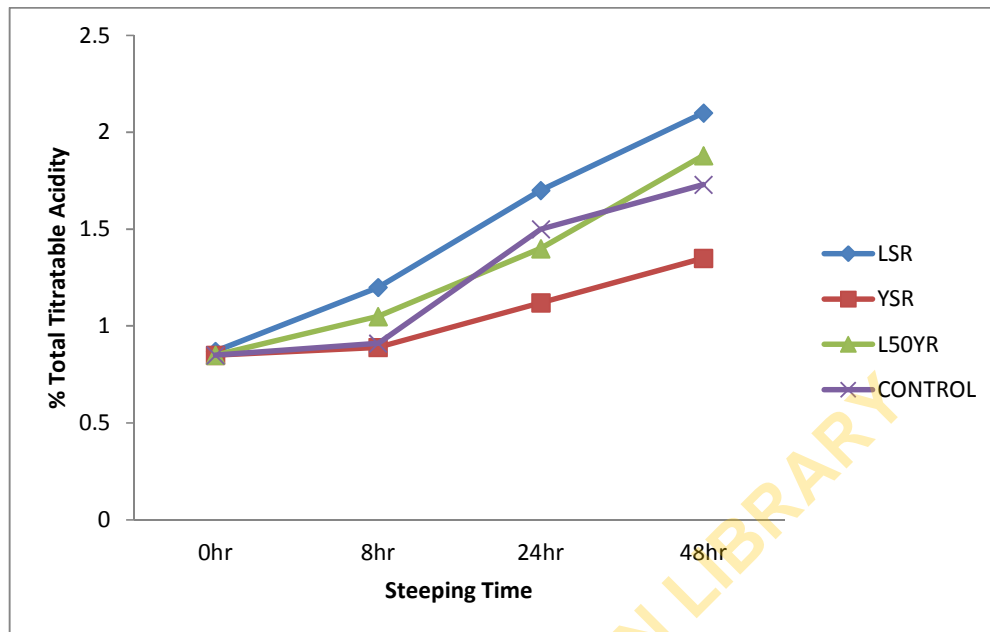
**Fig. 4. Profile of Yeast Count during steeping of experimental and control sorghum in water for 48h**

Key: Control = Sorghum steep without microbial culture  
 LSR = Sorghum steep with *Lactobacillus plantarum* CLB8  
 L50YR = Sorghum steep with equal portions of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1  
 YSR = Sorghum steep with *Saccharomyces cerevisiae* CYT1



**Fig. 5. Changes in pH during steeping of experimental and control sorghum in water for 48h**

Key: Control Red S = Sorghum steep without microbial culture  
 LSR = Sorghum steep with *Lactobacillus plantarum* CLB8  
 L50YR = Sorghum steep with equal portions of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1  
 YSR = Sorghum steep with *Saccharomyces cerevisiae* CYT1



**Fig. 6. Changes in Total Titratable Acidity during steeping of experimental and control Sorghum in water for 48h**

Key: Control = Sorghum steep without microbial culture

LSR = Sorghum steep with *Lactobacillus plantarum* CLB8

L50YR = Sorghum steep with equal portions of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1

YSR = Sorghum steep with *Saccharomyces cerevisiae* CYT1

**Table 2. Anti-nutritional factors concentration in treated and untreated sorghum during steeping**

	Polyphenol	Phytate
CWT (grain)	0.87±0.01 <sup>e</sup>	1.58±0.02 <sup>e</sup>
YSR	0.71±0.15 <sup>d</sup>	1.36±0.01 <sup>d</sup>
CONTROL	0.52±0.01 <sup>b</sup>	1.22±0.01 <sup>c</sup>
L50YR	0.62±0.01 <sup>c</sup>	0.94±0.02 <sup>b</sup>
LSR	0.47±0.15 <sup>a</sup>	0.84±0.01 <sup>a</sup>

Mean values in the same column followed with different lower case letters are statistically significantly different at  $p < 0.05$

Key: Control = Sorghum steep without microbial culture

LSR = Sorghum steep with *Lactobacillus plantarum* CLB8

L50YR = Sorghum steep with equal portions of *Lactobacillus plantarum* CLB8 and *Saccharomyces cerevisiae* CYT1

YSR = Sorghum steep with *Saccharomyces cerevisiae* CYT1

#### 4. CONCLUSION

In conclusion, the high demand by consumers for foods and drinks free of chemical preservatives

has led to increasing amounts of research to provide alternatives to these chemicals. LAB provides technologically practicable alternatives for the replacement of chemical preservatives. The option of selecting LAB as starter cultures or co-cultures in fermentation processes can improve the desired properties of malt, at the same time providing consumers with new chemical-free malt products

#### 5. RECOMMENDATION

Based on the results of this research, it is hereby recommended that biological control of moulds and enteric bacteria using cultures of lactic acid bacteria should be adopted during the steeping stage of malting. Therefore, optimisation of the process conditions to achieve improved results and possibly to develop genetically modified yeasts that can degrade the toxicity of mycotoxins should be conducted.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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