PROFILING OF FUNCTIONAL PROPERTIES OF *Lactiplantibacillus plantarum* ISOLATE AND ITS APPLICATION IN FERMENTATION OF FINGER MILLET-CASSAVA COMPLEMENTARY PORRIDGE

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DECLARATION AND RECOMMENDATION

Declaration

This research thesis is my original work and has not, wholly or in part, been presented for the award of a degree in any other university.

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Recommendation

This research thesis has been submitted with our recommendation as the official university supervisors.

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DEDICATION

I dedicate my work to my son; Gabriel Mumo and mother; Winfred Mbithe.

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ABSTRACT

In Kenya, children fed on high-energy foods, resulted in high levels of malnutrition, although they are rich in micronutrients. Controlled fermentation and malting are some of the processes that used to improve the nutritional and safety properties of food. This study aimed at developing a complementary food product with probiotic benefits and improved nutritional value. A *Lactiplantibacillus plantarum* strain that was previously isolated from spontaneously fermented milk analyzed for its probiotic properties, safety profile, and its application for product development. The strain was tested for temperature sensitivity pH tolerance and 0.4% phenol tolerance to observe its survival in the gastrointestinal tract of humans. For safety evaluation of the isolate, antagonistic activity against pathogenic strains, antibiotic susceptibility pattern examined using common antibiotics and hemolytic activity done using lamb blood agar. To test the isolate for product development, a blend of U15 variety of finger millet flour and a sweet variety of cassava (KME-2 variety) flour were used as ingredients. Finger millet was malted for 0 days, 1 day, and 2 days. The flours were mixed in three ratios, 50:50; 70:30, and 0:100, then made into porridge and cooled to 37°C, where the *L. Plantarum* isolate was inoculated and fermented for 0 h, 12 h, and 24 h. The porridge dried at 50°C to a moisture content of below 5%. The product analyzed for anti-nutritional content and descriptive sensory analysis. The results were recorded and analyzed using Excel and R studio software. The *L. plantarum* isolate had optimal growth at 37°C and demonstrated pH tolerance at pH 2.0 to 3.5. It was able to maintain~100% viability after exposure to 0.4% phenol. The selected isolate showed inhibition against the pathogens, with *S. typhi* having the largest (ZDI = 31.0 ± 1.73 mm) zone of diameter inhibition (ZDI) and *Candida albicans* having the least (ZDI = 18.0 ± 0.76 mm). Moreover, the strain exhibited γ - hemolytic activity hence safe for use as a starter culture and identified as a *Lactobacillus plantarum* strain Eger202111 based on 16S rRNA gene sequencing. Malting and fermentation had a significant (p=0.05) effect on phytates, tannins, and cyanide. There was a 14.6% and 49.5% decrease in phytates after 12hrs and 24hrs of fermentation respectively while after 24hrs of fermentation 88.2%, 60.3% and 27.3% decrease in tannins was observed. Cyanide decreased by 87.5%, 50% and 55% after 12hrs of fermentation. Loadings from principal component analysis (PCA) of 17 sensory attributes of porridge resulted in two principal components, which accounted for 97.3% of the total variability observed among the treatments. Malting and fermentation flavour and taste were evident in the samples. This study revealed *L. plantarum* isolate as an excellent probiotic starter culture for the fermented cassava-finger millet porridge, a nutritious complementary beverage for children.

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LIST OF ABBREVIATIONS/ACRONYMS

AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
ASLT	Accelerated Shelf Life Tests
FAO	Food and Agriculture Organization of the United Nations
FOS	Fructooligosaccharides
GRAS	Generally Recognized As Safe
HCN	Hydrogen Cyanide
IgG	Immunoglobulin G
IMO	Isomaltooligosaccharide
KALRO	Kenya Agricultural & Livestock Research Organization
LAB	Lactic Acid Bacteria
MRS	Man Rogasa Sharpe
MSc	Master of Science
CLSI	Clinical and Laboratory Standards Institute
PCA	Plate count Agar
PDA	Potato Dextrose Agar
PPM	Parts Per Million
QPS	Qualified Presumption of Safety
RFOs	Raffinose Oligosacchades
TCC	Total coliform count
TPC	Total phenolic content
USDA	United States Department of Agriculture
WFP	World Food Program
WHO	World Health Organization
XOS	Xylooligosaccharide

CHAPTER ONE

1.1 Background Information

Complementary foods are foods introduced to infants between 4 and 6 months (USDA, 2009). This is an important part of an infant's life extending from 6 months to 3 years (Bloss *et al.,* 2002). In developing countries, Kenya included, complementary foods for infants are the high-energy food such as tubers and cereal grains, the most important one being finger millet, mostly referred to as *wimbi* in Swahili language (Gewa & Leslie, 2015). Cassava (*Manihot esculanta* Crantz), a very important food in sub-Saharan Africa and second staple food in western Kenya has high utilization mainly as porridge for weaning children (Nungo *et al.*, 2014).

Nungo *et al*. (2014) established that the use of cassava, millet and sorghum has led to high levels of malnutrition in Nambale community in western Kenya where, 26.6% of children are stunted, 13.9% underweight, and 10.1% are wasting. Early childhood malnutrition has adverse effects on the physical and cognitive development and immunity of children. Furthermore, most of these foods are prepared under unhygienic conditions, hence contamination by pathogens leading to diarrhea cases among children. For example, in Nyanza, 54% of children had acute diarrhea (Kawakatsu *et al*., 2017).

Cassava is energy dense, with the lowest protein-energy ratio compared to other staple foods. It has a limited amount of methionine, which is sulfur-containing amino acids (Montagnac *et al*., 2009). Nungo *et al.* (2014) recommended blending of cassava with locally available nutrient-dense foods to improve its nutritional quality.

Finger millet (*Eleusine coracana*) is a food security crop due to its drought resistance and rich nutrient composition (Adebiyi *et al.,* 2018). Millet grains contain protein, energy, vitamins and minerals making them nutritionally comparable or even superior to other cereals (Jaybhaye *et al.,* 2014). Finger millet also contains methionine (Thapliyal & Singh, 2015), which is insufficient in cassava. Millet has high fiber content, which makes it have a hypoglycemic effect (Singh & Raghuvanshi, 2012). Finger millet also has good malting characteristics compared to other tropical cereals (Shobana *et al*., 2013). This makes finger millet suitable for blending with cassava because it can be malted to produce enzymes (Cethan *et al*., 2008), that can be used to pre-digest cassava starch for easy assimilation by infants. Studies have shown that there is insufficient availability of sugar to support rapid production of lactic acid, when non-malted cereal are fermented; this is due to low amylase

activity by LABs (Oguntoyinbo & Narbad, 2015). Hence, there is need for malting of finger millet before fermentation to produce amylolytic enzymes.

Though cassava and finger millet are nutritious, they contain anti-nutritional compounds that limit the utilization of their nutrient potential due to presence of phytates, phenols, tannins and enzyme inhibitors, which bind to nutrients in finger millet (Singh & Raghuvanshi, 2012). Cassava also contains cyanogenic glycosides, which are toxic; limits the bioavailability of sulfur-containing amino acids, and inhibits uptake of iodine by thyroid hence deficiency, which can seriously affect children (Kasankala & Kaitira, 2019).

Some economical traditional technologies such as fermentation and malting are able to boost the nutritional profile of millet and can detoxify cassava. Through fermentation, 90% of total cyanogen in cassava can be removed (Ndubuisi & Chidiebere, 2018). Additionally, according to Adebiyi *et al.* (2018) fermentation reduces the anti-nutritional compounds in the finger millet and enhances nutritional content by improving the protein content, mineral biovailability and palatability hence making the nutrients to be available to the body (Nkhata *et al*., 2018). For enhancement of human health and nutritional value of African traditional foods, fermented foods have been advised (Bell *et al.,* 2017). Fermentation improves the digestibility of raw materials and functional qualities available to local communities (Stefano *et al*., 2017). It further improves organoleptic quality, removes toxic compounds and reduces cooking energy (Anal, 2019).

Most of the African traditional cereal fermented foods and beverages, are produced spontaneously without inoculation with starter cultures (Achi & Asamudo, 2018). Traditionally, in Kenya most fermented foods are produced and utilized in small scale at home, which have not been scaled up for industrial production to meet the growing demand (Nduko *et al*., 2017). Spontaneous fermentation results in low yield and poor quality outcomes, since it is unpredictable and uncontrollable because of the diverse microbiota initially present (Achi & Asamudo, 2018). There are safety concerns associated with spontaneous fermented products due to poor handling and improper fermentation, which leads to contamination with pathogenic bacteria and production of chemical intoxicants (Bell *et al*., 2017). Hence, there is a need for standardization of the fermentation processes by using starter cultures.

Probiotics are viable microorganisms that confer health benefits to the host when consumed in appropriate concentrations (Mantzourani *et al*., 2019). The probiotics inhibit growth of pathogenic bacteria, which mostly cause diarrhoea in children, through competition

for attachment sites with pathogens, production of antimicrobials like bacteriocins, organic acid and hydrogen peroxide (Ma *et al.*, 2018).

In a previous study, *L. plantarum* was isolated in traditionally fermented milk, *Amabere amaruranu* (Sichangi *et al*., 2020). The isolate was able to grow at 6.5% NaCl, lower acidity, and cause clotting of milk, indicating potential applications in product development. *Lactobacillus plantarum* strains have generally been recognized as safe (GRAS) status and have qualified presumption of safety (QPS) (Behera *et al*., 2018), hence can be used for product development. Thus, the study of *L. plantarum* for use as starter culture for fermented porridge will be useful in making a safe and nutritious food product that is affordable and commercially available hence will increase its utilization, diversification and consequent consumption.

1.2 Statement of Problem

The local weaning foods that contain cassava and finger millet have anti-nutritional factors, which bind to the food nutrients such as minerals like iron, proteins especially the sulfur-containing amino acids, and enzymes necessary for digestion hence causing malnutrition in children. Fermentation helps alleviate this problem, though most African fermented foods are spontaneously fermented which makes them unsafe for consumption especially to children who are highly affected by diarrhoea cases and each diarrhoea episode deprives the child of the necessary nutrients for growth. In addition, spontaneous fermentation leads to inconsistency in the quality of the product and reduced shelf life, hence the necessity for pure starter cultures. The imported conventional starter cultures available are expensive and inaccessible to most rural communities. Our indigenous isolates can be able to do better since most microorganisms involved in traditional fermented foods have been isolated but not made into starter cultures. Therefore, there is a need to assess the functionality of our local isolated *Lactiplantibacillus plantarum* in fermented porridge to be able to commercialize the African fermented foods like porridge and be able to extract the probiotics for commercial use.

1.3 Objectives

1.3.1 General Objective

Contribute to food and nutrition security in Kenya by assessing the functional properties of locally probiotic isolated; *L. plantarum* and applying it in development of complementary beverage, from local food sources of nutrients of finger millet and cassava for children below five years.

1.3.2 Specific Objectives

i. To determine the functional properties of *Lactiplantibacillus plantarum* in fermenting complementary porridge of finger millet and cassava.
ii. To determine the effect of *Lactiplantibacillus plantarum* fermentation on anti-nutritional content of the complementary porridge.
iii. To determine the effect of *Lactiplantibacillus plantarum* fermentation on the sensory properties of fermented complementary porridge.

1.4 Hypothesis

i. No significant functional properties of *Lactiplantibacillus plantarum* used in the fermentation of the complementary porridge.
ii. *Lactiplantibacillus plantarum* fermentation has no significant effect on anti-nutritional contents of the complementary porridge.
iii. *Lactiplantibacillus plantarum* fermentation has no significant influence on the sensory properties of fermented complementary porridge.

1.5 Justification

According to Kenya Demographic and Health Survey (2017), in Kenya 26% of the children under the age of five are stunted, 4% are wasted and 11% are underweight. In a study of 1146 children admitted with moderate to severe malnutrition, in a hospital in Western Kenya (2005-2007) risk of death increased four times following an episode of diarrhoea. A malnourished child is susceptible to infectious diseases and every episode of diarrhoea deprives that child the necessary nutrients for growth. The use of readily available and less expensive fermented food products as vehicles of probiotics might play an important role in improving nutrition, thereby reducing malnutrition and infectious diseases especially in children in the rural areas in developing countries. The development of an African starter culture will result to modernization, industrialization and commercialization of fermented porridge, which will go a long way in adding value to the raw materials used in production and alleviation of malnutrition and diarrhoea disease among children in the rural areas that consume this fermented food. This would assist the government in accomplishing the sustainable development goal of eradicating malnutrition by 2030 by promotion of lactic acid fermented weaning foods. The development of an indigenous starter culture of *Lactiplantibacillus plantarum* as a probiotic in the market would make it easily accessible and affordable.

CHAPTER TWO
LITERATURE REVIEW

2.1 Malnutrition in children

Good nutrition allows for healthy growth and development. Globally malnutrition affects one in three people. Across Africa, 36% of children under the age of five are chronically undernourished, and 45% of all the deaths in this group are due to undernutrition (Hoddinott, 2016). During the weaning periods, children are most vulnerable to poor nutritional status when both macro and micronutrients are inadequate for growth maintenance and development, leading to malnutrition (Konyole *et al*., 2012). The nutritional status of infants and children continues to be troubling, regardless of important economic upgrade, a substantial proportion of children under the age of 3 and 5 years are stunted, wasted and underweight (Pai *et al*., 2018). According to the Kenya Demographic and Health Survey (2017), 26% of children in Kenya, under the age of five are stunted, 4% are wasted and 11% are underweight. However, the levels of malnutrition of under-five children have declined noticeably from 2003-2014 (World Food Programme, 2016).

2.2 Diarrhoea in Children

Annually, diarrhoeal disease is accountable for taking lives of around 525,000 children, hence the second most predominant mortality cause in under five years' children (World Health Organization, 2017). The consumption of contaminated water and food results to this disease. Seven hundred and eighty million persons have no access to improved drinking water and 2.5 billion lack-improved sanitation across the globe. Diarrhea due to infection is widespread throughout developing countries. Every diarrhea occurrence in children impoverishes them with nutrients needed for growth, hence main cause of malnutrition. Children with malnourishment are more likely to suffer from diarrhea (WHO, 2017) since poor nutritional status is associated with micronutrient deficiencies, hence impaired immunity (Pai *et al*., 2018).

The diarrhoeal disease is accountable for over 90% under five children mortality rate in Africa and especially sub-Saharan Africa (WHO, 2017). In Kenya, a research done by Kawakatsu *et al.* (2017), which involved 278 mothers in the former Nyanza province, revealed that 150 (54%) children had acute diarrhea. The diarrhea occurrence was 20.9%, 25%, and 8.5% amidst children under 1, 1–2 years and 4-5 years old respectively. It established that the most common etiological microorganism was *Escherichia coli* in Bondo District in Kenya (Torheim *et al*., 2010).

2.3 Introduction to finger millet and cassava

2.3.1 Finger millet

The world's important food supply includes cereals, which have a vital role in the human diet throughout the world. In both developed and developing nations, cereal grains provide nutrients and non-nutrients in significant amounts in the diet of the population all over the world (Adebiyi *et al.,* 2018). For countless number of people in underdeveloped countries millets are one of the major cereals, an important source of protein and energy, besides maize, rice and wheat, since they can grow in adverse weather conditions like limited rainfall (Thapliyal & Singh, 2015). They have a short growing season (Hejazi & Orsat, 2016). Finger millet is one of the staple foods in the semi-arid tropics of Africa and Asia (Siwela *et al.,* 2014). Traditionally, in preparing weaning foods, finger millet is used (Hejazi & Orsat, 2016).

2.3.2 Nutritional Composition of finger millet

Foods loaded with calories, proper amounts of good quality protein, minerals and vitamins, devoid of anti-nutritional factors and minimum content of indigestible fiber; for a standard weaning food product, is required (Sajilata *et al*., 2002). Guidelines recommend the use of gluten-free cereals for weaning infants below 6 months. Finger millet is gluten-free and a major source of carbohydrates and minerals (Hejazi & Orsat, 2016). Millets have high fiber content which makes them have a hypoglycemic effect; therefore, the complex carbohydrates and fiber content are digested slowly, which leads to a reduction in postprandial glucose (Thapliyal & Singh, 2015).

Compared to the FAO. (1991) reference protein of 33.9%, finger millet holds 44.7% of the total amino acid (Mbithi *et al*., 2000). Among the millets, finger millet contains more threonine, lysine and valine, which makes it relatively balanced (Thapliyal & Singh, 2015). Finger millet protein characterization depicts that the main storage protein is prolamin and lysine highly limited amino acid followed by cysteine, though methionine is slightly more in millets (Jaybhaye *et al.,* 2014). Finger millet is nutritionally superior to cassava as shown in Table 2.1 of proximate composition.

2.3.3 Anti-nutritional Properties of Finger Millet

The presence anti-nutrients such as, tannins, phenols, enzyme inhibitors and phytates, hinders the highest potential of nutrient utilization in millet. Tannins bind both endogenous and exogenous proteins including digestive tract enzymes hence, affecting the utilization of

proteins (Singh & Raghuvanshi, 2012). An investigation performed by Shibairo *et al*. (2014) depicted that tannins negatively correlates with zinc and iron contents. Phytic acid combines with dietary minerals for instance, zinc, magnesium, calcium and iron, forming complexes that are inaccessible to the body (Shibairo *et al.*, 2014), since they are insoluble at the body pH in human (Makokha *et al.*, 2002).

Rao (1994) reported finger millet to be loaded with high tannins content (0.04% -3.74% catechin equivalents) among the millets and 149 to 150 mg/100g phytate (cited in Singh & Raghuvanshi, 2012). It was recorded by Ramachandra *et al*. (1997) that in comparison, the white varieties of finger millet grains were observed to have low tannin content of 0.05% as opposed to brown and dark brown varieties which had 0.61%, also Parida *et al.* (1989) observed that the white varieties had very low phenol and tannin levels (Kumar *et al*., 2016). Siwela *et al*. (2007) suggested that the grain colour was largely attributed to phenolic compounds and condensed tannins in finger millet contributed the larger proportion of total phenolic content.

The primary phenolic compounds are tannins existing in whole grains that are water-soluble, categorized into hydrolysable that is gallotannins and ellagitannins, while the non-hydrolysable constituting condensed tannins e.g. proanthocyanidins). Proanthocyanidins are plenty in millets (Adebo & Meza, 2020; Altop, 2018; Samtiya *et al.*, 2020). The structure of condensed tannins consists of a C6-C3-C6 carbon backbone with two aromatic rings as shown in Figure 5.1 (Watrelot & Norton, 2020). They are polymers and oligomers of flava-3-ol.

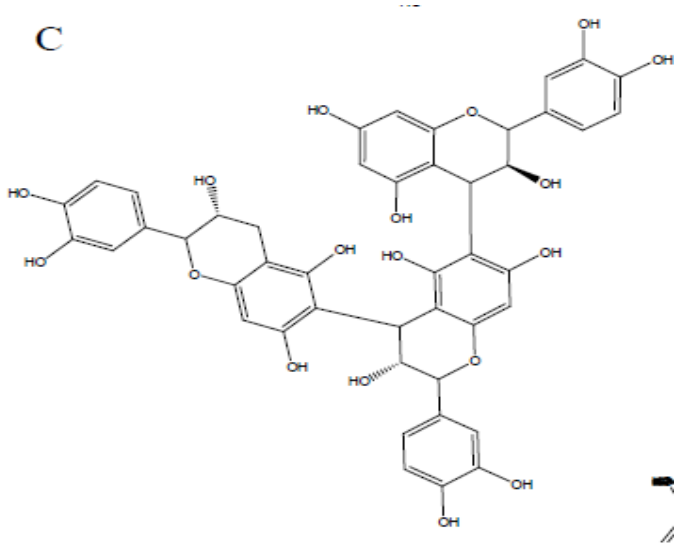


Figure 2.1: condensed tannin including trimer of catechin and epicatechin linked in C4-C6

(Watrelot & Norton, 2020).

Tannins usually are bound to proteins structures that have are open and flexible with abundant proline and that are hydrophobic and relatively large (Samtiya *et al*., 2020).

2.3.4 Malting of Finger Millet

Its uncomplicated traditional processing technique, which improves nutrients quality in cereals (Hejazi & Orsat, 2016). Finger millet has good malting characteristics compared to other tropical cereals (Shobana *et al*., 2013). Malting decreases anti-nutrients content in finger millet. A study done by Syeunda *et al*. (2019) showed a decreased of 50%, 44%, and 41%, of tannin, phytic acid, and phenol content respectively, when malted. This reduction may be due to the leaching of phytates and tannins during steeping, enzymatic breakdown into low molecular weight compounds (tannins into flavanols and phytate into inorganic phosphorus and inositol) and hydrophobic association of tannins with seed proteins (Baranwal *et al*. 2014; Syeunda *et al*. 2019).

Forty-eight hours of germinating finger millet at thirty degrees Celsius, increased protein amount by 17%, resistant starch decreased by 70% and a slight increase in energy by 10% maximized at 26°C, hence the optimum temperature for germinating finger millet is 30°C (Hejazi & Orsat, 2016). Malting for 48 hours had a better impact on the mineral composition (Udeh & Duodu, 2018). Longer than 48 hours of germination results in dry matter loss without further improvement in nutritional quality (Camp *et al*., 2000). Besides the malt of millet, being a great source of β-amylase it is also rich in α-amylases (Chethan *et al.*, 2008). Alpha amylases are the most predominant enzymes in malted finger millet among the other carbohydrate-degrading enzymes (Nirmala & Muralikrishna, 2003). To boost the texture and nutritional density of weaning food products, the millet amylases are used (Chethan *et al*., 2008).

Table 2.1 Proximate composition of raw cassava and finger millet

Component	Raw cassava (g/100gm, dry basis)	Finger millet (Native grain) (g/100gm, dry basis)
Moisture content	59.7	13.1
Proteins	1.4	7.7
Ash	0.62	2.6
Fat	0.3	1.5
Carbohydrates	38.1	72.6
Crude fibre	1.8	3.6
Mineral (mg)		
Calcium	16	350
Iron	0.3	3.9
Phosphorus	27	283
Vitamin (mg)		
Thiamin	0.087	0.42
Niacin	0.854	1.1
Riboflavin	0.048	0.19

Raw cassava nutritional data adopted from the USDA National Nutrient Database for standard references (http://www.nal.usda.gov/fnic/foodcomp/search/). Nutrient values and weights are for the edible portion (Salvador *et al.*, 2014)**.** Finger millet nutritional data adopted from USDA National Nutrient Database for Standard References, Release 28 (2016) (Cited in Jaybhaye *et al.* 2014)

2.3.5Cassava

2.3.6 Cassava utilization

In the Western region of Kenya, the first ranked and most utilized food crop relative to other food crops is cassava. In addition, the Western region is the leading region in the adoption of the new and improved varieties of cassava with 77% compared to other regions in Kenya (Githunguri & Gatheru, 2017). In comparison to cereals, cassava is more low soil fertility and drought resistant. The ranked cassava variety was KME-1 first in terms of sensory evaluation compared to other varieties (Wambua *et al*., 2020). The KME-1 had 1.08-6.4 mg of cyanide per 100 grams of sample hence a sweet variety (Wambua *et al*., 2020). Enzymes digest cyanogenic glycosides present to liberate hydrogen cyanide that posse a great concern in food and industrial utilization of cassava roots as a raw material (Ndubuisi & Chidiebere, 2018). Most areas that grow cassava have widespread micronutrient deficiency, especially in Africa; hence, there is a need to improve the cassava nutritional potential to mitigate hidden hunger (Montagnac *et al*., 2009).

2.3.7 Nutrient Value of Cassava Root

It is an energy-dense food, having a high carbohydrate content ranging from 80%-90%, but is low in protein (1% to 3%) on a DM basis, lipid (0.1% to 0.3%) on fresh weight (FW) basis (Montagnac *et al*., 2009). Cassava is usually low in minerals and vitamins as compared to sorghum, maize and finger millet (Montagnac *et al*., 2009).

2.3.8 Cyanide in Cassava

Cassava root tubers are associated with cyanogenic glucosides, which limit their consumption (Onyango *et al*., 2020). Cyanogenic glycosides are a group of nitrile-containing plant secondary metabolites enzymatically broken down to yield cyanide (cyanogenesis) (Ndubuisi & Chidiebere, 2018). The role of cyanogenic compounds in plants is to act as a defense against insects, animals and pathogens. Both cassava roots and leaves contain cyanogenic glycosides that are the most potent substances (Salvador *et al*., 2014), with linamarin being the most predominant cyanoglycoside in cassava (Ndubuisi & Chidiebere, 2018). Three forms of cyanogens exist in cassava; cyanogenic glucosides (93% linamarin and 7% lotaustratin) (Hawashi *et al*., 2018), free cyanide and cyanohydrins (Etsuyankpa *et al*., 2015; Kuliahsari *et al*., 2021). According to the chemical structure of cyanide, it has a group of C≡N attached to the hydrogen atom and a carbon atom attached to the nitrogen atom (Figure 5.2) (Kuliahsari *et al*., 2021). Dhurrin found in millet and sorghum when broken down, produces cyanide, a sugar and a ketone or aldehyde (Figure 5.2) (Chebet *et al*., 2018).

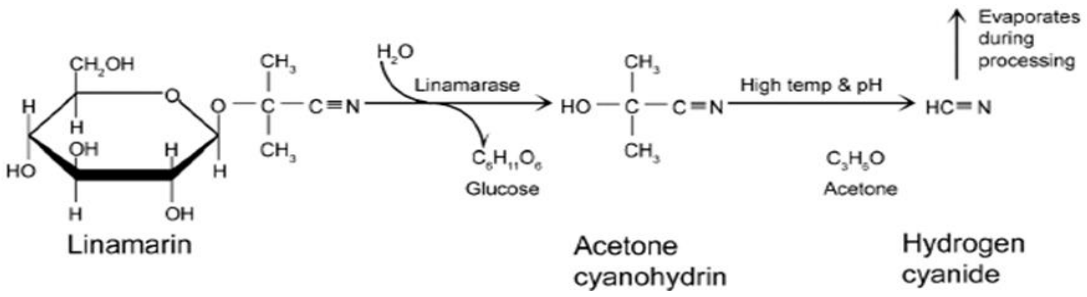


Figure 2.0.1: Hydrolysis of Linamarin

To the extent which cyanogenic glucoside (hence hydrogen cyanide) are present, cassava cultivars can either be classified as “bitter” or “sweet”. Bitter varieties have values ranging from 15-400 mg of hydrogen cyanide per kilogram of fresh-weight of cassava roots. Sweet varieties of cassava (low cyanide content) typically contain approximately 15-50 mg hydrogen cyanide/ kg fresh cassava (Ndubuisi & Chidiebere, 2018).

Cyanide is a highly toxic anion, its ingestion could cause nausea, stomachache, diarrhea, headache, dizziness, mental confusion, seizures, hypertension, paralysis, tachycardia, neurological disorder (cretinism, ataxic neuropathy, and xerophthalmia) and goiter with continuous consumption (Chebet *et al.,* 2018; Kuliahsari *et al.,* 2021; Qin *et al.,* 2021). The glucosidases and sulfur transferase enzymes can convert glucosides to thiocyanate, which hinders the absorption of iodine causing goiter (Chebet *et al*., 2018). In addition, the production of ethyl carbamate, which is a potential carcinogen, is associated with cyanide (Shen *et al*., 2021).

The FAO/WHO (1991) recommends less than 10mg or 10-ppm cyanide in cassava flour (HCN) equivalent/kg to prevent acute toxicity in humans. Peeling and roasting, baking or boiling are satisfactory cassava processing methods for sweet cassava varieties, while bitter varieties need a more sophisticated method like drying and fermentation due to high cyanide content (Ndubuisi & Chidiebere, 2018; Salvador *et al.*, 2014). Ndubuisi and Chidiebere (2018), stated that some cassava cultivars especially the bitter variety with high quantities of cyanide need to be done value addition; converting them into finished products ready for consumption to alleviate toxicity in humans and animals. Also, a reduction in cyanide prolongs shelf life and increases the utility and product value (Kuliahsari *et al.,* 2021).

2.4 Probiotics, Prebiotics, Synbiotics and Post biotics

Fermented foods with live probiotics cultures are functional foods (Ukwuru & Ohaegbu, 2018). Foods that adequately affect, one or more target body functions, past the benefits of

nutrients, applicable in a way to enhanced health status and well-being and /or a reduction in disease susceptibility are functional foods (Ghosh *et al.*, 2019).

As suggested, probiotics prevent and cure some types of diarrhea (Mandal & Sahi, 2017). Probiotics have therapeutic effects such as anti-cancer (Chuah *et al*., 2019), serum cholesterol decline (Yadav *et al*., 2016; Huang *et al*., 2013), prevent intestinal infections (Campana *et al.* 2017), antioxidant, immunomodulatory, hypoglycemic properties, and antihypertensive properties (Abushelaibi *et al*., 2017). They have also been demonstrated to be effective in preventing several digestive disorders including necrotizing enter colitis, antibiotic-associated diarrhea, and irritable bowel disease (Mantzourani *et al*., 2019). Probiotics utilized in both animal and human beings are members of the lactic acid bacteria (LAB) including genus: *Enterococcus, Lactobacillus, Bifidobacterium, Pediococcus, Streptococcus* and the yeast *Saccharomyces*. Lactic acid and bacteriocins secreted by LABs prevent food spoilage and pathogenic microorganisms' contamination therefore ensuring food safety and food preservation (Chuah *et al*., 2019).

There is a rapidly increase to attention of probiotics and prebiotics over the current years in the public, healthcare and scientific arenas (Cunningham *et al.,* 2021). Microbiome research has helped in making public knowledgeable and improved adoption of probiotics and prebiotics, beyond the norm of disease-causing microorganisms that should be avoided (Cunningham *et al*., 2021). The probiotic and prebiotic industries have an estimated annual growth rate of 7% and 12.7% respectively, over the next 8 years (Cunningham *et al*., 2021).

The human gastrointestinal tract microbiome includes Achea, fungi and viruses, which account for more than 100 trillion bacteria in the gut microbiota (Tilg & Mosch, 2015). The human intestine is colonized by microbiota up to 1011 bacteria/g density of luminal content, which harbors 100-fold genes; genetic information more than the human genome, hence influencing life at numerous functions levels of human life including host immunity and metabolic (Tilg & Mosch, 2015).

The foremost bacterial to colonize the huge gastrointestinal tract of a young child are facultative anaerobes such as *Escherichia coli* and *streptococcus* sp. Oxygen in the infant's gut is metabolized by these species to create an anaerobic environment, the diet of the infant and environment (hygiene) plays a vital role in determining the other subsequent gut colonization (Varankovich *et al*., 2015). Wen and Duffy (2017) showed that the mode of birth, type of infant feeding, medication especially antibiotics, illness and diet affect gastrointestinal colonization. Children three years of age have a gut microbiota similar to that

of an adult with genera such as *Eubacterium, Fusobacterium Bifidobacterium, Clostridium, Bacteroides, Lactobacillus,* and various Gram-positive cocci (Varankovich *et al.,* 2015).

There are more studies documenting the isolation and characterization of microorganisms involved in traditionally fermented porridge. Lactic acid bacteria predominate the probiotic market with the *Lactobacillus* genus being predominant (Tajabadi *et al.,* 2013). The most studied probiotic species of lactobacillus include *Lactobacillus casei, L. plantarum, L. paracasei,* and *L. rhamnosus* (Mantzourani *et al.,* 2019*). Lactobacillus plantarum* has been widely studied and used in fermented foods production, since it is highly versatile and sustainable species (biosynthesis and flexibility capability), which exists in numerous food products that are fermented (Behera *et al.,* 2018; Jin *et al.,* 2018). *Lactobacillus plantarum* has been isolated from different ecological niches such as cereals, fruits, meat, vegetables, wine, and dairy products (Tajabadi *et al.,* 2013; Wang *et al.,* 2018). The habitats contrast result in different types of isolates. Cognizant of this fact, several scientific studies have been involved in the isolation of new strains with specific and different functional properties, which could expand the probability of getting stains with advanced functional properties.

2.4.1 Probiotics

The origin of the term "probiotic" originated from the use of several microorganisms for treatment of diseases. The first developed probiotic drug was in the treatment of *E. coli* infection in pigs. Orrhage discovered that people consuming yoghurts fermented with *lactobacillillus* spp. had low blood cholesterol and the same was observed in infants consuming *Lactobacillus acidophilus* had reduced serum cholesterol; this convinced WHO that probiotics were the next important immune defense system (Yadav *et al.,* 2022). Traditionally probiotics used include lactobacillus, bifidobacterium and other lactic acid–forming microorganisms, initially fecal microbial organism and dairy products isolate (Cunningham *et al.,* 2021).

Probiotics are beneficial to human health as they act as antimicrobials, immunity stimulators, ulcer treatment, diarrheal disease treatment, used by lactose intolerant people, food preservation, cancer treatment and others (Yadav *et al.,* 2022). A study done in 2017 by Santarmaki reported that *L. plantarum* reduced the levels of A (IgA) serum levels and increased immunoglobulin G (IgG) (Birch & Bonwick, 2018). A mixture of *Bifidobacterium lactis* LMG P-28149 and *Lactobacillus rhamnosus* LMG S-28148 can regulate obesity

related microbiota and restore *Akkermansia muciniphila* and *Rikenellaceace* load while reducing *Lactobacillaceace* load (Li *et al.,* 2021).

The microencapsulation technique of using biopolymers prepared to withstand the stomach acid environment is one of the strategies used to aid probiotics transits through the gut (Birch & Bonwick, 2018). Several ways that probiotics deploy to pass health benefits to the host body, is by preventing food borne diseases and infections. Firstly, they colonize the gut, secondly synthesize several inhibitory substances such as short-chain fatty acids, organic acids, carbon dioxide, hydrogen peroxide, acetaldehyde, acetone, diacetyl, bacteriocins and bacteriocin-like inhibitory substances that prevent the proliferation of pathogenic microorganisms, thirdly they competitively block the adhering sites of the epithelial surface of the intestine and reduces the interaction between pathogens. Also, they compete for nutrients, surprisingly probiotics inhibit pathogens that ingest the nutrients and deny the host (Yadav *et al.,* 2022). As a prescription to improve immunity against SARS_COVID_19, probiotics need to be part of a healthy diet (Zhang *et al*., 2021).

2.4.2 Prebiotics

A relatively latest idea of non-digestible food constituents. They selectively fermented by gut microorganisms for their beneficial effects (Shehata *et al*., 2022). They are short-chain fatty acids that evade digestion and are used selectively as substrates by colon bacteria for growth, hence modulation the levels of the resident microbiome to maintain an equilibrium when homeostasis is altered, also they do not contain bacteria in their composition (Oniszczuk *et al.,* 2021; Yadav *et al.,* 2022). Non-digestible carbohydrates and oligosaccharides are food components considered to have prebiotic properties (Farias *et al*., 2019). Mostly include Oligosaccharides, lactose, fructo-oligosaccharides and inulin; they have been widely used in the manufacturing of functional foods and as an add-on treatment for dysbiosis (Rinninella *et al*., 2022). There are three main groups of established prebiotics namely; 1) polyols (lactulose, xylitol, mannitol, lactiol), 2) oligosaccharides (fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), isomaltooligosaccharides (IMO), raffinose oligosaccharides (RFOs), isomaltulose, inulin and others), 3) fibers (cellulose, dextrins, pectins, beta- glucans) (Farias *et al*., 2019). FOS (inulin and neosugar) promote the growth of *Bifidobacterium, Enterococcus feacalis, E. faecium, Bacteroides vulgatus, B. thetaiotaomicron, B. ovatus, B. fragilis and lactobacillus acidophilus.* FOS are dietary fibers with health benefits, XOS a forth coming prebiotic is available in fruits, vegetables, bamboo shoots, milk honey and others, it is metabolized

by *Bifidobacterium adolescentis* while *Lactococcus lactis,* and *Lactobacillus rhamnosus* and *L. plantarum* which efficiently utilizes oat beta glucooligosaccharides. Fermented foods such as miso, soy sauce and honey contain IMO, metabolized by bifidobacterium and the Bacteroides groups (Yadav *et al*., 2022).

Unlike probiotics, prebiotics help the proliferation of the already existing gut bacteria in the gastrointestinal tract; they stimulate the growth of beneficial bacteria that prevent the growth of pathogenic microorganisms (Farias *et al*., 2019). Prebiotics increase the intestinal length in elderly humans, change the colonic bacteria and influence gut metabolism that is increase protein metabolism, energy metabolism, fiber digestion, absorption and gut maturation in Leptin-Resistant Mice (Shehata *et al*., 2022). Also, prebiotics regulate the expression of intestinal anorectic hormones such as tyrosine, tyrosine peptide, the glucagons-1 peptide and leptin and decrease levels of orogenic hormones, such as ghrelin, which in turn influence metabolism and obesity (Farias *et al*., 2019). The short-chain fatty acids produced after prebiotic metabolism regulate a range of ex-gut and gut functions, including gut epithelial and mucus barrier function hence maintaining intestinal membrane integrity, it also regulates immunity, inflammation, glucose and lipid metabolism, energy expenditure and satiety (Cunningham *et al.,* 2021).

In order to boost the growth of probiotics in the gut, prebiotics are administered together with probiotics; they have a symbiotic relationship (Scarpellini *et al.,* 2022). In presence of prebiotics such as maltodextrin, pectin and fructose, they facilitate probiotics enhancement hence, are included in practical drinks and baby formulas. A test done on 100 healthy volunteers where by, they daily consumed 480g of fermented milk with two probiotic strains of *Bifidobacterium Lactis BI-07* and *Lactobacillus acidophilus* together with prebiotic (Isomaltooligosaccharide) for two weeks. As discovered all the participants had an evident higher concentration of Lactobacilli and *Bifidobacteria* than the control sample to the top of it, *Enterobacilli* had decreased significantly (Jagtiani & Adsare, 2022).

2.4.3 Synbiotics

A new frontier that refers to a combination of both prebiotics and probiotics for the improvement of human or animal health (Yadav *et al.,* 2022). The International Scientific Association for Probiotics and Prebiotics, updated the concept of synbiotics through a panel of experts, which stated that synbiotics are of two types that is a complementary and a synergistic symbiotic. A complementary symbiotic consist of a probiotic and a prebiotic that together confer one or more health benefits but do not require co-dependent functions while a

synergistic symbiotic contains a substrate that is selectively utilized by co-administering microorganism(s) (Swanson *et al.*,2020; Yadav *et al.*, 2021). Synbiotics amplify the colonization of probiotics in the gut by stimulating their growth and survival in the presence of selective prebiotic substrate and facilitate the apoptotic response to carcinogen-induced DNA damage in the colon, increase SCF synthesis and immunomodulation (Jagtian & Adsare 2022).

A mixture of the omega-3 fatty acids and alive probiotics consisting of Bifidobacterium, *Lactobacillus, Lactococcus a*nd *Propionibacterium* showed a more distinct reduction in hepatic steatosis and lipid accumulation compared to probiotics alone. Synbiotics also reduce obesity (Li *et al.*, 2021). A synergistic anti-obesity effect on obese mice observed, as resulted due to mixing of *L. plantarum* PMO 08 with chia seeds and this led the mice to have a more favourable intestinal microenvironment for *L. plantarum* growth (Li *et al.*, 2021). An important feature here to consider for the regulation of the gut microbiome is using specific prebiotics in order for bifidobacteria to be able to break down these prebiotics (species-dependent), for example, *Bifidobacterium adolescentis* GI reacts with fructo-oligomers rather than inulin (Yadav *et al.*, 2022).

2.4.4 Post biotics

They are defined as beneficial substances also referred to as 'inactivated probiotic's or 'ghost probiotics', resulting from microbiota metabolism eliciting beneficial effects to the microflora as well as the host (Birch & Bonwick *et al.*, 2018; Scarpellin *et al.*, 2022). Examples of post-biotics include; cell-free supernatants, short-chain fatty acids, bacterial lysates, exopolysaccharides, enzymes, cell fragments and gut microbiota metabolites (Scarpellin *et al.*, 2022). Exopolysaccharides interact with dendric cells and macrophages, inhibit cholesterol absorption and enhance immune response. Enzymes act as antioxidants, microbiome modulators and protect against pathogens (Scarpellin *et al.*, 2022). Short-chain fatty acids act as energy sources and immunosuppressants especially butyrate Lee *et al.* (2017), fat deposition reducers, inhibitors of cholesterol synthesis and used for energy harvesting. The bacterial lysates stimulate dendric cells and activate T and b-lymphocytes, which in turn reduce asthma and wheezing episodes, prevent viral and bacterial disease in both children and adults (Scarpellin *et al.*, 2022). Supernatants have antibacterial activity, anti-inflammatory and antioxidant properties undo the impaired intestinal peristalsis induced by stress, prevent invasion of colon cancer cells and trophic action on the intestinal barrier (Scarpellin *et al.*, 2022).

Lactobacillus plantarum supernatant has a trophic effect on the intestinal membrane wall structure; it leads to improve intestinal absorption in young lambs and decreased pathogens in the intestines (Izuddin *et al.*, 2019). Metabolites such as vitamins, phenols, aromatic amino acids and bioactive peptides cause increased production of folate, B12, K vitamin and biopeptides. This in turn lowers the risk of reduces anemia, stroke, acceleration or reversal of carcinogenesis in risk subjects or colon cancer patients, coagulation modulation, continuation of chronic kidney disease, weight loss and improve insulin resistance, high carotid arterial stiffness reduction, cholesterol profile improvement. Elevated mineral density for the whole body in postmenopausal women, immune-modulation and ant-allergic properties (Scarpellin *et al.*, 2022). Lactobacillus acidophilus added to a yoghurt matrix has been associated with increased synthesis of vitamin B12 (Mohammad *et al.*, 2006). Post-biotics could be the best alternative for use as functional food products regarding food safety since there is a minimal chance of transferring antibiotic-resistant genes to the host microbiome (Birch & Bonwick *et al.*, 2018). The gut microbiome modulation with probiotics affects and counteracts the inflammatory system of COVID-19 and replication of SARS-CoV-2. Hence, gut modulation through post-biotics could have the same effect and be used in arresting SARS-CoV-2 infection in pre-disposed humans (Scarpellin *et al.*, 2022).

2.5 Fermentation

Fermentation is a biochemical process that modifies the majorly food matrix due to by microorganisms and their synthesized enzymes, which is desirable (Nkhata *et al.*, 2018). Through fermentation, protein has been observed to increase and hydrogen cyanide levels to decrease (Damayanti *et al.*, 2021; Isa *et al.*, 2021). Primarily fermentation was done to increase shelf life of food by production of metabolites such as organic acids, bacteriocins and ethanol together with reduced water activity. Although, studies have shown that fermentation improves food safety through pathogenic growth hindrance or detoxification of toxic compounds, nutrient profile and organoleptic quality enhancement of the food (Anal, 2019).

2.5.1 Fermentation of Cassava

The lactic acid bacteria fermentation of food is a method commonly used in Africa (Ndubuisi & Chidiebere, 2018). Examples of African cassava-fermented products include *gari* in Western Africa, *fufu* in Southern Nigeria, *attieke* and *placali* in Ivory Coast, *chickwanghe* in Zaire and *Kivunde* in Tanzania (Ray & Sivakumar, 2009). A study by Lyayi and Losel (2001), showed an increase in protein content via solid-state fermentation

by *Aspergillus niger* while another study showed a significant decrease in cyanogen content by up to 95% (Montagnac *et al.,* 2009). Fermentation of roots soaked in water is more effective in the detoxifying cyanide compared to grating roots as 90% of total cyanide removed after three days of fermentation (Ndubuisi & Chidiebere, 2018).

2.5.2 Fermentation of Cereals

Cereal fermentation is an old and cheap way of preserving food, which is a traditional and cultural exercise within local communities in Africa and most developing countries (Achi & Asamudo, 2018). Cereals account for 60% of the world's food production with 73% of the total world's harvest area. They contain vitamins, dietary fiber, minerals, proteins and mostly carbohydrates (Sandhu & Punia, 2017).

Cereals constitute a major source of dietary nutrients although they are deficient in some components like essential amino acids. Therefore, fermentation may be a simple and economical way of improving their nutritional value, sensory properties and functional properties (Blandino *et al*., 2003). Fermented cereals have a higher content of riboflavin, thiamine, niacin, and lysine (Stefano *et al.,* 2017).

Most of indigenous fermented cereal-based foods in Africa, are naturally or spontaneously fermented hence, the increment of lysine (Ukwuru & Ohaegbu, 2018). Fermented cereals make porridges and children's weaning foods. In Kenya, fermented porridge (*uji*) or sour milk is used to wean a child. In Kigezi, Uganda, millet is malted and then fermented before children are given as gruel and In West Africa, *ogi* and *koko* are weaning foods (Kunyanga *et al.,* 2009). In Ghana people consume *koko,* which is millet porridge, and the fermented top-layer liquid is used for the treatment of stomach upsets or as a refreshing drink (Blandino *et al*., 2003). *Chichi* and *masa* are widely consumed fermented foods from maize in South American countries and *nshima* consumed in central and southern Africa (Nkhata *et al.,* 2018).

2.5.3 Lactic Acid Fermentation

Lactic acid bacteria (LAB) are the most commonly used microorganisms in the production of fermented foods as starter cultures (Marko *et al*., 2014). The LAB consists of genera *Bifidobacterium*, *Lactococcus* and *Lactobacillus* spp. They are gram-positive, non-spore former, catalase-negative, acid-producing and acid-tolerant rods and cocci (Marko *et al.,* 2014).

The Lactobacillus genus is the most predominant, with *L. plantarum* being the most versatile and adaptable species (metabolic flexibility and biosynthesis ability) found in many

fermented food products (Behera *et al.,* 2018; Jin *et al.,* 2018). *Lactobacillus plantarum* is extensively used for the industrial production of fermented foods and is generally recognized as safe (GRAS) and has a qualified presumption of safety (QPS) (Behera *et al.,* 2018). *Lactobacillus plantarum* has been isolated from cassava starch and is the most predominant microorganism involved in the fermentation of *Ogi* (a traditional fermented cereal such as sorghum, millet or maize) Behera *et al*. (2018) and *Kirario* (a Kenyan fermented porridge based on green maize and millet Nkirote (2006) and has been isolated from *amabere amaruranu* (Sichangi *et al.,* 2020).

2.5.4 Functional Properties of *Lactobacillus plantarum*

Fermented foods with live probiotics as cultures are functional foods (Ukwuru & Ohaegbu, 2018). Probiotics are defined by FAO & WHO as a term used to describe, life microorganisms that when administered in adequate amounts confer health benefits to the host. The amount required is a minimum of 106 viable probiotic cells per milliliter during storage until the expiry date. Presently probiotics serve the purpose of preservation and flavour improvement of food products, improving immune health and inhibiting pathogens (Ukwuru & Ohaegbu, 2018).

2.5.5 Probiotic properties

A probiotic must be able to survive the gastrointestinal tract, adhere and colonize gut epithelial cells. Therefore, they should be acid and bile-tolerant (Yadav *et al.*, 2016). A study by Kalui *et al*. (2009), showed that all the *Lactobacillus plantarum* strains isolated from a Kenyan traditional fermented maize porridge (*ikii*), were highly viable after the first hour of exposure to pH 2 but decreased in the consecutive hours to a level of 5 and 7 log cfu/ml after 3 hours (Kalui *et al*., 2009). There was an increase in viable counts of some stains of *Lactobacillus plantarum* cultured in broth supplemented with 0.3% bile after exposure to acid (Kalui *et al*., 2009).

Lactobacillus plantarum isolate RYPR1 and RYPR9 from *Raabadi*, an indigenous fermented beverage in India showed good survival in acid and bile (Yadav *et al.,* 2016). The acid-tolerant *L. plantarum* CH3 and CH41 isolated from cocoa fermentation had the shortest adaptation time of approximately one hour in bile media compared to *L. brevis* SAU105 that had four hours (Lacerda *et al*., 2013). Among the 20 strain isolates, *L. Plantarum* code 63 isolated from *Ikii* was able to survive and grow in low pH and bile conditions in the duodenum (Kalui *et al.,* 2009).

2.5.6 Antimicrobial properties

Many studies have reported that probiotics have inhibitory effects against pathogens (antagonistic activity) (Bernborn *et al*., 2006). Probiotics can inhibit pathogenic multiplication through a number of pathways as follows:

i) Snythesis of antimicrobial or harmful substances; hydrogen peroxide, organic acids and bacteriocins, ii)

ii) Limited nutrient and energy competition with disease causing microorganism,

iii) Adhesion sites competition against the pathogens, and

iv) Increasing acid content through production of acetic acid and lactic acid hence, lowering the gut pH (Ma *et al*., 2018).

Karimi *et al*. (2018) reported that *L. plantarum* had an inhibitory effect on *Escherichia coli* 0157:H7, the intestinal pathogenic *E. coli* that causes 8-10% of the diarrhea cases in children. *Lactobacillus* species compete with pathogens for limited adhesion sites and *L. plantarum* induces MUC3 mucins, which reduce the adherence of *E. coli* (Ma *et al*., 2018). Production of lactic acid lowers the pH, which inhibits the growth of pathogens such as *Salmonella* and *E. coli* (Ma *et al*., 2018). Messi *et al*. (2001) reported that *L. plantarum* produces a high-activity bacteriocin with a wide range of antimicrobial activity; *Staphylococcus aureus*, *acidophilus hydrophila* and *Listeriamonocytogenes* (Karimi *et al*., 2018). Except for one *Lactobacillus plantarum* (code 186), all isolates showed inhibition against *E. faecalis*, followed by *S. aureus* and the least inhibition against *E. coli* (Kalui *et al*., 2009).

2.5.7 Ability to break down anti-nutritional compounds

Lactic acid bacteria fermentation to reduce phytic acids and tannins hence improving protein availability and digestion in various cereals such as maize, sorghum and finger millet (Stefano *et al*., 2017). The decrease in phytate could be due to the activity of native phytase in fermentative microflora and the increase in acidity during fermentation optimizes the environment for microbial phytase activity (Asres *et al*., 2018). The reduction in tannins during fermentation has been due to tannase activity by *Lactobacillus* that breaks down tannin complexes with proteins (Simwaka *et al*., 2016). Tannins contribute to a larger proportion of the polyphenols; hence, total phenols are higher in millet than tannins. The activity of polyphenol oxidase of the fermenting microorganism causes a decrease in polyphenols during fermentation (Simwaka *et al*., 2016). In addition, *L. plantarum* produces

linamarase enzyme (0.0416 Nmol/ml/nmol) which degrades cyanogenic glycoside in cassava (Ahaotu *et al*., 2011).

2.5.8 Antibiotic Susceptibility

Antibiotic resistance does not constitute a major risk factor in probiotics (Gueimonde *et al*., 2013), but they should not contain transmissible antibiotic resistance determinants; hence they must be safe for consumption (Guidone *et al*., 2013; Gueimonde *et al*., 2013). However, antibiotic resistance could be beneficial in restoring the gut microbiota after antibiotic therapy (Gueimonde *et al*., 2013). According to EFSA (2008) guidelines, the strains of *L. plantarum* are either classified as antibiotic resistant or susceptible antimicrobials depending on whether they are hindered or not, at breakpoint levels for a particular antimicrobial (Gueimonde *et al*., 2013). Resistance against tetracycline, kanamycin, nalidxic, gentamicin, pencillin G, vancomycin and ciprofloxacin by *L. plantarum* isolate RYPR1showed the maximum number of resistance against the antibiotics tested (Yadav *et al*., 2016). In another study, *L. plantarum* strain B23 showed resistance against vancomycin and this resistance is intrinsic, not acquired nor transmissible (Yadav *et al*., 2016).

L. plantarum subsp. *Plantarum* C17 strain was resistant to tetracycline and erythromycin (Guidone *et al*., 2013).

2.5.9 Ability to improve the organoleptic properties of food

Lactobacillus plantarum fermentation has a profound effect on the flavor and texture of foods due to degradation, acidification and production of aromatic compounds (Todorov & Franco, 2010). The acidification is due to the production of acetic acid and lactic acid which are the main organic acids produced during lactic acid fermentation (Salmeron *et al.*, 2015). Lactic acid production is strongly associated with *L. plantarum* growth (Demir *et al.*, 2006). A study on the fermentation of carrot juice concluded that *L. plantarum* as a starter culture produced proteolytic enzymes, which softened vegetable tissues and may have increased the juice yield (Demir *et al.*, 2006). The incorporation of *L. plantarum* as a starter culture in *fufu* (fermented cassava) affected its pasting properties and reduced its characteristic odour, hence enhancing the wider acceptability of the *fufu* compared to traditional *fufu* samples (Sobowale *et al.*, 2007). Salmeron *et al.* (2015), reported that high amounts of acetaldehyde were detected in malt beverages inoculated with *L. plantarum* (1.36 mg/L), which is a major flavour component in yoghurt and other yoghurt related milk hence a unique ‘yoghurt flavour’. Smaller amounts of acetic acid are favourable especially on the

overall flavour since it has unpleasant flavor attributes described as ‘vinegar’, ‘sour’ and ‘pungent’ (Salmeron *et al.*, 2015).

2.5.10 Use of Fermented Complementary Foods

Research done by Chelule *et al.* (2014) in South Africa showed that caregivers had limited knowledge of the nutritional value of fermented foods since they were not comfortable feeding them to their children. Onubi *et al.* (2015) reported a significant growth in children in the probiotic group (elaborate on the probiotic group) who were undernourished compared to the control. Probiotic-fermented foods exhibit a reduction malnutrition and childhood diarrhea disease, through prevention of infections and improved absorption of micronutrients (Onubi *et al.*, 2015). Long-term consumption of live probiotics in fermented foods demonstrate not only to be safe but also well tolerated by infants and young children, which leads to adequate growth and development (Saavedra *et al.*, 2004).

2.5.11 Lyophilization

The preservation of sensitive biomaterials like proteins, liposomes and microorganisms with minimum losses in quality is one of the major challenges of drying research currently (Aschenbrenner *et al.*, 2015). Lyophilization is also known as freeze-drying or cryodesiccation, whereby the food or suspension medium is quickly frozen, resulting in the formation of small ice crystals, while at the same time concentrating the non-frozen phase (Guergoletto *et al.*, 2012).

Lyophilization involves four major steps: freezing, vacuum, sublimation and condensation (Aschenbrenner *et al.*, 2015). The reduction in volume during lyophilization is minimal (5 to 15%) as compared to conventional drying (close to 80%) (Guergoletto *et al.*, 2012). Though it is slow and the most expensive (Guergoletto *et al.*, 2012), freeze-drying has an advantage in that it maintains the probiotic cells at low temperatures to limit damage to the cells’ structure and metabolites (Fenster, 2019). The viability and activity of probiotics are important to give beneficial effects to the consumer (Guergoletto *et al.*, 2012). The water content of about 2 to 5% seemed to cause the highest stability because oxidation reactions showed the highest reaction rates close to zero water contents and a certain amount of residual water seemed to protect against oxidation (Aschenbrenner *et al.*, 2015). The dehydration temperature for freeze-dried bacteria is very important and should be done above the membrane phase transition temperature; the optimum rehydration temperature may be above 40^{o}C (Aschenbrenner *et al.*, 2015).

2.6 Research Gap

Few cases are reporting *L. plantarum* in raw milk (Quigley *et al*., 2013). Although strains with probiotic properties have been isolated in dairy-related niches, for example, Dahunsi *et al*. (2017) isolated *L. plantarum* from camel milk as cited by Behera *et al*. (2018) and Nyambane *et al*. (2014) isolated it from *Amabere amaruranu* (bovine milk) as cited by Nduko *et al*. (2017), its utilization for product development has not been explored. The use of probiotic strains to enrich traditional fermented food systems is presently being investigated as a way of recommending it to the rural community to resolve worsening health conditions (Behera *et al*., 2018). Optimization of local indigenous technologies would go a long way to sustain the health and functional benefits of such starch/ nondairy-based fermented food/beverage in developing countries. Furthermore, the aspect of microbial proliferation which is the most difficult part of quality assurance in the reconstitution of dried complementary foods (Sajilata *et al*., 2002), can be overcome by LAB fermentation thereby reducing the pH and preventing the growth of pathogenic microorganisms.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Research Site

This work was carried out at the Dairy and Food Science and Technology Department (DAFTECH), Egerton University and Kenya Agricultural and Livestock Research Organization (KALRO), Njoro.

3.2 Raw Materials Aqusition

The finger millet (*Eleusine coracana*) U-15 variety, and the sweet variety of cassava; KME-2 (*Manihot esculanta* Crantz) were obtained from KALRO, Njoro. The cassava variety used has a wide adaptation region, is early maturing, resistant to cassava mosaic disease, is tolerant to cassava termites, is high yielding (45T/Ha) and has low cyanide content (KARLO, 2019). The *Lactiplantibacillus plantarum* strain used in this study was a laboratory isolate from Kenyan spontaneously fermented milk (Sichangi *et al.*, 2020).

3.3 Experimental Design

A completely randomized design was employed in the experimentation with two replications in objective one while in objective two a factor experiment in a completely randomized design was employed in the experimentation with two replications. The experimental design was a 5*3 factorial experiment where;

Factor 1: The first factor was fermentation time in 0 h, 12 h, and 24 h.

Factor 2: The second factor was malting of finger millet at 0, 1 and 2 days.

Factor 3: The third factor was the proportion of finger millet and cassava used that is 50:50, 70:30 and 100:0.

The 0 hour fermentation and 0 day malting acted as control for objective three a completely randomized design (CRD) was employed. Four treatments replicated thrice were used. The treatments included; unfermented and unmalted porridge, fermented and unmalted porridge, unfermented and malted porridge and fermented and malted porridge.

3.4 Objective One: To Determine Functional Properties of *Lactiplantibacillus plantarum* from Fermented Milk.

3.4.1 Test Organism Strain

The *Lactiplantibacillus plantarum* strain used in this study was an isolate from *amabere amaruranu*, a Kenyan spontaneously fermented milk product (Sichangi *et al.*, 2020). The organism stored in 6% sucrose solution was first cultured in Man Rogosa Sharpe (MRS) broth, sub cultured, and then used for studies.

3.4.2 Temperature sensitivity assay

The isolate was cultured in MRS broth for 16 h at 37°C. From the 16 h culture, decimal dilutions of the sample were made using maximum recovery diluent (Oxoid) and 1 ml was taken from 10^{-6}, 10^{-7} and 10^{-8} was drawn and inoculated in MRS broth incubated at 20°C, 30°C, 37°C and 45°C for 1 and 2 h. From the cultures, 0.1 ml was surface-plated in triplicates on 25 ml of MRS agar. The plates were incubated at 37°C for 48 h anaerobically using anaerobic jars. Viable counts were determined by counting number of colonies from the plates and logarithmic colony forming units per milliliter (log cfu/ml) was determined from the average (Čanak *et al*., 2018).

3.4.3 Acid tolerance determination

Acid tolerance of *Lactiplantibacillus plantarum* was determined according to Mantzourani *et al*. (2019) with few modifications. *Lactiplantibacillus plantarum* was grown for 16 h in MRS broth at 37°C. From the16 h old culture, decimal dilutions of the sample were made using maximum recovery diluent (Oxoid) and 1 ml was taken from 10^{-6}, 10^{-7} and 10^{-8} and inoculated into MRS broth acidified to pH 2.0, 2.5, 3.0, and 3.5 using 1N Hydrochloric acid (HCl). The MRS broth with pH of 6.5 was used as a control. Samples (0.1 ml) were drawn after 0, 2, and 4 h then surface-plated in duplicates on 25 ml of MRS agar. The plates were incubated at 37°C for 48 h anaerobically using anaerobic jars. Viable counts were determined by counting the number of colonies from plates and logarithmic colony forming units per milliliter (log cfu/ml) was determined from the average.

3.4.4 Resistance to 0.4%Phenol

The ability to tolerate and grow in the presence of phenol was determined according to the method described by Rajoka *et al*. (2017) using MRS broth, supplemented with 0.4% (w/v) phenol. Cell viability was enumerated using plate count method after surface plating on MRS agar at 0 h and 24 h of incubation at 37°C.

3.4.5 Antagonistic activity

Agar overlay method as outlined by Halder *et al*. (2017) was used with few modifications to determine the antagonistic activity of the *L. plantarum* isolate. The isolate was cultivated on MRS broth at 37°C for 24 h then using a loopful ($\approx 10^5$ CFU/spot) of the MRS broth culture, it was spot-inoculated on the MRS agar plates, which were incubated at 37°C for 24 h. The MRS agar plates containing *Lactobacilli* in spot form (5 mm diameter) thereafter were overlaid with soft Muller-Hinton agar (0.8% agar) pre-mixed with 10^8 CFU of the indicator strains (one on each MRS agar plate). After solidification of the overlaid agar medium, at

37°C for 24 h, they were incubated. Muller-Hinton agar plates pre-mixed with 10^8 CFU of the indicator strains were overlaid with MRS agar plates without *L. plantarum* were prepared under similar conditions as controls. The zone diameter of inhibition (ZDI) values obtained were measured and interpreted as the ZDI >20 mm, 10–20 mm, and <10 mm for strong, intermediate, and weak inhibitions, respectively. All the tests were replicated and the data was presented as mean ± SD (standard deviation).

3.4.6 Safety Profiling

The safety profile of *Lactiplantibacillus plantarum* isolate was determined by their hemolytic activity and antibiotic susceptibility.

3.4.7 Hemolytic Activity

For hemolytic activity, the overnight grown MRS broth culture of the lactobacilli strains were streaked on a blood agar plate supplemented with 5% sheep blood and incubated at 37 °C for 48 h. Thereafter, the plates were observed for the hemolytic action (Angmo *et al*., 2016). The formation of any clean (β-hemolysis), greenish (α-hemolysis) hemolytic zones, or no such zone (γ-hemolysis) around the *L. plantarum* colonies was recorded.

3.4.8 Antibiotic Susceptibility

The antibiotic susceptibility test was performed following disc diffusion method (Bauer *et al*., 1996). As described by Halder *et al.* (2017), *L. plantarum* isolate was inoculated on MRS broth for 24 h at 37°C. Using a sterile cotton swab, the bacteria on MRS broth culture were spread on the surface of MRS agar (plate approximately 10^8 CFU inocula), and the antibiotic discs were placed on the surface of the agar plates. Afterwards they were incubated for 24 h at 37°C. The susceptibility was tested against seven antibiotic discs including tetracycline (TE: 30 mcg/disc), gentamicin (GEN: 10mcg/disc), ampicillin (AMP: 10µg/disc), nalidixic acid (NA: 30µg/disc), azithromycin (AZ: 15µg/disc), ciprofloxacin (CIP: 30µg/disc), and chloramphenicol (CM: 30µg/disc). The ZDI values obtained were interpreted according to CLSI 2009 and classified as; resistant (ZDI: ≤ 15 mm), sensitive (ZDI: ≥ 21 mm), or intermediately susceptible (ZDI: 16–20 mm) (Liasi *et al.*, 2009; Vlková *et al.*, 2006). The measurements were replicated thrice and data recorded as mean ± SD (standard deviation).

3.5 Molecular Characterization of the Lactic Acid Bacteria Isolate

Sichangi *et al*. (2020) previously described genomic DNA extraction from the isolate and handling. Samples of dissolved DNA were sent to Inqaba biotechnical industries Ltd, Pretoria, South Africa, for 16S rRNA partial gene sequencing using primer pairs; 907R

(5’CCGTCAATTCCTTT(AG)AGTTT3’) and 1492R (5’GG(CT)TACCTTGTTACGACTT3’). The partial 16S rRNA gene sequence data was aligned and analyzed to find the closest homologous organisms in the nucleotide databases using BLASTN program that is available from the National Center for Biotechnology Information (NCBI, 2014) and retrieved from Gene Bank database and the Nomenclature proposed by Zheng *et al*. (2020). The consensus sequence was deposited in the gene databank (GenBank) (Accession No: MW843637).

3.6 Objective Two: To Determine the Effect of *Lactiplantibacillus plantarum* Fermentation on Anti-nutritional Content of the Complementary Porridge.

3.6.1 Preparation of the Finger Millet and Cassava Flour

The finger millet grain was mechanically threshed then hand-cleaned to remove the glumes followed by further cleaning to remove broken kernels and foreign matter during which the pericarps might also be peeled off (Siwela *et al*., 2007). Malting of finger millet was done as described by Chethan *et al*. (2008). The clean whole grain was steeped for 24 h, and then germinated on moist cloth at 25°C for 2 and 3 days. The seeds were drawn from the germination bed at intervals of 48 and 72 h. Then it dried at 50°C for 24 h in an air oven. For fermentation, the grain was finely ground into flour after polishing to remove the rootlets. The grains were milled to a fine flour with a laboratory hammer mill fitted with a 0.8 mm screen for chemical analysis (Siwela *et al.*, 2007). For cassava, it was washed and peeled. Then sliced with a kitchen machine, blanched for 5 minutes in boiling water and then oven dried the same way as millet and then milled into flour (Udensi *et al.*, 2005).

3.6.2 Preparation of the Composite Flour Porridge

The flour (finger millet and cassava) was blended into three rations: 50:50, 70:30, 100:0 respectively, according to Wafula *et al*. (2016). Then the porridge was made from the composite flour (Figure 3.1). Porridge preparation method was modified from Syeunda *et al*. (2019). The composite flour (30g) was mixed with clean cold water to a thick batter, 100 ml of water was boiled and the batter added with continuous stirring to form a thick gruel. Then steamed at 70°C for 30 minutes, which is the optimal temperature and time for partial cassava starch liquefaction by alpha amylases produced during malting of finger millet (GI *et al*., 2018). The porridge was pasteurized at 90°C for 30 minutes (Rouweler *et al.*, 2015). Subsequently, the temperature was lowered to inoculation temperatures of *Lactiplantibacillus plantarum*, which previous studies have shown to work best at a temperature of 37°C (Sichangi *et al*., 2020; Wardani, *et al*., 2017). The starter cultures were prepared as outlined

by Onyimba *et al.* (2017), where McFarland standard was used to standardize the cfu/ml of the culture; a dense suspension of *L. plantarum* was prepared in 1ml of sterile saline whose turbidity was similar to that of McFarland standard ($1.8 *10^9$) was centrifuged at 4500rpm for 10 mins. The culture was mixed with 100g of flour and incubated at 37°C for 24 h to make the starter culture. For fermentation, 1% of the culture was used. After fermentation, the porridge was oven dried up to below 5% moisture content.

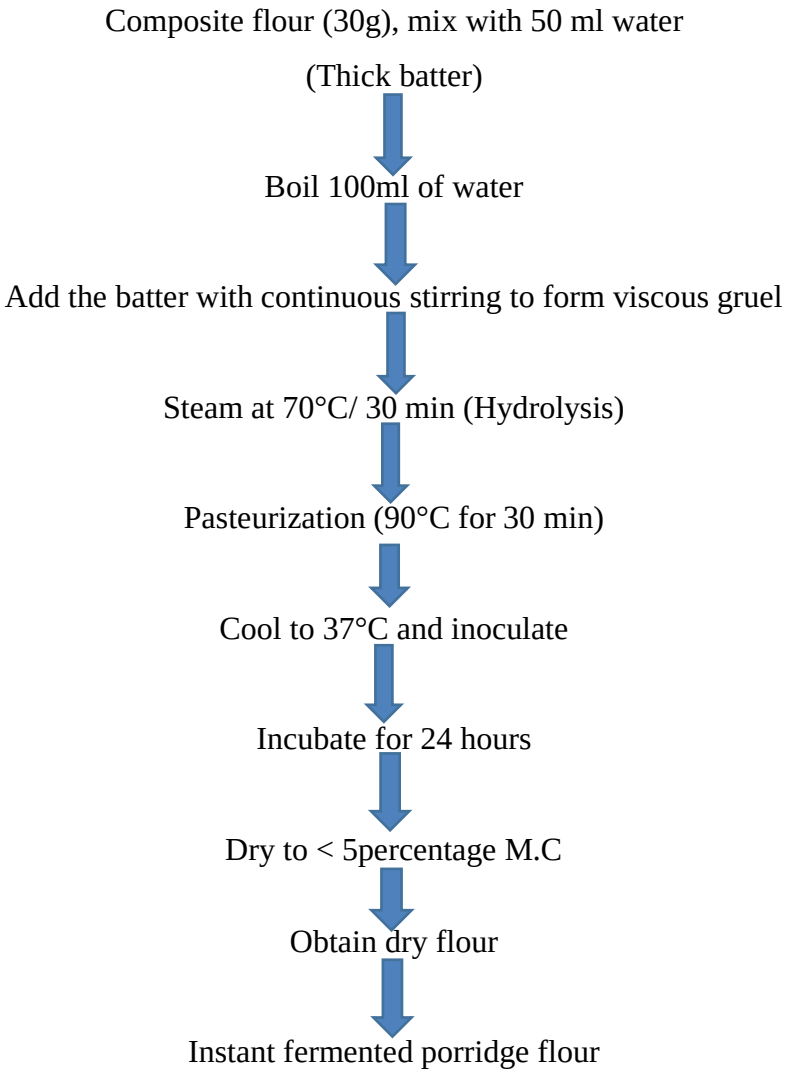


Figure 3.0.1: Porridge preparation

3.6.3 Determination of Condensed Tannin in Finger Millet

The Vanillin-HCL method was used to determine the condensed tannin content in finger millet (Price *et al.*, 1978) with modifications as described by Syeunda *et al.* (2019). Ground samples (section 3.7.1) of 0.25g were weighed into 50 ml Erlenmeyer flask and extracted with 10 ml of 4% HCL in ethanol. Then shaken for 20 minutes using Ratek Orbital Incubator (Boronia, Victoria, Australia) at room temperature. Centrifugation of samples at 12600 ×g for

20 minutes using temperature at room temperature using Eppendorf centrifuge to obtain three supernatants was done. Five (5) ml of vanillin reagent mixed with 1 ml sample extracts in the test tube and then maintained at 30°C in the water bath for 20 minutes. Sample blanks with vanillin reagent replaced by 4% HCL in methanol was used. Finally, Absorbance at 500nm was measured with Catechin as a standard using UV/VIS spectrophotometer (model Pharmaspec UV-1700 Shimadzu, Japan) exactly after 20 minutes. For setting the calorimeter, 1 ml of blank (1% HCL in methanol) was used and condensed tannin was expressed as mg catechin equivalent per 100mg sample.

3.6.4 Phytic Acid Determination

The method was based on precipitation of phytates. For extraction, 500mg of the sample (section 3.7.1) was weighed and 50 mL of 3% trichloroacetic acid added. It was shaken on a magnetic stirrer for 30 min or with occasional swirling by hand for 45 min. The suspension was centrifuged (3000 g, 10 minutes) and transferred a 10-mL aliquot of the supernatant to a 40-mL conical centrifuge tube. Rapidly 4 mL of $FeCl_3$ solution was added to the aliquot in the centrifuge tubes and the contents heated in a boiling water bath for 45 min. To the samples that were not clear one or two drops of 3% sodium sulfate in 3% TCA was added and continued heating. The samples were again centrifuged (3000 g, 10–15 min) and carefully decanted the clear supernatant. The precipitate was washed twice by dispersing it well in 20 to 25 mL 3% TCA, then heat it in boiling water for 5 to 10 min and then centrifuged (3000 g, 10 min). The washing of the precipitate was repeated with distilled water. The precipitate was dispersed in a few milliliters of water and 3 mL of 1.5N NaOH added with mixing. The volume was approximately brought to 30 mL with distilled water and heated in boiling water for 30 min. It was quantitatively filtered hot through a moderately retentive paper (Whatman No. 1). The precipitate was washed with 60 to 70 mL of hot distilled water and discarded the filtrate. Then the precipitate was transferred and dissolved from the filter paper into the 100 mL volumetric flask containing 40 mL of hot 3.2N HNO3. The paper was washed with several portions of distilled water and the washings collected in the same flask. The flask was cooled to room temperature and the volume brought to 100 mL with distilled water. Five (5) mL aliquot was transferred into another 100-mL volumetric flask and diluted to approximately 70 mL with distilled water. Then 20 mL of 1.5M KSCN was added and the volume brought to 100 mL with distilled water, and the color read immediately (within 1 minute) at 480 nm using a spectrophotometer. Distilled water was used as a reagent blank.

Preparation of Fe (NO3)3 Calibration Curve

From the stock Fe(NO3)3 solution, 2.5-, 5-, 10-, 15- and 20-mL aliquots were pipetted into a series of 100-mL volumetric flasks and dilute them to approximately 70 mL with distilled water. Then 20 mL of 1.5M KSCN was added and the volume brought to 100 mL with distilled water, and the color read immediately (within 1 min) at 480 nm using a spectrophotometer (model Pharmaspec UV-1700 Shimadzu, Japan).

Calculation

To determine the micrograms of iron present in the test from the calibration curve, the phytate P was calculated as per the following equation:

Phytate P mg/100 g sample = [Fe (μg) × 15]/Weight of sample in g

Correct the values obtained for dry matter of the sample.

This analysis will be done according to the simple and sensitive method outlined by Agostinho *et al*. (2016).

3.6.5 Determination of Cyanide in Cassava

The total cyanide content in the complementary porridge flour was analyzed using modified picrate paper kit method. Sodium picrate solution used was prepared by mixing 25g anhydrous sodium carbonate and 5g moist picrate acid in 1 liter of distilled water. The picrate paper was prepared by dipping 0.3 mm thick filter paper into a 2.5% (w/v) picrate solution then dried in a fume chamber. The dried papers were cut into 3 cm by 1 cm rectangle and attached to the plastic strip (size 5 cm by 1 cm, 1 mm thickness). A sample of 1g was placed inside the test tube and five drops of toluene was added into the tube. The strip carrying a picrate paper saturated with alkaline picrate was suspended above the sample by holding it with a plastic cap. The contact between the strip and the sample inside was avoided. The sample was left at 30°C for 24 h and color intensity noted afterwards. For the blank, a picrate paper suspended in a vial without sample was used (Ayele *et al.,* 2021).

3.7 Objective Three: Determination of Descriptive Sensory Analysis of the Complementary Porridge.

3.7.1 Preparation of the instant complementary porridge

A series of test was done to determine the reconstitution amount of water. For sensory evaluation of porridge made from the composite flours, the dried flour was reconstituted with boiled water at 85°C. To 100 grams of dried flour, 350 ml of water was added and stirred

continuously to make a viscous gruel. Then the porridge was cooled and maintained at 50°C in a water bath prior to tasting. Samples that had a cyanide level of <10ppm were used.

Table 3.1: Porridge flour formulations

Formulation Number	Fermentation Days	Malting Days
1	0	0
2	0	2
3	0	3
4	12	0
5	24	0
6	24	2
7	24	3

3.7.2 Microbial Analysis

Microbial analysis was done to ensure the product was safe for consumption. For microbial analysis, 25 g of the porridge samples (Figure 3.1) were homogenized in 225 mL peptone water, serially diluted and inoculated on plates in replicates. Total viable counts (TVC) was obtained by pour plating on Plate Count Agar (PCA) then incubating at 37°C for 48 h. Total Coliform Counts (TCC) was enumerated by pour plating on MacConkey agar then incubating at 37°C for 24 h. Yeast and Molds were determined on Potato Dextrose Agar (PDA) and incubated at 25°C for 5-7 days (Spiteals *et al*., 2014).

3.7.3 Descriptive Sensory Analysis

The composite porridge (Figure 3.1) was subjected to sensory evaluation to determine the descriptive evaluation of the product based on appearance, flavor (aroma and taste) and texture. The appearance was evaluated through visual observation and aroma through short sniffs of the porridge. A spoon full of porridge was swallowed in the mouth for flavour and texture. The test employed 12-trained panelists of the post-graduate students of Egerton University with the experience of descriptive analysis. They were screened through the filling of the pre-screening forms in appendix 1 and passed. After training, every panelist was involved in the discussion to come up with the descriptive terms and scale for the complementary porridge, which was defined and agreed by all. They evaluated and filled the sensory evaluation forms in sensory evaluation booths. Each panelist was provided with serviette and two cups of warm water for rinsing their mouths and spoons before and in

between testing of samples. Seven samples each coded differently and randomly placed were served to each panelist. The profile of the porridge was determined by the spectrum method where the food characteristics were subjected to a subjective method on a scale based on the descriptive method for the different formulations on the characteristics (Meilgaard *et al*., 2006). A quantitative descriptive analysis with a nine-point scale rating used. The descriptive sensory analysis questionnaire in the appendix 2 was used.

The twelve-trained panelist came up with seventeen descriptive terms (lexicon) grouped under appearance, flavour (aroma and taste) and flavour, which was used for sensory evaluation of the complementary porridge (Table 3.2).

Table 3.2: Lexicon for descriptive sensory evaluation for the complementary porridge

Descriptor	Definition	Referencing Material Rating	Scale
Appearance			
Colour	Degree of brownness	1-craft paper 9- Coca cola	1-light 9-Dark
Consistency	Degree of visual uniformity/ Level of smoothness	1-Mala 9-Honey	1-Least smooth 9-Very smooth
Syneresis	Degree of separation	1-Soda 9-Jam	1-Less separation 9-More separation
Glossiness	Degree of shininess at the surface	1-Brown bread 9-Leather	1-Dull 9-Glossy
Flow ability	The easiness of movement from the spoon	1-Tomato paste 9-water	1-Least flow able 9-Highly flow able
Texture			
Coarseness	Extend of grittiness	1- Blue band 9- Weetabix	1- Not Coarse 9- very coarse
Lumpiness	Amount of lumps	1- Ice cream 9-Mala/ mrusik	1- Least lumpy 9- Very lumpy
Presence of residue	Amount of particles left in the mouth after swallowing	1-Yoghurt 9-cookies	1- Low 9- High

Flavour			
Aroma			
Fermentation aroma	Aroma associated with fermented foods	1-Tea 9-Cheese	1-Least perceivable 9-Very perceivable
Malty aroma	Aroma associated with malted foods	1-Mango Juice 9-Alvaro	1-Least intense 9- Very intense
Starchy aroma	Aroma associated with high starchy foods	1-Beef 9-Cassava	1-Least starchy 9-Very starchy
Rancid aroma	Off odours/ unpleasant smells due to malting and fermentation	1- Fresh fish 9-Over stayed fish	1-Least Intense 9-Very intense
Finger millet Aroma	Associated with uncooked finger millet	1-Cooked finger millet 9-Uncooked finger millet	1-Least intense 9-Very intense
Taste			
Sugary	Degree of sweetness due to malting and fermentation	1-Lemon 9-Table sugar	1-Least sweet 9-Very sweet
Sourness	Degree of sourness due to fermentation	1-Table sugar 9-Tamarind	1-Least intense 9-Very intense
Aftertaste	How long the taste lasts/lingers in the mouth	1-Rice 9-Uncooked ground nuts	1-Least intense 9-Very intense
Astringency	Due to Pickering/dry feeling in the mouth after swallowing	1-Crisps 9-Unripe Mango	1-Least intense 9-Veary intense

3.8 Statistical Data Analysis

The statistical model for objective was as per the following equation; $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ where by Y_{ij} was the functional property of ith treatment (product) and jth replication, α_i was the ith treatment effect and ε_{ijk} was the random error term. The statistical model for objective

two was as follows; $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$ where by Y_{ijk} was the response in terms of the functional properties and the amount of anti-nutrient from the i^{th} level of fermentation, the j^{th} level of finger millet malting and the kth level of the error component, μ was the overall mean, α_i was the i^{th} level of fermentation, β_j was the effect on the j^{th} level of finger millet malting, $\alpha\beta_{ij}$ was the interaction between the i^{th} level of fermentation and the j^{th} level of finger millet malting and ε_{ijk} was the random error component. The statistical model for objective three was $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ where; Y_{ij} was the sensory observation of i^{th} treatment (product) and j^{th} replication, α_i was the i^{th} treatment effect and ε_{ijk} was the random error term.

The data was analyzed using Microsoft excel 2016 and SPSS 20^{th} edition. For microbial analysis, the microbiological counts were expressed as colony forming unit per ml and were transformed into $\log_{10}$ before analysis. Data obtained from anti-nutritional content analysis and sensory analysis was analyzed by R Studio version 1.3.1093-1 (R Core Team, 2020) statistical software for Analysis of Variance (ANOVA). Principal Component Analysis (PCA) was performed on sensory attributes. Mean separation was done using Turkeys Honestly Significant Difference (HSD) method at $p \leq 0.05$, based on studentized range test.

$HSD = q[\alpha, p, fe] * \sqrt{2MSE/n}$

Where: α = is the p-value of the level of significance (0.05); p = is the number of treatments in the experiment, fe = is the degree of freedom of the experiment, MSE = mean square error, obtained from the ANOVA test and n = number of scores used to calculate the means.

CHAPTER FOUR

RESULTS

4.1 Objective One: To Determine Functional Properties of *Lactiplantibacillus plantarum* from Fermented Milk.

4.1.1 *Lactiplantibacillus plantarum* identification results

Morphologically, the *L. plantarum* isolate was observed to form white, smooth and disc like colonies. The isolate was non-spore forming, non-motile. Microscopically, the isolate was Gram-positive hence recognized as a member of the genera *Lactobacillus.* The BLAST-search for homology of the 16S rDNA sequences of the isolate with known sequences in the NCBI database indicated that the isolate was a strain of *Lactiplantibacillus plantarum*. The strain aligned most closely (100% identity) with *Lactiplantibacillus plantarum* strains in the Gene Bank. The strain sequence with the accession number (OK569795.1) was deposited in the gene bank with the strain name *Lactiplantibacillus plantarum* EGER41.

4.1.2 Temperature Sensitivity of *L. plantarum* EGER 41

In this study, the temperature tolerance of *Lactiplantibacillus plantarum* EGER41 isolate was examined by the determination of its growth at different temperatures (Figure 1). The *L. plantarum* isolate survived well at 30°C and 37°C. Poor growth was observed at 20°C and 45°C where the cell numbers per ml decreased after 1 and 2 h of incubation. At 30°C and 37°C the organism had significant ($p<0.05$) increase in its cell numbers per ml in the first one and slightly afterwards up to two hours.

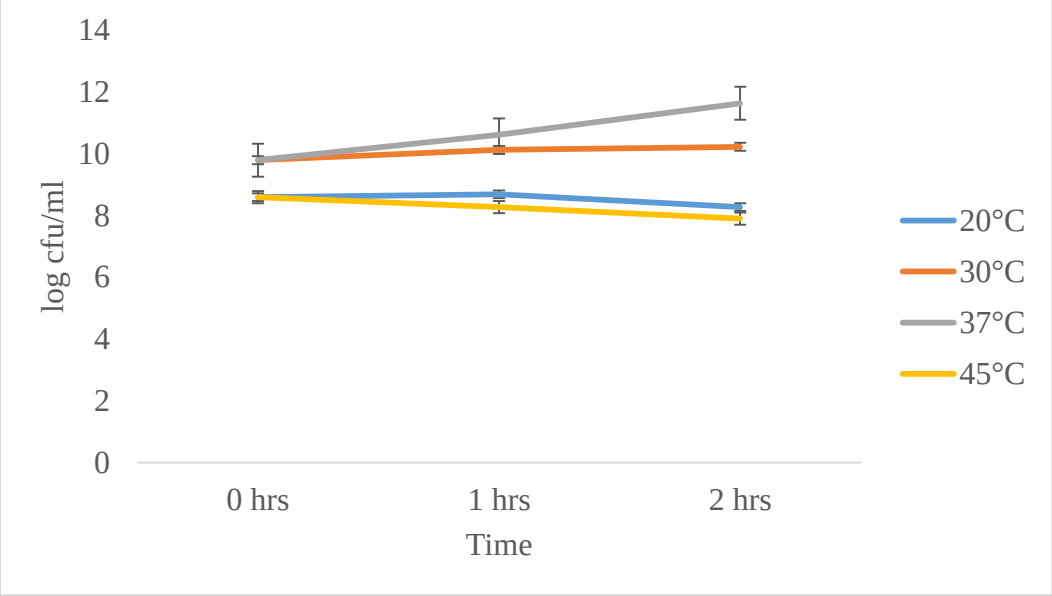


Figure 4.1: Temperature sensitivity of *L. plantarum* EGER 41 isolate cultivated on MRS broth at various temperatures.

4.1.3 Acid tolerance results

Figure 3 shows acid tolerance profile of the *L. plantarum* EGER41 isolate at acidic pH of 2.0, 2.5, 3.0, 3.5 and pH 6.5 as control after 0 h, 2 h, and 4 h of incubation at 37°C. The survival of the microbial isolate on acidic pH was significantly ($p<0.05$) affected by the acidic pH (2.0 – 3.5) compared with the control pH (pH 6.5), whereby microbial cell counts were not affected ($p>0.05$) after 2 and 4 hour of pH exposures. The highest growth (cfu/ml) was observed at pH 3.0 and pH 3.5 compared to pH 2.0 and pH 2.5 which had the least growth. After 4 h of exposure to acid, *L. plantarum* EGER41 had the lowest count at pH 2.0 of 5.6×10^2 cfu/ml and highest count at pH 3.5 of log cfu/ml 5.78. There was a steady decrease in growth at pH 2.5 with incubation time from 7.16 to 3.53 log cfu/ml. Generally, growth decreased with incubation time but according to the test, the *Lactiplantibacillus plantarum* EGER41 isolate exhibited resistance to acidic conditions.

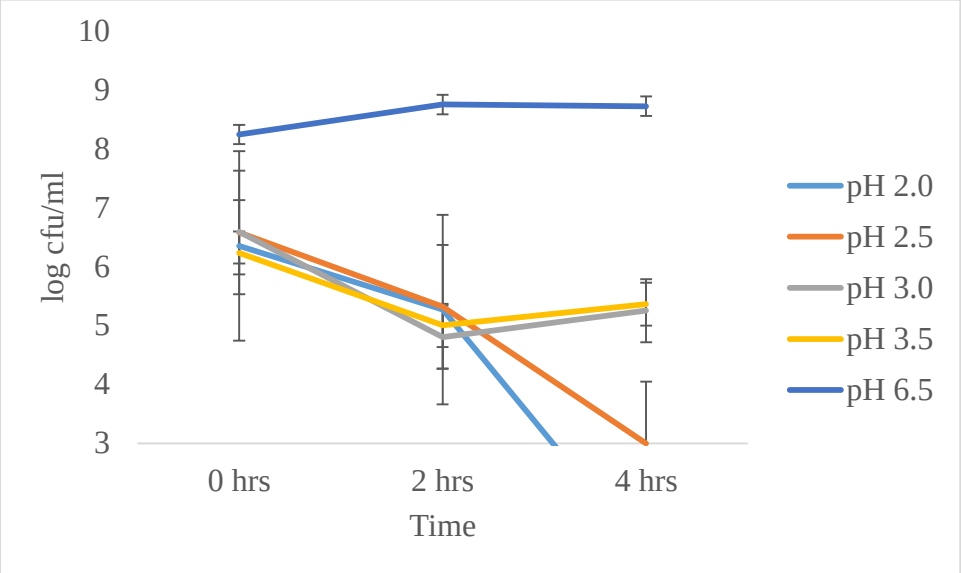


Figure 4.2**:** Acidic pH tolerance of *Lactiplantibacillus plantarum* EGER 41 isolate

4.1.4 Resistance to 0.4% phenol of *Lactiplantibacillus plantarum* EGER 41

The isolated *Lactiplantibacillus plantarum* EGER 41 strain exhibited significant ($p<0.05$) tolerance towards 0.4% (w/v) phenol (Figure 4.3); where after 24 h of incubation there was no significant difference between the control and 0.4% phenol. This was observed through the viable colony counts on the MRS agar plates after 24 h incubation at 37°C. At 0 h, the colony counts (Log_{10} = 11.33 cfu/ml) for 0.4 % phenol treatment were not significantly ($p>0.05$) different compared to the colony counts (Log_{10} = 11.20 cfu/ml) of the control treatment (No phenol). Phenol was resisted by the isolate since colony counts after 24 h

culture in 0.4% phenol treatment (Log_{10} = 16.17 cfu/ml) was similar to the control (Log_{10} = 16.46 cfu/ml) at p=0.05.

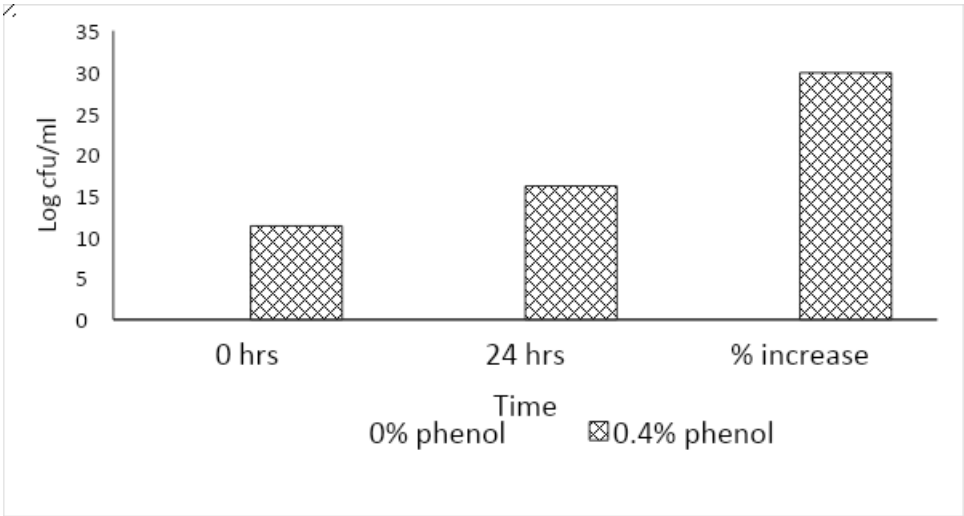


Figure 4.3: Phenol tolerance of the *Lactiplantibacillus plantarum* EGER41 isolate

4.1.5 Antagonistic activity of *Lactiplantibacillus plantarum* EGER41

A halo of growth inhibition in the agar overlay method by the *L. plantarum* EGER41 isolate against pathogenic organisms (*Salmonella enterica* serovar Typhi, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*) showed the results of the antagonistic activity, while for controls without *L. plantarum* there was no zone of inhibition (Table 4.1). The *L. plantarum* EGER41 isolate had strong antagonistic activity against all the pathogenic bacteria (ZDI>20 mm), while the antagonistic activity was lowest towards the *Candida albicans* (ZDI = 18 mm). Strong inhibition was highest towards *Salmonella enterica* serovar Typhi (ZDI = 31 mm) and *E. coli* whereas, *S. aureus* (ZDI = 25 mm) had the least inhibition among the pathogenic bacteria recruited in the study.

Table 4.1: Antimicrobial activity of *Lactiplantibacillus plantarum* EGER41 isolate against select indicator pathogenic organisms

Pathogenic microorganism	Zone diameter of inhibition (mm)
Salmonella enterica serovar Typhi	31.0±1.73[a]
Escherichia coli	28.0±1.00[ab]
Staphylococcus aureus	25.0±1.52[b]
Candida albicans	18.0±0.76[c]
Controls (Without *L. plantarum*)	0*

The values are means ± standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are not significantly different at p=0.05. *There was no inhibition zone for indicator organisms without *Lactiplantibacillus plantarum* EGER41.

4.1.6 Hemolytic Activity of *Lactiplantibacillus plantarum* EGER41

The haemolytic activity of the *L. plantarum* EGER41 isolate was evaluated on 5% defribrinated sheep blood agar plates. The result in Figure 4.4 showed no clear transparent or greenish zone on the streaked area of the blood agar plates surrounding the colonies, indicating that the isolated *L. plantarum* isolate as non-haemolytic or classified as γ-haemolytic after 48 h of incubation.

Figure 4.4: Haemolytic activity of *Lactiplantibacillus plantarum* EGER41 isolate

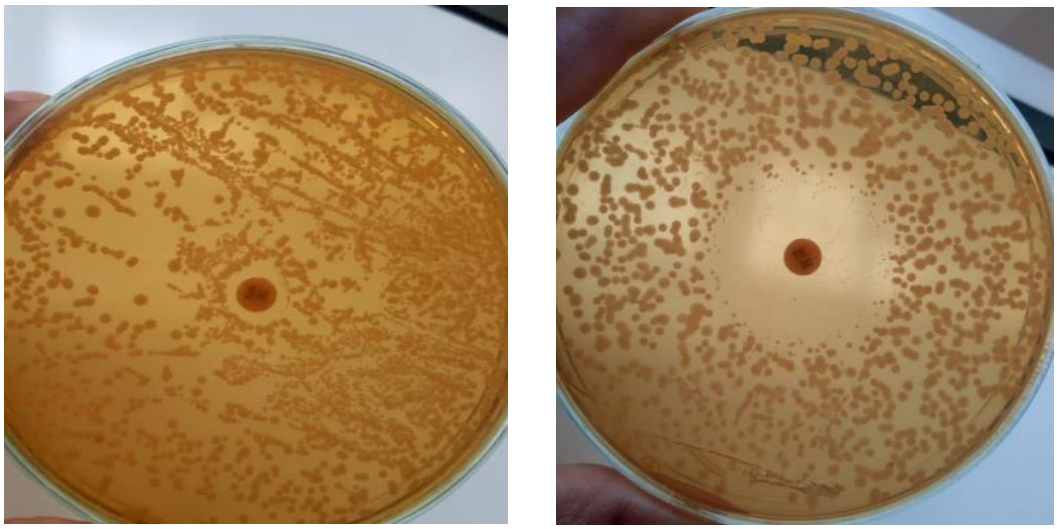
4.1.7 Antibiotic Susceptibility Assay of *Lactiplantibacillus plantarum* EGER41

The antibiotic susceptibility of the *L. plantarum* EGER41 isolate was performed using different commonly used antibiotics and is depicted in Figure 4.5. The figure showed that *L. plantarum* EGER41 was resistant to nalidixic acid (A) and sensitive to tetracycline (B). The results presented in the Table 4.2 revealed that the isolate was sensitive to azithromycin, tetracycline, and chloramphenicol (ZDI: >21mm), whereas it was resistant to nalidixic acid, ampicillin, and ciprofloxacin (ZDI <15 mm) and exhibited intermediate susceptibility towards gentamycin (ZDI: 16-20 mm).

Table 4.2*:* Antibiotic susceptibility test results for *Lactiplantibacillus plantarum* EGER41

Antibiotics	Concentration (μ/disc)	Zone diameter of inhibition (mm)	Susceptibility
Nalidixic Acid	30	6.0 ± 1.41^{f}	Resistant
Ampicillin	10	13.5 ± 0.71^{e}	Resistant
Azithromycin	15	35.5 ± 0.71^{a}	Sensitive
Ciprofloxacin	30	8.0 ± 0.1^{f}	Resistant
Tetracycline	30	25.5 ± 2.12^{b}	sensitive
Gentamycin	10	17.0 ± 1.41^{d}	Intermediately Sensitive
Chloramphenicol	30	21.5 ± 0.71^{c}	Sensitive

The values are means ± standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are not significantly different at p=0.05.



A **B**

Figure 4.5**:** Antibiotic resistance (Nalidixic acid) (A), Tetracycline (B) of *Lactiplantibacillus plantarum* EGER41

4.2 Objective Two: To Determine the Effect of *Lactiplantibacillus plantarum* EGER41Fermentation on Anti-nutritional Content of the Complementary Porridge

4.2.1 Effects of Malting and fermentation with *Lactiplantibacillus plantarum* EGER41on phytates in complementary porridge

The control, which were samples that were neither fermented nor malted. The level of phytates differed across the samples, with the highest being the sample 3 that was fermented and malted for 24 h and 3 days respectively. While the lowest was sample 1 and 3 that were not malted and were fermented for 24 h. Substitution level 3 had the highest amount of phytates for the control sample. According to the analysis of variance, all the factors significantly affected the amount of phytates in the complementary porridge (Appendix 3). The phytates increased after two days of malting then it decreased. The decrease observed was by 51% and 50.9% for sample 1 and 2, respectively. The 100% finger millet sample three had more phytates compared to the other mixed samples and its content increased throughout the malting process. The same observations were made for the process of fermentation by *L. plantarum* EGER41. There was a decrease from 12 h to 24 h of fermentation by 56.0% and 26.9% for sample 1 and 2, respectively. Interactively, with both fermentation and malting, the phytate content of the complementary porridge flours increased continuously except for sample 1. There was a significant decrease in phytate content for sample 1 after 2 days of malting in both 12 and 24 h of fermentation; 14.6% decrease in 12 h

fermentation and 49.5% decrease in 24 h fermentation. While for sample, two and three the phytates continued to increase throughout the processes.

Table 4.3: Effects of malting and fermentation using *Lactiplantibacillus plantarum* EGER41 on phytates in complementary porridge

Porridge flour		Phytate P mg/100g (db)		
Fermentation (hrs)	Malting(days)	Substitution level 1	Substitution level 2	Substitution level 3
0	0 (Control)	540.00±94.28^{h}	506.67±47.14^{h}	706.67±47.14^{g}
0	2	1373.33±141.42^{c}	1506.67±47.14^{b}	733.33±47.14^{g}
0	3	673.3±94.28^{gh}	740.00±94.28^{g}	940.00±94.28^{ef}
12	0	773.33±141.42^{g}	1240.00±47.14^{d}	873.33±94.28^{fg}
12	2	1140.00±0.00^{de}	1040.00±47.14^{e}	940.00±0.00^{ef}
12	3	973.33±47.14^{ef}	1140.00±0.00^{de}	1006.67±0.00^{e}
24	0	340.00±94.28^{i}	906.67±47.14^{f}	117.33±141.42^{i}
24	2	573.33±47.14^{h}	706.67±47.14^{g}	1373.33±141.42^{a}
24	3	906.67±47.14^{f}	973.33±47.14^{ef}	1673.33±94.28^{a}

Values are means ± standard deviation, Substitution level 1: 50% finger millet flour and 50% Cassava flour, Substitution level 2: 70% finger millet flour and 30% Cassava flour, Substitution level 3: 100% finger millet flour, db-dry weight basis.

4.2.2 Effects of malting and fermentation with *Lactiplantibacillus plantarum* EGER41 on tannins in complementary porridge.

Highest levels of tannin were recorded for the substitution level 2 with 10.37±0.33 which was malted and fermented for 3 days and 24 h, and 10.29±0.65; malted for 3 days and fermented for 12 h. While the lowest was observed for sample: 0.18±0.01, which was malted for 2 days and fermented for 24 h. Substitution level 3, had the lowest amount of phytates for the control sample. According to the analysis of variance (Appendix 3), all the factors significantly affected the amount of tannins at alpha level of 0.05%. Also, the means were

significantly different for each factor as shown in Appendix 3. The control which were samples that were neither fermented nor malted; sample one, had the highest amounts tannins. Most of the samples that were malted and fermented had increased amounts of tannins. After 2 days of malting, there was an increase in tannins content, then after 3 days, the tannin content decreased except for sample 2, which had an increase. The decrease in tannin content was 31.18% and 18.39% for sample 1 and 3, respectively. It was observed that after 12 h of fermentation, there was an increase in tannin content for all the samples, but after 24 h, there a 58.36% decrease for Sample 1, but between 12 and 24 h of fermentation there was 88.2%, 60.3% and 27.27% decrease in tannin content for sample 1, 2, and 3, respectively. However, the tannin content was slightly higher after 24 h of fermentation compared to the initial content at the start of fermentation.

Table 4.4: Effects of malting and fermentation with *Lactiplantibacillus plantarum* EGER41 on Tannins in complementary porridge

Porridge flour		Condensed tannin as mg CE / 100(db)		
Fermentation(hrs)	Malting(days)	Sample 1	Sample 2	Sample 3
0	0(control)	2.67 ± 0.07^{k}	2.14 ± 0.01^{l}	0.67 ± 0.03^{o}
0	2	4.33 ± 0.14^{h}	3.28 ± 0.02^{i}	5.71 ± 0.32^{f}
0	3	2.98 ± 0.24^{j}	4.43 ± 0.07^{gh}	4.66 ± 0.24^{g}
12	0	9.50 ± 0.14^{a}	7.38 ± 0.24^{e}	1.65 ± 0.01^{n}
12	2	1.41 ± 0.08^{n}	7.55 ± 0.44^{b}	8.34 ± 0.02^{c}
12	3	4.16 ± 0.16^{h}	10.29 ± 0.65^{a}	4.10 ± 0.18^{h}
24	0	1.12 ± 0.15^{n}	2.93 ± 0.06^{jk}	1.20 ± 0.01^{n}
24	2	0.18 ± 0.01^{p}	0.92 ± 0.09^{no}	2.86 ± 0.06^{jk}
24	3	1.51 ± 0.11^{n}	10.37 ± 0.33^{a}	4.10 ± 0.08^{h}

Values are means ± standard deviation, Sample 1: 500% finger millet flour and 50% Cassava flour, Sample 2: 70% finger millet flour and 30% Cassava flour, Sample 3: 100% finger millet flour, CE-catechin equivalent, db-dry weight basis.

4.2.3 Effects of Malting and fermentation with *Lactiplantibacillus plantarum* EGER41 on cyanide in complementary porridge flour.

As observed substitution level 1, had the highest amount of cyanide content compared to the rest of the substitution levels. The control sample for substitution level 1 had the highest

cyanide content of 20 ppm while the lowest was for the substitution level 3 of 5.5 ppm. The samples with the lowest amount of cyanide had 2.5-ppm cyanide content. The cyanide content decreased during the malting period where it decreased by 75% for substitution level 1, 2, and 9% for substitution level 3. After 12 h of fermentation, the cyanide levels decreased by 87.5%, 50% and 55% for sample one, two, and three, respectively. There was a higher decrease in cyanide levels after 12 h of fermentation as compared to 24 h of fermentation. The decrease observed after 24 h of fermentation was 75%, 50% and 9% for sample 1, 2, and 3, respectively. Comparing between fermentation and malting, 12 h of fermentation with *Lactiplantibacillus plantarum* EGER41 was adequate in reducing cyanide content in the complementary porridge flour. There was no more decrease in cyanide content for sample 3 after two and three days of malting; it remains constant. The decrease observed after three days of malting is by 50%, 45% and 9% for sample 1, 2 and 3, respectively.

Table 4.5: Effects of Malting and fermentation with *Lactiplantibacillus plantarum* EGER41 on cyanide content of the complementary

Fermentation (hrs)	Malting (days)	Substitution level 1	Substitution level 2	Substitution level 3
0	0(control)	20^{a}	10^{ab}	5.5^{b}
0	2	5.5^{b}	5^{b}	5.5^{b}
0	3	5^{b}	2.5^{b}	5^{b}
12	0	2.5^{b}	5^{b}	2.5^{b}
12	2	10^{ab}	5^{b}	5^{b}
12	3	10^{ab}	5.5b	5^{b}
24	0	5^{b}	2.5^{b}	5^{b}
24	2	20^{a}	5^{b}	5^{b}
24	3	5^{b}	5^{b}	2.5^{b}

Substitution level 1: 50% finger millet flour and 50% Cassava flour, Substitution level 2: 70% finger millet flour and 30% Cassava flour, Substitution level 3: 100% finger millet flour.

4.3 Objective Three: Descriptive Sensory Analysis of the Complementary Porridge

4.3.1 Microbial analysis of the complementary porridge

The table below shows microbial quality for the seven tested samples in descriptive sensory analysis, which according to the KEBS standards were below the acceptable limits. The dried complementary porridge flour showed no growth in total coliform count and total

viable count, while the yeast and moulds were within acceptable limits of the KEBS standards.

Table 4.6: Microbial quality of the complementary flour formulations

Porridge flour			Microbe		
Formulation	Fermented	Malted	Total viable count	Total coliform count	Yeast and moulds
1	0	0	nd	nd	2.2×10^2
2	0	2	nd	nd	nd
3	0	3	nd	nd	2.1×10^2
4	12	0	nd	nd	2.5×10^2
5	24	0	nd	nd	1.5×10^2
6	24	2	nd	nd	1.4×10^2
7	24	3	nd	nd	8.7×10^2
Limits[1]			1.0×10^5	Nil	1.0×10^5

nd- not detected; [1]Kenyan Bureau of Standards (KEBS 2008)

4.3.2 Sensory Attributes

4.3.3 Appearance of the complementary porridge

The Descriptive scores for the appearance of the porridges formulated from different composite flours are presented in Table 4.7. There were no significant (p=0.05) differences in descriptive scores for the seven different formulations of porridge in terms of colour and glossiness, but for consistency and syneresis, there were significant differences between the samples.

Table 4.7: Appearance of porridge from different cassava-finger millet composite flour formulations

Formulation	colour	Consistency	Syneresis	Glossiness
1	5.55±2.07[a]	6.91±1.45[b]	4.18±2.82[b]	5.82±2.27[a]
2	5.0±1.73[a]	5.0±2.53[ab]	4.45±2.30[b]	4.91±2.21[a]
3	5.27±1.90[a]	5.09±2.34[ab]	5.45±1.51[ab]	4.82±2.14[a]
4	6.27±1.85[a]	6.64±1.86[b]	2.82±2.4[b]	6.0±1.18[a]
5	6.5±1.98[a]	6.5±2.611[b]	4.5±2.2[b]	6.5±2.02[a]
6	4.64±2.50[a]	3.55±1.75[a]	5.91±2.51[ab]	3.91±2.02[a]
7	4.2±2.20[a]	4.0±2.21[ab]	6.7±2.45[a]	4.1±2.28[a]

The values are means ± standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are not significantly different at p=0.05.

4.3.4 Texture of the complementary porridge

The flow ability and lumpiness was the same for the seven samples except for coarseness and presence of residues where the sample hedonic means differed (Table 4.8).

Table 4.8: Texture of porridge from different cassava-finger millet composite flour formulations

Formulations	Flow ability	Coarseness	Lumpiness	Presence of residues
1	2.45±1.13[a]	6.36±1.96[bc]	5.27±2.33[a]	6.8±1.69[c]
2	3.45±1.21[a]	6.45±1.75[c]	5.36±2.38[a]	6.36±1.86[bc]
3	6.73±1.35[b]	4.45±2.02[abc]	3.82±2.14[a]	4.27±1.79[ab]
4	3.36±2.87[a]	4.73±1.62[abc]	4.91±2.21[a]	5.09±2.07[abc]
5	6.67±1.88[b]	2.83±1.19[a]	3.42±1.93[a]	3.33±1.83[a]
6	7.27±1.95[b]	4.18±2.18[ab]	3.82±1.83[a]	4.91±2.26[abc]
7	8.2±0.79[b]	3.9±1.85[a]	3.1±1.97[a]	4.2±1.69[abc]

The values are means ± standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are not significantly different at p=0.05.

4.3.5 Flavour (Aroma) of the complementary porridge

There was a significant difference between the samples for the fermentation and malty aroma but all the samples had the same starchy aroma, finger millet aroma and rancidity aroma at p=0.05 (Table 4.9).

Table 4.9: Flavor (aroma) of porridge from different cassava-finger millet composite flour formulations

Formulations	Fermentation aroma	Malty aroma	Starchy aroma	Rancidity aroma	Finger millet aroma
1	2.82±1.72[a]	3.27±2.054[a]	5.18±2.27[a]	3.64±2.50[a]	6.09±2.51[a]
2	2.55±1.97[a]	3.00±2[a]	5.09±2.47[a]	3.36±1.91[a]	6.09±2.63[a]
3	3.45±1.75[a]	4.09±1.58[a]	5.55±1.92[a]	3.27±1.85[a]	5.18±2.52[a]
4	6.36±2.20[b]	6.64±1.36[b]	5.73±1.79[a]	4.91±2.3[a]	4.82±1.66[a]
5	7±1.28[b]	6.67±1.92[b]	4.83±1.34[a]	5.67±2.67[a]	5.08±2.15[a]
6	7.64±1.36[b]	6.73±2.01[b]	4.55±1.64[a]	5.64±2.77[a]	4.91±2.34[a]
7	7.6±1.08[b]	7.2±1.87[b]	4.8±1.69[a]	6.2±2.66[a]	4.8±2.25[a]

The values are means ± standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are not significantly different at p=0.05.

4.3.6 Flavour (Taste) of the complementary porridge

All the samples differed in taste scores with the different definitions of the samples (Table 4.10). The sourness, after taste, and astringency were significantly different with minimal score differences. On the other hand, the sugary taste varied markedly among the samples at p=0.005.

Table 4.10: Descriptive scores on flavor (Taste) of porridge from different cassava-finger millet composite flour formulations

Formulation	Sugary taste	Sourness	After taste	Astringency
1	5.09±2.07^{bcd}	3±1.67^{a}	3.27±2.24^{a}	3.18±1.78^{a}
2	5.36±1.86^{cd}	3.09±2.3^{a}	4±2.57^{ab}	3.09±2.02^{a}
3	6.45±1.86^{d}	3.36±2.11^{a}	3.55±1.97^{ab}	2.73±2.01^{a}
4	2.91±2.02^{ab}	7.18±1.54^{ab}	5.45±1.37^{ab}	6.55±2.34^{b}
5	2.58±1.56^{a}	7.75±0.97^{ab}	5.17±1.64^{ab}	7.0±1.86^{b}
6	2.73±1.42^{ab}	7.73±1.09^{ab}	5.91±2.12^{b}	7.45±1.21^{b}
7	3.20±2.39^{abc}	7.20±1.23^{b}	6.20±1.4^{b}	7.2±1.23^{b}

The values are means ± standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are not significantly different at p=0.05.

4.3.7 Principal component analysis (PCA)

A scree test conducted on the principal components by plotting a scree plot to determine the number of principal components to retain in the PCA. According to the scree plot in Figure 4.6, at least two factors are related and retained as being significant.

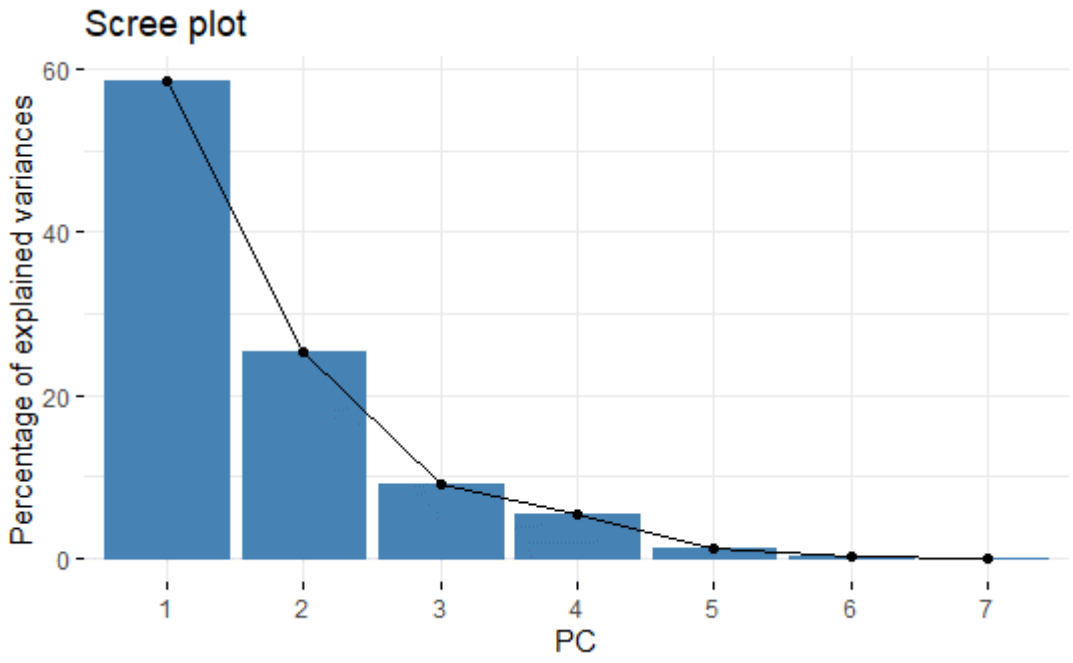


Figure 4.6: Scree plot

Figure 4.8 and 4.9 illustrates PCA results; the samples are located near the descriptors that characterize them. In this study, two principal components obtained and accounted for 97.3% of the total variability observed among the treatments. The first PC explained 58.5% and PC 2 explained 25.2 % of the variation. Figure 4.7 shows that formulation 1, 2, 3, 7 and 6 were highly represented on the principal components because of the high Cos 2 values while formulation 5 was medially represented with formulation 4 being poorly represented.

As shown by figure 4.8 and 4.9, sample 1, which was the control sample: neither malted nor fermented had a significant effect on the sensory characteristics such as glossiness consistency, starchy aroma and lumpiness, which contributed to the first quadrant. Sample 5 and 6, which were fermented for 24 h and malted for 0 days and 3 days respectively, had a significant effect on sensory attributes such as odour, sourness, astringency rancidity aroma and malty aroma, which contributed on the second quadrant. Sample three and seven, which were fermented for 0 hours and 24 h; and malted for 3 days and 2 days respectively, had a significant effect on sensory attributes such as syneresis, flow ability, fermentation aroma and after taste which contributed on the third quadrant. Sample 2 and 4, which were fermented for 0 h and 24 h; and malted for 2 days and 0 days respectively, had a significant effect on sensory attributes such as finger millet aroma, coarseness, presence of residues and sugary taste, which contributed on the fourth quadrant.

Most attributes like glossiness, colour, consistency, sourness, astringency, rancidity aroma, malty aroma, fermentation aroma, flow ability and syneresis made a high contribution to PC1. Lumpiness, finger millet aroma, presence of residues, sugary taste, after taste and coarseness attributes made medium contributions to PC2 as starchy aroma made the least of the contributions to the PCs as shown in figures 4.8 and 4.9.

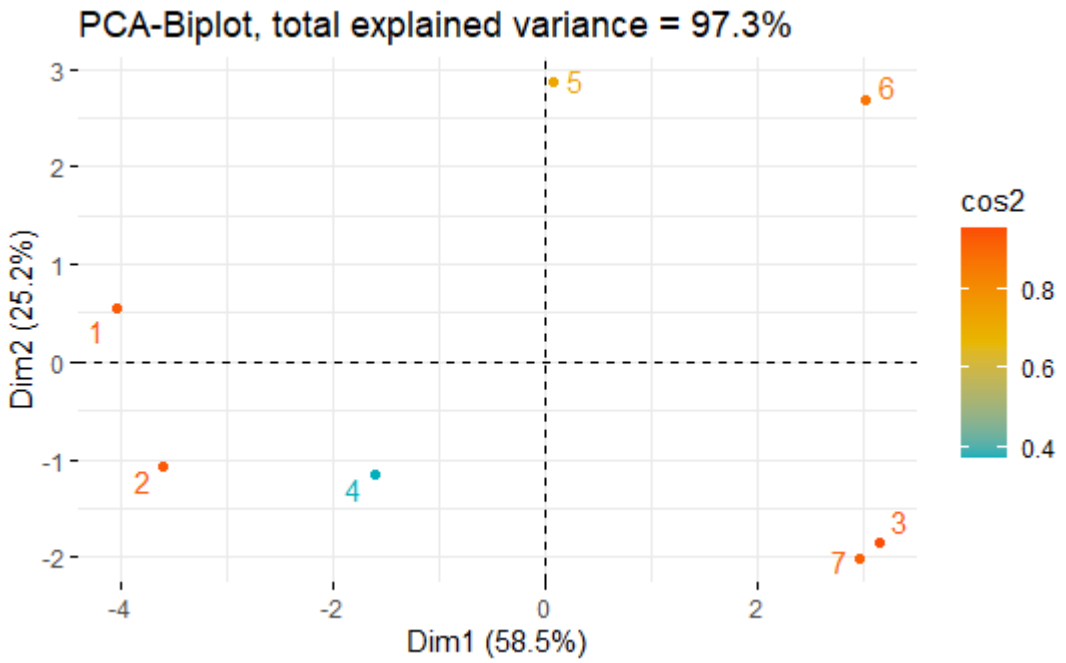


Figure 4.7: Quality of representation of the formulations on factor map (Cos2/ square cosine)

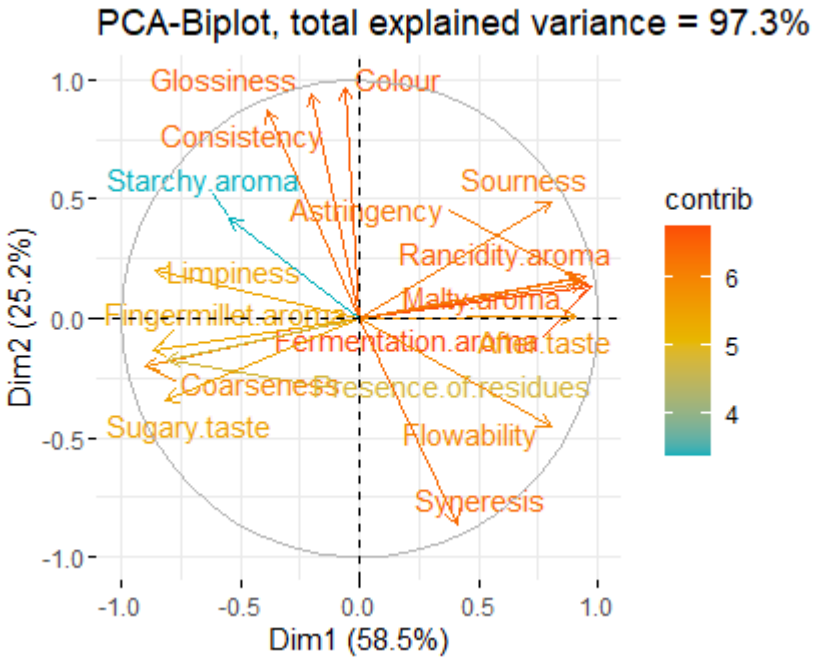


Figure 4.8: Contribution of each attribute/variable to the PCs.

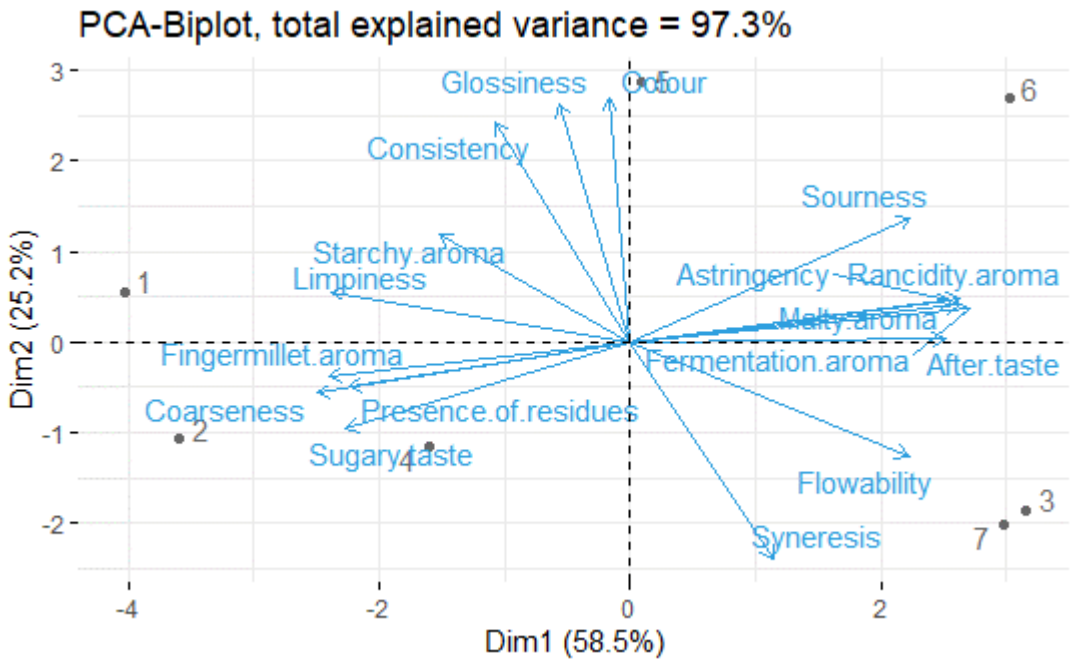


Figure 4.9: Principal Component analysis of sensory data of complementary porridge samples. Principal loadings 1 and 2, including all evaluated descriptors with treatments 1 to 7.

CHAPTER FIVE

DISCUSSION

5.1 Objective One: To Characterize Functional Properties of *L. plantarum* from Fermented Milk

Majority of lactic acid bacteria have been enhanced and utilized as probiotics for improvement of both animals and human beings' health (Rajoka *et al*., 2017). Attempts to screen and isolate new LAB bacteria from different foods is in progress to obtain those with significant probiotic properties for commercialization of foods with functionality and scientific purposes. The objective of the present study was to evaluate the probiotic potential of *L. plantarum* strain isolated from traditionally fermented milk, *amabere amaruranu* (Sichangi *et al*., 2020). This was to assess the probiotic and safety properties of *L. plantarum* isolate, whether it could overcome the hurdles in the gut, colonize and pass its beneficial health effect to the consumer.

Survival and growth in the human gastrointestinal tract temperature (37°C) is an essential factor in determining whether the isolated isolate could survive within the normal body temperature ranges and industrial production conditions. This determines the effectiveness of probiotics hence, considered during selection (Dixit *et al*., 2013). In this study, the *L. plantarum* isolate survived well at temperature of 30°C and 37°C, whereas its survival was poor at temperatures of 45°C, results which are similar to those of other Lactobacillus strains was reported by Kim *et al*. (2019). At 20°C, the low survival rate perhaps was due to reduced metabolic activity. The survival ability of *L. plantarum* at different temperatures indicated that it could retain its viability throughout the human gastro intestinal tract movement and conditions for industrial processes hence, its applicability as a probiotic after accomplishing other standards (Essayas *et al*., 2020; Kim *et al*., 2019).

Acid tolerance is important for the assay of probiotic bacteria because it is vital for its survival in the harsh environment of the stomach during passage (Gupta *et al*., 2020). In this study, there was no viability loss of *L. plantarum* when exposed to pH of 2.0, 2.5, 3.0 and 3.5. These results were in agreement with Srinu *et al*. (2013), who reported good survival abilities of *L. plantarum* at different low pH ranges. As observed, at pH 2.0 there was a remarkable decrease in the viability of *L. plantarum*, which is in unison with Angmo *et al.* (2016), who recorded a significant decrease in survival rates of LABs at pH 2.0. Soliman *et al*. (2015) also observed no loss of viability after 3 h of exposure to pH 3.0, hence high levels of resistance by our *L. plantarum* isolate. The standard pH for this investigation is 3.0 (Halder

et al., 2017). The property of acid resistance by Lactobacillus is due to presence of F_0F_1-ATPase activity (Angmo *et al.,* 2016). The low pH in the stomach is essential for preventing entry of bacterial into the intestinal tract (Gupta *et al*., 2020). During fasting, the stomach pH lowers to 1.0 and after a meal, it increases to 4.5 and it takes three hours for food to be ingested (Mohammad *et al.,* 2020; Soliman *et al.,* 2015). It is important for the isolates to foods such as fruits (Citrus fruits like oranges and lemons) and fermented food products with low pH. (Angmo *et al.,* 2016; Srinu *et al.,* 2013). The study demonstrates that the *L. plantarum* isolate could tolerate the low stomach pH environment and provide health benefits to the host after transit.

New probiotics should be resistant to phenols since they are harmful metabolites formed in the intestine as regular byproducts of aromatic amino acids (derived from dietary and endogenous) deamination by bacteria (Forhad *et al.,* 2015; Soliman *et al.,* 2015). Phenols have *in vitro* bacteriostatic effect and could inhibit the establishment and growth of probiotic bacteria (Gupta *et al*., 2020). The *L. plantarum* isolate exhibited excellent resistance towards 0.4% phenol, which is important as it indicates that the isolate could get through gastrointestinal setting to exert benefits to the health host.

For novel probiotics, screening for safety is paramount to ensure toxins, virulence factors, transmission of antibiotic resistance genes and hemolytic potential are non-existent in them. (Angmo *et al.,* 2016; Yasmin *et al.,* 2020). The assessment done for safety purposes of the isolate included hemolytic action, antibiotic tolerance and antagonistic activity of the isolate. Probiotics should not harbor resistant gene material transferable to the intestinal microbiota (Gupta *et al*., 2020). The *L. plantarum* isolate was resistant to nalidixic acid, ampicillin, and ciprofloxacin. Soliman *et al*. (2015) had demonstrated resistance of *L. plantarum* isolates against nalidixic acid, ciprofloxime, kanamycin, clindamycine, cefotaxine, vancomycin, and gentamycin. Yadav *et al*. (2016) also found out the four indigenous isolates of *L. plantarum* isolates from raabadi a fermented beverage to be resistant to ciprofloxacin, vancomycin, and nalidixic acid. This indicates that resistance to nalidixic acid, ampicillin and ciprofloxacin could be widespread among *L. plantarum* strains, which harbor antibiotic resistance genes, transferable plasmids, and conjugative transposons. Resistance to antibiotics could enable the strains to be resistant to antibiotics in the case of administration of antibiotics for therapeutic healing of intestinal disorders or their avoidance. Nonetheless, the resistant genetic genes pose a threat to human health, when transferred to human pathogens making them resistant to anti-biotics (Soliman *et al*., 2015). The *L. plantarum* isolate was sensitive to most of the

antibiotics such as, azithromycin, tetracycline, and chloramphenicol and intermediately sensitive to gentamicin. Halder *et al*. (2017) found *L. plantarum* isolates that were intermediately susceptible to gentamicin but sensitive to ampicillin and gentamicin. Hoque *et al*. (2010) also reported *Lactobacillus* spp. isolated from Bogra yoghurt of Bangladesh that was slightly sensitive to gentamycin. These results indicate Lactobacillus strains are sensitive to antibiotics.

Antagonistic activity against pathogens is crucial for safeguarding the host from intestinal pathogenic infection (Wang *et al*., 2018), maintenance of a healthy microbial balance in the gut (Halder *et al*., 2017), and preventing food spoilage and extend shelf life (Jampaphaeng *et al*., 2017). The naturally fermented milk isolate of *L. plantarum* exhibited an extended antibacterial spectrum against pathogenic indicator microorganisms. Maximum inhibition was against *S. typhi*, followed by good inhibition against *E. coli* and moderate inhibition against *S. aureus,* while the least inhibition was against *Candida albicans*. Similar inhibition trends had been reported by Halder *et al*. (2017) following agar overlay method that showed that *L. plantarum* LMEM7 isolate had high growth inhibitory activity against *Acinetobacter baumanii, E. coli,* and *Proteus vulgaris.* The ZDI of *L. plantarum* LMEM7 isolated by Halder *et al*. (2017) was 30.00 ± 1.71mm with *E. coli,* which is similar to a ZDI of 28 mm against *E. coli* that we recorded with our isolate in this study. Wang *et al*. (2018) reported that their isolate; *L. plantarum* PIC33 had strong antagonistic activity towards *S. aureus, S. enterica* and *S. dysentriae,* while Olatunde *et al.* (2018) recorded significant inhibition of *S. aureus, S. typhimurium* and *E. coli* by selected strains of LABs. The antagonistic activity of Lactobacillus is due to secretion of anti-microbial compounds such as organic acid mainly (lactic acid), hydrogen peroxide, secondary metabolites, and bacteriocins (Halder *et al.,* 2017).

Probiotic strains; newly isolated should be incapable of haemolysing blood. The hemolytic action of the *L. plantarum* isolate in this research employed 5% defibrinated sheep blood agar plates. The isolate exhibited γ-hemolytic or no hemolytic activity and did not cause α- or β-hemolysis. The γ-hemolytic activity is important because this implies that the strain is has no virulence factors hence no pathogenicity. Wang *et al*. (2018) found similar results and Mohammad *et al.* (2020) recorded that all their LAB isolates had γ-hemolytic activity. These results indicate the *L. plantarum* isolate can prepare probiotic food products for human consumption that are safe.

5.2 Objective Two: To Determine the Effect of *L. plantarum* Fermentation on Anti-nutritional Content of the Complementary Porridge

Foods containing cereals have low bioavailability of minerals, which is a critical problem for young children and infants since they are quickly growing mentally and physically in developing countries, where there is limited availability to animal-source foods and presence of anti-nutritional factors; phenolic compounds, phytates and fibers (Ares *et al*., 2018; Vishakha & Boora, 2016). Fermentation with lactic acid bacteria (LAB) as starter cultures has been observed to improve the nutritional quality of foods by making minerals to be bioavailable, increasing protein digestibility and boosting the amounts of vitamin B and C especially in weaning foods (Achi & Asamudo, 2018; Adeyeye *et al.*, 2019).

5.2.1 Phytic Acid

After malting for 72 and 96 hours, there was a considerable decrease in the phytic acid (Samtiya *et al*., 2020). The phytic acid decrease in this study could be attributed to the presence of native phytases and fermenting microorganism's phytases (Ares, 2018; Osman, 2011). Germination of cereals improves the activity of phytases as compared to non-malted cereals (Vashishth *et al*., 2017).

The significant decrease in phytic acid after 24 hour of fermentation could have be due to by elevated pH levels, which might be optimum for microbial phytases, as Ares (2018) observed that after 72 hours, the decrease in phytic acid was reported to be higher. The phytase enzyme breaks down phytates into to myoinositol, phosphorus, and its intermediate forms (Feyera, 2020). Gabaza *et al*. (2018) made similar observations where in red variety 2 of finger millet no change observed in phytic acid after fermentation, while in red variety 1 and white variety 1 of finger millet, slight increase observed. The increase of phytates after fermentation is unascertained but it might be the conversion of nutrients or plant metabolites in the solution to phytates or phytate- like products as suggested (Uduro *et al*., 2021).

The decrease in phytates during 12 to 24 hours of fermentation by *L. plantarum* is as attributed by presence of amylases in the malted finger millet that break down the cassava starch into simple sugars. Hence, enabling the fermenting microbes to grow and synthesize phytase enzyme as compared to the sample 3 with finger millet only where reduction in phytic acid was minimal. As Oguntoyinbo and Narbad (2015), observed that fermenting non-malted cereal grains results in low availability of fermentable sugars for the rapid growth of fermenting microflora. Hashem *et al*. (2018) also reported maximum decrease in phytic acid as observed at the pH range 4.5-5.5 after 12-16 hours of *L. plantarum* subsp. *plantarum.*

According to Hashem *et al*. (2019), *L. plantarum* subsp *plantarum* had the highest phytase activity of 2.77 U/mg compared to *L. casei* subsp. *casei* and *L. fermentum* which had 2.12 and 2.38 U/mg.

5.2.2 Condensed Tannins

The tannins might be bound to cellulose and proteins; hence, when enzymes produced during malting and fermentation with Lactobacillus strain break them down, they are released (Adebo & Meza, 2020; Altop, 2018). Additionally, the conversion of condensed tannins to proanthocyanidin might have caused its reduction (Osman 2011). Osman (2011) made similar observations during traditional fermentation of pearl millet in preparation of Lohoh. A study done by Hashemi *et al.* (2019), showed an increase in total phenolic content after 12 hours of fermentation of the dough by *Lactobacillus plantarum* subsp. *plantarum* compared to other *Lactobacillus* strains. This is due to production of lactic acid that improves total phenol extraction.

The decrease of tannin content observed could be due to rearrangement of the structures during fermentation due to acid production, self-polymerization leading to reduced extractability, and /or binding with other macromolecules such as amino acids and starch (Adebo *et al*., 2018). Increase in malting and fermentation time can also decrease the anti-nutrients (Nkhata *et al*., 2018).

5.2.3 Cyanide

In order to detoxify a food product, the aim is to reduce the cyanogenic glycosides such as lotaustralin and linamarin, and dhurrin (Kuliahsari *et al*., 2021). As shown in figure 5.3, when the cell wall is damaged the vacuole is opened the cyanogenic glycosides are exposed to enzymes that break it to form cyanohydrin which naturally reduces to hydrogen cyanide. The free hydrogen cyanide boils and evaporates at 26°C (Kuliahsari *et al*., 2021).

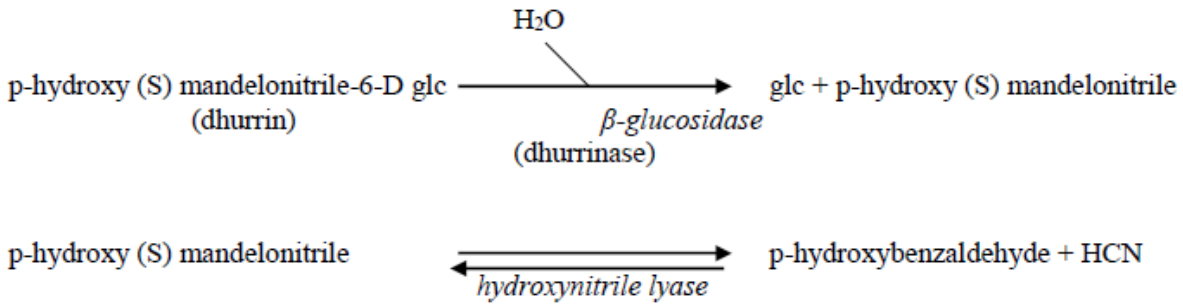


Figure 5.1: Dhurrinase (β-glucosidase) enzyme hydrolysis of dhurrin to hydrogen cyanide (Kuliahsari *et al.*, 2021).

The inherent β-glucosidase enzyme hydrolyzes dhurrin into p-hyroxy (S) mandelonitrile which in turn dissociates into p-hydroxybenzaldehyde and hydrogen cyanide as shown in figure 24 (Kuliahsari *et al.,* 2021). There are four pathways for biological breakdown in cyanide documented; 1) Cyanidase or cyanide hydratase breaks down cyanide to form ammonia and formate or amide respectively, 2) Cyanide is reduced to ammonia and carbon dioxide by monooxygenase and cyanase, 3) Cyanoalanine synthase converts cyanide to β-cyanoalanine, 4) its catalyzed to ammonia and methane by nitrogenase enzyme (Shen *et al*., 2021).

A study done by Ojha *et al*. (2017) on reduction of anti-nutrients in sorghum depicted an increase in cyanide content of the malted grain compared to the fermented one. This finding were almost similar to our findings where 12 hours of fermentation decreased more cyanide compared to malting. This could be due to dhurrin produced during steeping of the seeds and cyanogenic glucosides biosynthesized from amino acid precursor during malting (Ojha *et al*., 2017). In another recent study done to improve the quality of modified cassava through fermentation by Damayanti *et al.* (2021) showed 52.06% decrease in hydrogen cyanide due to fermentation using yeast Tr7 and LAB G6, which also had the highest amylolytic activity compared to various types of traditional fermented cassava isolates. Qin *et al.* (2021) recorded the highest decrease in cyanide content of 81.5 % after scarification and fermentation of cassava flour, hence the reduction of cyanide in our studies after malting the finger millet.

5.3 Objective Three: Determination of Descriptive Sensory Analysis of the Complementary Porridge

According to the microbial results, the porridge flours were safe for consumption since the microbial load was within the acceptable limits as per KEBS standard. (2008). Moreover, the samples with high cyanide content of > 10 ppm were eliminated and only those with low cyanide content were used for sensory evaluation. The drying process led to low microbial load, which decreased the moisture content of the flours. The absence of coliforms showed that the pasteurization process was effective and that the product processing was hygienic. Availability of coliform in food makes the food unfit for human consumption. It was essential to carry out the microbial analysis for the porridge flour to determine its safety for human consumption especially the vulnerable children below five years of age (Kinyuru *et al*., 2021).

PCA was performed on the sensory data from the complementary porridge of cassava and finger millet, since it summarizes complex data into lower dimensions called principal components (PCs), where the PCs are uncorrelated with each other (Lever *et al*., 2017). According to the scree plot (Figure 4.7), at least two factors are related. KMO and Bartlett's test showed that the data was adequate for performing PCA and that at least one component was principal. Figure 19 illustrates PCA results; the samples are located near the descriptors that characterize them.

The 1, 2 and 4 formulations were characterized by the descriptors starchy aroma, lumpiness, finger millet aroma coarseness, sugary taste and presence of residues while formulation's 7,3,6 and 5 were characterized by syneresis, flow ability, fermentation aroma, after taste, malty aroma, astringency, sourness, rancidity aroma and odour.

Most of the fermented samples were located near the fermented aroma and sour taste, which showed that fermentation, influenced the sensory attributes of the porridge. The formulations that had been fermented and malted were near the malty and fermentation aroma, syneresis and flow ability. The syneresis observed in these formulations was because of starch retrogredation. During the pasteurization of the porridge the starch present in cassava and finger millet gelatinized, on cooling the starch chains reassociate to form partially ordered structures different from the original granules (Wang *et al*., 2015). This caused the instant porridge flour to separate with water when reconstituted, but as the amount flour increased during reconstitution with hot water, the syneresis ceased; it may be that the flour partially underwent starch retrogredation. *Lactobacillus plantarum* being a homo fermenter producing only lactic acid caused sourness as identified in all the fermented porridge formulation. Due to breakdown of starch by amylases produced during malting in the hydrolysis stage of making the flour and during fermentation, caused the porridge to be highly flow able. Although some samples were not flowing easily (sticky) and were astringent due to high level of bitter tannins and polyphenols (Syeunda *et al*., 2019) in the flour especially the control sample that was neither malted nor fermented. The sugary taste observed, was due to malting because of starch breakdown into simple sugars by amylases. The glossier appearance of the porridge was due to the clarity of the aqueous starch gel in the porridge (Onyeoziri *et al*., 2021).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

This study demonstrated *Lactiplantibacillus plantarum* EGER 41 isolate as an excellent probiotic candidate. It exhibited high probiotic potential owing to its high resistance to pH, temperature and phenol and antibiotic susceptibility. The isolate also displayed strong antagonistic activity towards human pathogens, was sensitive to the most commonly used antibiotics, and had no hemolytic activity. Since the complementary food product may contain post-metabolites released by the probiotic culture during fermentation hence considered a functional food product. The aggregated benefits of the *Lactiplantibacillus plantarum* EGER 41 indicate its capacity to produce safe functional foods.

i. *Lactiplantibacillus plantarum* EGER 41 as a starter culture was effective in detoxifying the complementary porridge of its anti-nutrients. Fermentation was more effective in reducing the cyanide content compared to malting. Fermenting for 12 hours reduced the cyanide by 87.5%, 50% and 55% for sample 1, 2 and 3, respectively. Fermentation was able to lower cyanide levels as low as 2.5 ppm, which is within the acceptable limits set by FAO.

ii. Fermentation using a pure probiotic starter culture was effective in formulation of fermented finger millet- cassava based complementary porridge. Fermentation and malting improved the flavour of the porridge. With the aid of malting *Lactiplantibacillus, plantarum* EGER 41 effectively fermented the porridge in a short span of time. In addition, controlled fermentation ensured the microbial safety of the fermented porridge and hence possibility of commercialization and large-scale production.

6.1 Recommendations

i. Use more locally isolated microorganism from spontaneous fermented foods as probiotic starter cultures after investigation for industrial production to promote the use of locally indigenous starter cultures.

ii. However, more probiotic parameters and *in vivo* activity of the *Lactiplantibacillus plantarum* EGER 41 isolate need investigation to validate its immunomodulatory, nutritional, and health benefits.

iii. It will be of importance to consider analyzing for resistant starch probably found in the porridge flour after processing as this could add more health benefits to consuming the product.

iv. The analysis for the metabolites produced by *Lactiplantibacillus plantarum* EGER 41 during fermentation.

v. More production of novel products using *Lactiplantibacillus plantarum* EGER 41 isolate.

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APPENDICES

APPENDIX 1

Pre-screening Questionnaire

Name: _____

1. Phone Number: _____
2. Are there any weekdays (M–F) that you will not be available on a regular basis?

   _____

   ___
3. Do you have any of the following? Dentures _____ Diabetes

   _____ Oral or gum disease _____ Hypoglycemia

   _____ Food allergies _____ Hypertension _____
4. Do you take any medications which affect your senses, especially taste and smell?

   _____

   ___
5. Are you currently on a restricted diet? If yes, explain.

   _____

   ___
6. How often in a month do you eat fermented foods? _____
7. How often in a month do you eat malted foods? _____
8. What is (are) your favourite food(s)?

   _____

   _____

   ___
9. What is (are) your least favourite food(s)?

   _____

   _____

   ___
10. What foods can you not eat?

    _____

_____

___

11. What foods do you not like to eat?

_____

_____

___

12. Is your ability to distinguish smell and tastes

Above average _____ _____ Below average _____

13. If a recipe calls for vinegar and there is none available, what would you substitute?

_____

___

14. What are some of the malted and fermented products that you know?

_____

___

15. Are you familiar with food products termed as ‘complementary porridge’?

_____

16. How would you describe the difference between flavour and aroma?

_____

_____

___

17. How would you describe the difference between flavour and texture?

_____

_____

___

18. What is the best one- or two-word description of porridge?

_____

_____

__

19. Describe some of the noticeable flavours in

a) Finger millet _____

b) Cassava_____

20. Describe some of the noticeable flavours in malted cereals.

_____
___

21. Describe some of the noticeable flavours in fermented foods.

_____

_____
___

22. Is your sensitivity to textural characteristics in foods

Above average _____ Average _____ Below average _____

23. Describe some of the textural properties of foods in general.

_____

_____
___

24. Describe some of the particles one finds in foods. _____

_____
___

25. Describe the differences between spongy and rubbery.

_____

_____
___

26. What are some textural properties of cookies? _____

_____
___

27. What are some textural properties of porridge?

_____
___

28. For what type of products is texture important?

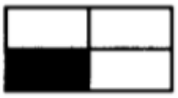
_____

_____
___

29. Instructions: mark on the line at the right to indicate the proportion of the area that is shaded.

EXAMPLES.

None ——/—— All



None ——/—— All

3. 

None —————— All

4. 

None —————— All

7. 

None —————— All

Contract

I _____ agree to be a panellist for **DESCRIPTIVE QUALITY SENSORY ANALYSIS OF SNACK BARS** for a period of one week.

I agree to comply with terms and conditions.

Signature ……

Date ……

APPENDIX 2

Sensory Evaluation

Descriptive test

Panellist number ……

Date …….

Gender: ……**…… Age Group……..……**

Instructions:

You are provided with 7 coded samples of porridge. You are required to rate the complementary porridge in caps as per the threshold of the attributes listed on top of the table in the appropriate box base on a 9-point hedonic scale.

Note:

- **Please rinse your mouth before starting and also in between when evaluating the samples.**
- **Evaluate the porridge in front of you by looking at it, feeling it and tasting it.**
- **Assign an appropriate score (with 1 being the least and 9 being the very) for each of the listed parameters/components.**

Complementary porridge evaluation

APPEARANCE

Colour: Degree of Brownness

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Light**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Dark(**

Consistency: Degree of visual uniformity of the sample/level of smoothness

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least Smooth**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Smooth**

Syneresis: Degree of separation.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Less**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **More**

	separation								**separation**

Glossiness: Degree of shininess at the surface of the porridge

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW									
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Dull**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Glossy**

Flow ability: Degree of movement of the porridge

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Highly**

TEXTURE

Coarseness: Extend of grittiness or granules of porridge caused by small particles

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Not Coarse**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Coarse**

Lumpiness: Amount of lumps in the porridge

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very**

Presence of residue: Amount of particles left in the mouth after swallowing

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Low**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **High**

FLAVOUR

Aroma

Aroma associated with fermented foods.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least perceivable**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Perceivable**

Aroma associated with malted foods.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**

XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least Intense**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Intense**

Starchy: Associated with high starch foods (cassava).

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least Starchy**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Starchy**

Rancid: Off odours/ unpleasant smells associated with malting and fermentation.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**

STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least Intense**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Intense**

Finger millet: Associated with uncooked finger millet.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Less Intense**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very intense**

TASTE

Sugary: Degree of sweetness due to malting and fermentation.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least Intense**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Intense**

Sourness: Degree of sourness due to fermentation.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least Intense**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Intense**

Aftertaste: how long the after-taste lasts/lingers in the mouth.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least Intense**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very Intense**

Astringency: due to puckering/shrinking of the tongue surface/ dry feeling in the mouth after swallowing.

Sample Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
BAC	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
CAB	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
PRQ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
STV	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
UVW	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
XYZ	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**
GHI	**[ ]** **Least**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]**	**[ ]** **Very**

	Intense								**Intense**

Comment:

APPENDIX 3

Analysis of Variance

Analysis of variance for phyates

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Ferme	2	274735.613	137367.807	20.05	<.0001
Malt	2	194730.798	97365.399	14.21	<.0001
Ferme*Malt	4	1262553.103	315638.276	46.07	<.0001
SubLevel	2	543375.712	271687.856	39.65	<.0001
Ferme*Sublevel	4	752427.893	188106.973	27.45	<.0001
Malt*Sublevel	4	300573.893	75143.473	10.97	<.0001
Ferme*Malt*Sublevel	8	1870276.947	233784.618	34.12	<.0001

Mean comparison (Tukey's HSD)

Fermentation	Means	Malting	Means	Substitution levels	Means
0	862.22[a]	0	862.22[a]	1	810.37[a]
12	936.30[b]	1	969.63[b]	2	973.33[b]
24	1036.30[c]	2	1002.96[b]	3	1051.11[c]

Analysis of variance for condensed tannin determination

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Ferme	2	174.6973444	87.3486722	2075.36	<.0001
Malt	2	19.0556333	9.5278167	226.38	<.0001
Ferme*Malt	4	34.2058556	8.5514639	203.18	<.0001
SubLevel	2	18.4865778	9.2432889	219.62	<.0001
Ferme*Sublevel	4	39.6246778	9.9061694	235.37	<.0001
Malt*Sublevel	4	77.5208889	19.3802222	460.46	<.000
Ferme*Malt*Sublevel	8	58.4910222	7.3113778	173.71	<.0001

Mean comparison (Tukey's HSD)

Fermentation	Means	Malting	Means	Substitution levels	Means
0	3.43[a]	0	3.25[a]	1	3.00[a]
12	6.11[b]	1	3.45[b]	2	3.87[b]
24	1.75[c]	2	4.6[c]	3	4.43[c]

APPENDIX 4

Nacosti Permit

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APPENDIX 5

Published Paper Abstract

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Preliminary evaluation of probiotic properties and safety profile of *Lactiplantibacillus plantarum* isolated from spontaneously fermented milk, *Amabere amaruranu*

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Department of Dairy and Food Science and Technology, Egerton University, P.O Box 536-20115, Egerton, Kenya

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Antagonistic activity
Lactic acid bacteria
Amabere amaruranu

ABSTRACT

The aim of this study was to assess the probiotic potential and safety profile of a *Lactiplantibacillus plantarum* EGER41 strain isolated from Kenyan spontaneously fermented milk, *Amabere amaruranu*. The *L. plantarum* EGER41 isolate was tested for temperature sensitivity (at 15 °C, 30 °C, 37 °C, and 45 °C), pH tolerance (at 2.0, 2.5, 3.0, 3.5, and 6.5 as control), and 0.4% phenol tolerance to observe its survival in the gastrointestinal tract of humans. For safety evaluation of the isolate, antagonistic activity was tested against pathogenic strains of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* serovar Typhi, and *Candida albicans*, while antibiotic susceptibility pattern was examined using nalidixic acid, ampicillin, azithromycin, ciprofloxacin, tetracycline, gentamicin, and chloramphenicol antibiotic discs and haemolytic activity was done using lamb blood agar. The *L. plantarum* isolate had an optimal growth at 37 °C, it also demonstrated low pH tolerance (2.0–3.5). It was able to maintain its viability (~100%) after exposure to 0.4% phenol. The selected isolate showed inhibition (antagonistic activity) against the pathogens with *S. typhi* having the largest (ZDI = 31.0 ± 1.73 mm) zone of diameter inhibition (ZDI) and *Candida albicans* having the least (ZDI = 18.0 ± 0.76 mm). *L. plantarum* isolate was sensitive to Azithromycin, tetracycline and chloramphenicol and was intermediately sensitive to gentamycin, while it was resistant to nalidixic acid, ampicillin, and ciprofloxacin. The isolate also exhibited γ-haemolytic activity hence safe for use as a starter culture and was identified as a *Lactiplantibacillus plantarum* EGER 41 strain based on 16S rRNA gene sequencing. The selected isolate can potentially be used as a starter culture and a probiotic since it had excellent probiotic properties.