BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF *Leuconostoc mesenteroides* ISOLATES AND THEIR BIO-DEGRADATION LEVELS OF SELECTED POST HARVESTED SUGARCANE (*Saccharum officinarum*) VARIETIES IN WESTERN KENYA

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A Thesis submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science in Microbiology of Masinde Muliro University of Science and Technology.

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DECLARATION

This thesis is my original research prepared with no other than indicated sources and has not been presented elsewhere for any other award.

Signature…… Date ……

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CERTIFICATION

We, the undersigned, certify that we have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled**'*Leuconostoc mesenteroides* isolates characterization and their bio-degradation levels of selected post-harvested sugar cane varieties.'**

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PLAGIARISM STATEMENT

DEDICATION

This work is dedicated to my late mother, Pilly Mughosi Khamasi, who constantly reminded me to work hard.

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ABSTRACT

Sugarcane (*Saccharum officinarum*) is a highly perishable crop that must be processed promptly after harvesting. In many countries that cultivate sugarcane, such as Kenya, there is typically a delay of three to seven days before milling occurs in sugar factories. This lag time results in the degradation of the sugar due to the action of the *Leuconostoc mesenteroides* bacterium. This bacterium converts sucrose into glucose and fructose, which cannot be crystallized, while also producing dextran, a branched glucose polymer. The presence of dextran in the sugar juice leads to specific problems, particularly in the crystallization process. The biodegradation of sugar in harvested sugarcane varies by variety. In Kenya, numerous sugarcane varieties are available to farmers, but the extent of their deterioration remains unclear. The use of sodium chloride in controlling sucrose deterioration in harvested sugarcane has been reported. However, the effective sodium chloride concentration for this purpose is not yet known. This study, therefore, sought to characterize three isolates of *Leuconostoc mesenteroides*, profile the biodegradation levels of selected post-harvested sugarcane, and determine a suitable concentration of sodium chloride for controlling the deterioration of harvested sugarcane. The three *Leuconostoc mesenteroides* isolates were collected from Kakamega, Nyando, and Sony sugar and sent to the Sugar Research Institute in Kisumu for isolation and identification on selective media. The confirmation test for the isolates was conducted by metabolizing different sugars. Four commonly adopted varieties, KEN 83-737, KEN 82-808, N14, and Co 421, were used to profile the deterioration levels by aseptically inoculating them with the three isolates in a closed bucket. Changes in deterioration parameters, namely Brix% juice, pol% juice, dextran, and pH, were measured over 10 days to facilitate evaluation. The results indicate that the Sony sugar isolate showed the highest levels of sugarcane deterioration, producing 1654.99 ppm of dextran. In contrast, the Kakamega and Nyando sugar isolates exhibited the least deterioration of sugarcane, producing 820.95 and 820.39 ppm of dextran, respectively. N14 and Co421 were the most and least degraded sugarcane varieties, losing 4.84 and 2.41 pounds of sucrose to dextran, respectively. Finally, Co421 and KEN83-737 varieties were specifically selected and used to determine the suitable sodium chloride concentration for controlling staleness in harvested sugarcane. The two varieties were treated with 6.5% NaCl, 13.0% NaCl, and a zero control supplemented with covering. Deterioration parameters were collected to evaluate sucrose loss. The results indicate that when harvested sugarcane is treated with 6.5% NaCl and 13.0% NaCl, sucrose loss is reduced by 18.04% and 18.31%, respectively. This study recommends prioritizing varieties susceptible to deterioration for milling to avoid sucrose loss. It also suggests that sugarcane milling after harvesting should be managed based on the region of harvest.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
CCS	Commercial cane sugar
COMESA	Common Market for Eastern and Southern Africa
ERS %	Estimated recoverable sugar
FAOSTAT	Food and Agriculture Corporate Statistical database
GDP	Gross domestic product.
ICUMSA	International Commission For Uniform Methods of Sugar Analysis
MMUST	Masinde Muliro University of Science and Technology
PPM	Parts per million
TCA	Trichloro-Acetic Acid
TCD	Tonnage Cane Daily

DEFINITION OF TERMS

Brix% % juice (symbol °Bx): This is a measure of the dissolved solids in a liquid and is commonly used to measure the dissolved sugar content of an aqueous solution.

Pol% juice: Also known as Sucrose percent, Pol of sugar is the resultant optical rotation of sucrose and other optically active substances, mainly glucose and fructose.

Dextran: An extracellular bacterial polysaccharide produced by lactic acid bacteria through the fermentation of sucrose.

pH: The pH scale expresses the acidity or alkalinity of a solution on a logarithmic scale, where seven is neutral. Lower values indicate a more acidic solution, while higher values indicate a more alkaline solution. The pH is calculated as $-\log_{10} c$, where c is the hydrogen ion concentration in moles per liter.

CHAPTER ONE

INTRODUCTION

1.0 Background of the study

Sugarcane (*Saccharum officinarum*) is an essential crop for daily life across numerous countries. Its wide-ranging applications and industrial uses significantly contribute to nutritional and economic development (Singh *et al*., 2019). The sugar cane industry is responsible for approximately 80% of the world's sugar production (de Matos *et al*., 2020). This means that nearly 28.3 million hectares across 90 nations are utilized for sugarcane farming, resulting in a global sugar output of about 1.69 billion tons(Singh *et al.*, 2025). Brazil and India lead in sugarcane production, with Brazil producing around 768,678,382 metric tons and India about 348,448,000 metric tons annually (Wani *et al.*, 2023). African countries produce only 5% of the world's sugar, with about 80% of their output originating from Sub-Saharan nations (Hess *et al*., 2016). The main sugarcane producers on the continent are South Africa, Sudan, Swaziland, Zambia, Mauritius, and Kenya, which together account for over half of Africa's total sugarcane production (Francis *et al*., 2020).

In Kenya, sugarcane is extensively planted in the western, rift valley, and the coastal region. The production of sugarcane in Kenya is one of the major agricultural activities contributing to national economic growth, alongside tea, coffee, horticultural crops, and maize (Yılmaz & Njora, 2021). The contribution of the Kenyan sugarcane sector to the total agricultural gross domestic product (GDP) is about 15%, with 25% of the Kenyan people relying directly and indirectly on sugarcane production for their living (Francis *et al.*, 2020).

Kenya can produce sufficient sugar to meet its domestic demand and even generate a surplus for export (Onyango, 2020). Currently, 16 sugarcane factories across the country have a combined milling capacity of 41,000 tons of cane daily (TCD), necessitating over 9.8 million tons annually (Kombo & Ndiema, 2022). This production could yield around 1.09 million tons of sugar, surpassing the annual demand for table sugar. However, the industry has struggled to meet local demands due to various challenges and inefficiencies within the value chain. This shortfall is addressed through imports from the Common Market for Eastern and Southern Africa (COMESA) region and other global sources (Tiema *et al*., 2024). Although the crop holds significant

importance, there has been limited research on primary varieties cultivated in the country regarding sucrose deterioration.In Kenya, numerous sugarcane varieties such as KEN 83-737, CO421, KEN82-808, and N14 are available to farmers, but the extent of their deterioration remains unclear. Consequently, assessing the bio-deterioration levels of these varieties will aid in minimizing sucrose loss by enabling better planning to shorten the time between harvesting and milling for each variety. This approach will allow for the recovery of a significant amount of sugar during processing, helping to address the gap of imported sugar needed to satisfy demand. The time lag between harvesting sugarcane and its processing is a critical issue for sugar millers (Misra *et al*., 2022), significantly affecting economic viability due to the decline in sugar content of the crop (Meghana & Shastri, 2020). This degradation begins immediately after harvesting, as the sugarcane is susceptible to microbial invasion, particularly by *Leuconostoc mesenteroides* (Van Tieghem, 1878) (*Lactobacillales, Lactobacillceae*) (Misra, Solomon, Hashem, *et al*., 2020). The bacterium in question utilizes sucrose as its primary energy source, converting it into compounds such as organic acids, reducing sugars, ethanol, and complex polysaccharides (Candeliere *et al.*, 2024). This process involves sucrose inversion, where the disaccharide is hydrolyzed into glucose and fructose, followed by the subsequent formation of polysaccharides (Asatkar & Basak, 2023). During this metabolic activity, the production of ethanol and organic acids increases, particularly during the staling period of sugarcane, which contributes to the crop's sugar content degradation (Misra *et al*., 2022). This study, therefore, sought to isolate and characterize three *Leuconostoc mesenteroides*isolates from three sugar cane growing regions: Kakamega, Nyando, and Sony. The isolates were then used to evaluate the deterioration levels of four popularly adopted sugar cane varieties under controlled conditions. Although the use of sodium chloride has been reported by Solomon *et al*., (2006) to control deterioration in harvested sugar cane, an effective sodium chloride concentration has not yet been determined. Therefore, two sugarcane varieties were purposefully selected and used to determine the effective sodium chloride concentration for use in controlling deterioration in harvested sugarcane.

1.1 Statement of the problem

Sugar cane is one of several perishable crops that should be processed immediately after harvesting. However, in Kenya, harvested sugar cane can take about three to seven days before milling (Nanjala *et al*., 2022). Immediately after sugarcane is harvested,

microorganisms invade through the cut ends of the cane. These microorganisms proliferate rapidly once inside the juice-rich area (Khan *et al.*, 2020). Among the various species that invade sugarcane, *Leuconostoc mesenteroides* is recognized as the primary agent of deterioration (Misra *et al*., 2022). This bacterium breaks down sucrose into glucose and fructose, which cannot be crystallized (Verma *et al*., 2021). Additionally, it produces dextran, a branched glucose polymer, which introduces several complications into the sugar extraction process (Bashari, 2023). The biodegradation of sugar in harvested sugarcane is based on the variety. In Kenya, numerous sugarcane varieties are available to farmers, but the extent of their deterioration remains unclear. The use of sodium chloride in controlling sucrose deterioration in harvested sugar cane has been reported by Solomon *et al* (2006). However, the effective sodium chloride concentration for use is not yet known. Consequently, assessing the bio-deterioration levels of these varieties and determining the effective sodium chloride concentration for use in controlling deterioration in harvested sugar cane will aid in minimizing sucrose loss.

1.2 Justification of the study

In many sugar cane-growing countries, including Kenya, sugar cane is harvested and milled within about 3 to 10 days, leading to an economic loss of sucrose(Nanjala *et al*., 2022). The delay poses a serious challenge as microorganisms, particularly *Leuconostoc mesenteroides*, invade and proliferate in the cane, breaking down the sucrose into glucose and fructose, which are less economical products that cannot crystallize. They also produce impurities that impede the entire sugar-processing process. If this problem is not solved and left to continue, the Kenyan sugar industry will continue sufferingfrom sucrose loss due to this bacterium. Use of sodium chloride to control deterioration in harvested sugarcane has been reported. However, the effective sodium chloride concentration is unknown. If the effective sodium concentration is determined, it will help alleviate the sucrose loss. This way, a lot of sugar will be recovered from harvested sugar cane and help bridge the gap in importing sugar. This study, therefore, seeks to isolate, characterize, and determine the deterioration levels of selected sugar cane varieties. The study also attempts to find a suitable sodium chloride concentration that complements the covering of harvested

sugar cane as a means of controlling the deterioration of sucrose in harvested sugar cane.

1.3 Objectives

1.3.1 General objective

To characterize *Leuconostoc mesenteroides* isolates and the biodegradation levels of selected post-harvest sugar cane, and to determine an appropriate concentration of sodium chloride for controlling the deterioration of harvested sugarcane.

1.3.2 Specific objectives

i. To isolate and characterize the biochemical characteristics of selected *Leuconostoc mesenteroides* isolates.
ii. To determine bio-degradation levels of selected post-harvested sugar cane varieties by *Leuconostoc mesenteroides* isolates under controlled conditions.
iii. To determine a suitable sodium chloride concentration in controlling deterioration in harvested sugar cane under covered and uncovered conditions.

1.4 Research Questions

i. What are the biochemical characteristics of selected *Leuconostoc mesenteroides* isolates?
ii. What are the bio-degradation levels of selected post-harvested sugar cane varieties by *Leuconostoc mesenteroides* isolates under controlled conditions?
iii. Which is the most suitable sodium chloride concentration for controlling the deterioration of harvested sugar cane under covered and uncovered conditions?

1.5 Significance of the study

Kenya can produce sufficient sugar to meet its domestic demand and even generate a surplus for export. However, the industry has struggled to fulfil local demands due to various challenges and inefficiencies within the value chain. Therefore, evaluating the bio-deterioration levels of primary sugarcane varieties is crucial for reducing sucrose loss. This assessment will facilitate improved planning to minimize the time between harvesting and milling for each variety. Additionally, the study aims to determine the

optimal sodium chloride concentration and covering to effectively reduce sugar degradation in harvested sugarcane. Ultimately, this study intends to assist millers in maximizing sugar recovery from harvested cane, addressing the shortfall currently met through imports.

CHAPTER TWO

LITERATURE REVIEW

2.0 Production and economic importance of sugar cane in Kenya

Sugarcane (*Saccharum officinarum*) is a significant agricultural crop in Kenya, primarily grown in the western, Rift Valley, and coastal regions. The crop has a long history in the country, dating back to its introduction by Indian settlers in the early 1900s for jaggery production (Amukoya *et al*., 2020). Before independence, the sugar industry in Kenya was dominated by private entrepreneurs (Oduor, 2019). Later, the Ministry of Agriculture conducted field experiments on farmers' fields in Kibos and Miwani and laboratory investigations at the National Agricultural Laboratories in Kabete, Nairobi (Amukoya *et al*., 2020). Large-scale production and processing began with the establishment of Miwani Sugar Mills in 1922 and expanded with the addition of Associated Sugar Mills at Ramisi in 1927 (Oduor, 2019). After independence, the Kenyan Government began to play a central role in the ownership and management of the sugar industry. The government established the following factories: Muhoroni (1966), Chemelil (1968), Mumias (1973), Nzoia (1978), and South Nyanza (1979) (Oduor, 2019). Private investors have also built sugar factories in West Kenya (1981), Soin (2006), Kibos (2007), Butali (2011), Transmara (2011), and Sukari (2012) (Jabuya, 2015). Production of sugarcane in Kenya is one of the major agricultural activities contributing to national economic growth alongside tea, coffee, horticultural crops, and maize (Yılmaz & Njora, 2021). The contribution of the Kenyan sugarcane sector to the total agricultural gross domestic product (GDP) is about 15%, with 25% of the Kenyan population relying directly and indirectly on sugarcane production for their livelihoods (Francis *et al*., 2020). Kenya has the capacity to produce enough sugar to meet its domestic demand and generate surplus for export (Onyango, 2020). However, challenges like outdated milling machines, delays in processing sugarcane after harvesting, and sucrose loss to dextran caused by *Leuconostoc mesenteroides* bacteria have resulted in insufficient sugar production in the country, despite its ability to produce enough. Currently, 16 sugarcane factories nationwide have a combined milling capacity of 41,000 tons of cane daily (TCD), requiring over 9.8 million tons per year. (Kombo and Ndiema, 2022). This production could yield around 1.09 million tons of sugar, surpassing the annual demand for table sugar. Sugarcane is grown commercially in 14 counties across the western, Nyanza, Rift Valley, and coastal

regions. The crop is cultivated in diverse agro-ecological zones at elevations ranging between 15 and 1,700m above sea level. Kenya's main sugarcane cultivars are Co 421, Co 617, Co 945, and N14 (Nasimiyu, 2022). Together, these occupy more than 85% of the total sugarcane acreage, while new varieties occupy about 10%. These older varieties are late maturing, low in sucrose content, and susceptible to major diseases such as smut, mosaic virus, and ratoon stunting diseases (Bhuiyan *et al*., 2022). Between 2002 and 2014, the Sugar Research Institute (SRI) developed and released 21 improved sugarcane varieties. Key attributes of these varieties include early maturity (harvesting between 12-16 months), resistance to smut, and high sugar and cane yields. The varieties include 19 KEN varieties, EAK, and imported varieties from Demerara, Guyana (Jamoza, 2005). This study considered four popularly adopted sugarcane varieties based on the Cane Census report by the Sugar Directorate in 2022. The attributes of these varieties are as follows: Co 421: This is a standard variety, imported from India in Coimbatore. It is susceptible to smut and mosaic virus, maturing between 20 and 22 months after planting (Ram *et al*., 2022). The sucrose content is 12, while the fiber content is 15, and it is grown in all sugarcane-growing regions. KEN 83-737 has the following attributes: Parentage: Co 421 x Co 1148, Year of release: 2002, Country of origin: Kenya. Appearance: Pale greenish to light pink stalks that turn yellow when exposed to sunlight. Expected Cane Yield (tonnes/ha): 114. Sucrose (Pol% %cane): 11.6. Fiber% %cane: 16. Disease reaction: Intermediate resistance to smut, resistant to sugarcane mosaic virus. Recommended harvest age (months): 16-18. Recommended zones: Nyando, Western, and South Nyanza (Jamoza, 2005). KEN 82-808 has the following attributes: Parentage: Co 6415 x Phil 5460, Year of release: 2002, Country of origin: Kenya. Appearance: Purplish pink, erect stalk with heavy tops. Expected Cane Yield (tonnes/ha): 118. Sucrose (Pol% %cane): 11.5. Fiber% %cane: 15. Disease reaction: Intermediate resistance to smut and sugarcane mosaic virus. Recommended harvest age (months): 15-19. Recommended zones: Nyando, Western, and South Nyanza. N14 has the following attributes: Parentage: Imported. Year of release: Imported. Country of origin: South Africa (Natal). Appearance: Greenish yellow stalks. Expected cane yield (tons/ha): 95-134. Sucrose (Pol% %cane): 13.4. Fiber% %cane: 16. Disease reaction: Resistant to smut, intermediate resistance to sugarcane mosaic virus. Recommended harvest age: (17-19 months). Recommended zones: Nyando, Western, South Nyanza. Besides sugar production, sugarcane juice is a popular beverage in many countries, including Kenya (Gaiti, 2021). Despite the

importance of the cane crop and the many varieties grown, studies have not been conducted on the main varieties available in the country concerning sucrose deterioration. Such comparative studies can shed light on differences among sugarcane genotypes and possible roles in mitigating post-harvest sucrose deterioration.

2.1 Sugar cane deterioration

Sucrose losses after cane harvest are among the most critical problems in sugarcane production, especially for millers and farmers. These losses significantly affect the sugar mills' economy. Crushing stale canes in sugar mills results in a sugar recovery loss of 12–50% (Solomon, 2000).This loss can be alleviated by properly profiling the available sugar cane genotypes in the country and establishing their deterioration levels for planned immediate milling based on each variety. If such a loss is controlled, the importation of sugar into the country is likely to reduce significantly. Therefore, it saves the country from foreign exchange. Various causes are responsible for post-harvest sugarcane deterioration, with storage conditions and the time lag between harvesting and crushing (staling) being the primary factors accountable for microbiological sucrose losses (Misra *etal.*, 2022). Deterioration due to microbial invasion and proliferation in harvested canes leads to a loss of 62 % (Misra *et al.*, 2020). Typically, freshly harvested canes are left in open fields in piles or transport vehicles for extended periods. This practice allows for significant microbial invasion, growth, and proliferation, leading to weight loss and sucrose content (Misra *et al*., 2020). This condition also occurs when canes are stored in cane centers or mill yards. This leads to deterioration in juice quality, thereby lowering recovery.

2.2 Factors responsible for cane deterioration

2.2.1 Sugarcane varieties

Sugarcane varieties significantly impact sugar recovery, with their effectiveness varying depending on climatic conditions and agricultural management practices (Tiema *et al*., 2024). Notably, there is a significant difference in the susceptibility of these varieties to post-harvest deterioration, which is especially critical in regions facing prolonged delays between harvesting and milling. Furthermore, the susceptibility of sugarcane to *Leuconostoc mesenteroides* infection may be influenced by its inversion behavior(Misra *et al*., 2019). Research has evaluated the post-harvest

performance of various sugarcane varieties in tropical and subtropical climates, showing that fibrous varieties experience a more significant reduction in sucrose levels compared to less fibrous types (Vasantha *et al*., 2022). Several factors contribute to these differences, including ambient temperature, humidity, variety characteristics, storage duration, and crop maturity status.

2.2.2. Crop maturity

Fully mature cane experiences a slower rate of deterioration than immature and over-mature cane. This deterioration tends to occur more quickly in hot weather, as this condition helps bacteria establish in harvested sugar cane and degrade the sucrose. The maturity of the cane plays a crucial role in determining the rate of microbial invasion after harvesting. As the level of maturity increases, the extent of deterioration decreases.(Solomon & Singh, 2024).

2.2.3 Green and Burnt Cane

Extensive research shows that whole green cane is less susceptible to post-harvest deterioration than chopped or burned cane (Mueangmontri *et al*., 2020). Numerous studies have explored the effects of cane burning on dextran production, indicating that burnt chopped cane deteriorates more rapidly than its green counterpart (Mueangmontri *et al*., 2020). Furthermore, delaying the harvest of standing burned cane or the supply of burned harvested cane can significantly reduce sugar yield.

2.2.4. Environmental factors

Weather conditions significantly impact the deterioration of harvested sugarcane, with increased temperature and humidity correlating with a more significant decline (Misra *et al.*, 2022). High moisture levels damage mechanically harvested sugarcane, prompting recommendations to pause harvesting during rainy weather to mitigate damage. While rainfall does not directly lead to deterioration or dextran formation, the muddy conditions resulting from heavy rain promote the growth of polysaccharide-producing bacteria like *Leuconostoc* spp., which thrive in the anaerobic environments created by mud (Solomon, 2000). The ambient temperature also plays a crucial role in the deterioration process. Elevated nighttime temperature can trigger dextran production in stored cane, exacerbating quality decline after harvest (Solomon & Singh, 2024). Although environmental factors are critical, the sugarcane variety also

influences the deterioration process, albeit to a lesser extent. The interaction between climatic conditions and the specific characteristics of different cultivars can affect how sugarcane ripens and deteriorates.

2.2.5. Mechanical harvesting

The adoption of mechanized harvesting and the subsequent chopping of green burnt cane results in a decline in quality and the formation of dextran, which complicates processing (Panigrahi *et al.*, 2021). Additionally, mechanical harvesting often delays transporting cane to the factories, which remains a significant challenge impacting the factory's efficiency and sugar quality.

2.3 Economic implications of cane deterioration

The decline in sugarcane quality after harvest has significant socioeconomic repercussions for farmers, millers, refiners, exporters, and consumers (Solomon & Singh, 2024). This post-harvest decline primarily impacts cane growers and millers. A swift moisture loss from harvested cane leads to decreased tonnage for growers. Additionally, the sugar industry suffers financial losses due to reduced sugar recovery from deteriorated cane (Bashari, 2023). Furthermore, the growth of bacteria and various chemical reactions produce undesirable compounds that hinder sugar processing. The low-quality sugar resulting from this deterioration negatively affects both consumers and exporters.

2.3.1 Cane growers

The deterioration in cane quality due to cut-to-crush delay affects cane growers and the sugar industry (Misra *et al*., 2022). Misra *et al*.(2022) observed weight loss between 7 and 10 percent under sub-tropical conditions within 72 hours after harvest. This delayed supply of harvested cane to sugar factories could lead to significant economic loss to cane growers.

2.3.2 Sugar Cane Millers

The impact of processing deteriorated cane is significant and has crippling effects on the sugar industry. Beyond the loss in sugar recovery, it affects machinery, processes, and the quality of the final product, sucrose. The sugar industry suffers financial losses because of low recovery from deteriorated cane (Eggleston *et al*., 2004). These losses may further escalate depending upon the variety, cut-to-crush delay, and

ambienttemperatures. Additionally, dextran that forms during cane deterioration from microbial activity complicates sugar processing and makes sugar manufacturing uneconomical. Firstly, it increases the viscosity of process material, which leads to a decrease in factory capacity as filtration, clarification, and boiling rates are reduced. Secondly, the crystallization rate of sucrose decreased by up to 50% with high levels of dextran compared to fresh syrup. Thirdly, dextran levels above 1% modify the growth of sucrose crystals, causing growth to be retarded on one face. This results in needle-shaped crystals, which are difficult to separate from molasses in the centrifugal machine (Misra *et al.*, 2022).

2.4 Biochemical basis of post-harvest sugar loss

The deterioration of harvested cane is primarily a biochemical process that aggravates with time (M. T. Khan *et al*., 2020). This is followed by bacterial invasion through the cut ends or damaged stalk sites. Therefore, the time lag between harvesting and milling is crucial to achieving maximum sugar recovery (Misra, Solomon, Hashem, *et al.,* 2020). Mehdi *et al*. (2023) observed an increase in the activity of both acid and neutral invertase after 72 hours of cane storage, along with a corresponding rise in the level of inverted sugars. Following this period, the decline in commercial cane sugar (CCS) became significant due to the increased acid invertase activity. This change in invertase activity in harvested cane is also associated with moisture loss from the cane. The behavior of endogenous invertase varies depending on the variety, storage conditions, and external temperature.

2.5 Microbiological aspects of cane deterioration

Of all the microorganisms invading harvested sugarcane stalks, the most crucial and devastating is the *Leuconostoc* bacterium, which belongs to the lactic acid bacteria group (Misra, *et al*., 2020). *Leuconostoc* is a soil-borne bacterium that readily expresses itself on sugarcane tissue, syrups with lower Brix, and cane juice. It invades sugarcane through cut ends or cracks and enters the juice-rich region, where it finds favorable conditions for its survival and proliferation. This bacterium consumes sucrose as an energy source and converts it into various compounds such as organic acids, reducing sugars, ethanol, and long, complex chain polymers (Misra *et al*., 2022). Mishandling of canes during mechanical harvesting, burning, and chopping into billets aggravates the inactivation of the phenol oxidase enzymes on cane stalks, which acts as a protective

or anti-bacterial layer. In harvested/stale sugarcane stalks, a slimy layer is usually seen, formed due to the presence of this microbe. Under favorable conditions, this bacterium undergoes multiplication to form nodular colonies. Harvested canes have been reported to be more infected with this bacterium. Studies have indicated that this bacterium and dextran formation are more common in stale than freshly harvested canes. This is so as the dextranase enzyme is secreted by *Leuconostoc mesenteroides/dextranicum* and is responsible for dextran production (Misra *et al*., 2022). It is this enzyme that catalyzes the transfer of glycosyl residues to a polymer of dextran. The difference in the formation of dextran in stale and freshly harvested canes is due to the time lag between harvesting and crushing. Another factor contributing to the proliferation of this bacterium is the change in the acidity of sugarcane due to the increase in time duration from cutting to crushing of cane. High sugar content (about 15 %) and initial pH ranging between 5.0 and 5.5 in cane juice make a perfectly congenial environment for this bacterium.

2.6 Biology of *Leuconostoc mesenteroides*

The genus *Leuconostoc* consists of Gram-positive, catalase-negative, non-motile bacteria with irregular coccoid shapes (Rama *et al*., 2024). Their optimal growth temperature ranges from 20 to 30 °C. They measure about 0.5 to 0.7 μm by 0.7 to 1.2 μm and form small grayish colonies that are usually less than 1.0 mm in diameter. *L. mesenteroides* is a facultative anaerobe and undergoes hetero-lactic fermentation under microaerophilic conditions (Heo *et al,* 2024). Morphologically, *Leuconostoc* generally appears as cocci that are similar in size and shape to lactococci, often occurring in pairs and forming short chains. Unlike lactococci, most *Leuconostocs* can grow on Rogosa agar and are heterofermentative, producing carbon dioxide from glucose and typically from fructose (Säde *et al*., 2024). *Leuconostoc* species are frequently found in sugar-processing liquors and fermented foods, such as olives, cucumbers, sauerkraut, wine, and cheese (Springham, 1999). These bacteria are desirable in most foods because of their involvement in flavor development and preservation (Shahidi & Hossain, 2022). However, they are unwanted in the sugar industry because they produce gums (dextrans) from sucrose that hinder further sugar extraction. In fermented dairy products, their primary function is to metabolize citrate (Cit+) into CO_2, which contributes to eye formation in many cheeses and diacetyl, a key flavor component in many products (Maslov Bandić *et al.*, 2023). Depending on which flavor-producing bacteria are present, mixed-strain mesophilic cultures are usefully divided into D

cultures, containing only Cit+ lactococci as flavor producers; L cultures, containing only *Leuconostoc* spp. as flavor producers; DL cultures, containing both Cit+ lactococci and *Leuconostoc* spp. as flavor producers; and cultures containing no flavor producers. The number of flavor producers in starter cultures varies between 1 and 10%, but the exact species of Leuconostoc found is unknown with any degree of certainty:*Leuconostoc mesenteroides* ssp. cremoris.

2.7 Sugar Metabolism by *Leuconostoc mesenteroides* bacteria

Like other LABs, *Leuconostoc* lacks a tricarboxylic acid cycle and cytochrome system, which prevents it from generating energy through oxidative phosphorylation. (Cogan & Jordan, 1994). Instead, it generates energy through substrate-level phosphorylation while converting sugars into lactic acid, ethanol, acetate, and CO_2. The pathway initiates with the initial release of CO_2 from the glucose molecule, followed by the division of the resulting pentose into two-carbon and three-carbon fragments in a phosphorolytic reaction catalyzed by phosphoketolase, producing glyceraldehyde-3-phosphate and acetyl phosphate, respectively (Brüssow, 2007). Due to this split, the pathway is referred to as the phosphoketolase pathway and is also present in heterofermentative lactobacilli. The three-carbon fragment is eventually transformed into lactate, similar to what occurs in the glycolytic sequence, while the two-carbon fragment is converted to ethanol (Candeliere *et al*., 2024). Lactate and ethanol are generated to regenerate NAD+ for the continuation of fermentation; 2 moles of NAD+ are required to convert three fructose molecules into one mannitol: 2 lactate, 0.5 acetate, 1.5 ethanol, and 2 CO_2. The oxidation of glucose-6-phosphate to ribulose-5-phosphate and 1 mole in the oxidation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate must be replenished. The overall stoichiometry is glucose----> 1 lactate + 1 ethanol + 1 C02 + 1 ATP. Because products other than lactate are produced, the pathway is also called the heterofermentative pathway to distinguish it from the homofermentative or glycolytic pathway found in lactococci, enterococci, and many lactobacilli (*strep bacteria* and t*hermobacteria*) in which only lactate is formed: 1 glucose----> 2 lactate + 2 ATP (Candeliere *et al*., 2024). In the phosphoketolase pathway, 1 mol of ATP is produced per mole of glucose metabolized, compared to 2 mol in the homofermentative pathway. Therefore, the fermentation of glucose through the phosphoketolase pathway is only half as efficient as that in the homofermentative pathway (Cogan & Jordan, 1994). Unlike glucose fermentation, fructose fermentation results in the production of

mannitol and acetate, in addition to lactate and ethanol $C0_2$ (Candeliere *et al.*, 2024). The fermentation balance approximates Mannitol, and NAD+ is formed from fructose and NADH in a reaction catalyzed by mannitol dehydrogenase. This reaction increases ATP production because less acetylphosphate is reduced to ethanol to regenerate the NAD+ required to continue the fermentation. Two moles of mannitol are formed for each mole of acetylphosphate that is not reduced to ethanol. Fructokinase and phosphoglucoisomerase are present in *leuconostoc* fermenting fructose, indicating that it is probably first phosphorylated to fructose-6-phosphate and then isomerized to glucose-6-phosphate. Lactic acid bacteria produce mainly either the L- or DL-isomers of lactate, but *leuconostoc* and a few species of lactobacilli (e.g., Lactobacillus delbrueckii) produce only the D-isomer. Isolates from dairy products can be presumptively identified as *Leuconostoc* spp. If catalase is negative, it produces D lactate and appears as cocci when examined microscopically. Pentoses. Pentoses are fermented by all heterofermentative LAB (30). In the fermentation of pentoses, no CO_2 is formed, and acetate is produced instead of ethanol: 1 pentose----> 1 lactate + 1 acetate + 2 ATP. The reason for this change in product formation becomes evident when the pathway is examined. When growing on pentoses, only I mol of NADH has to be oxidized, which is done in the pathway's three-carbon (glycolytic) leg. There is, therefore, no need to form acetaldehyde and ethanol; instead, an extra mole of ATP and acetate are formed from acetylphosphate. Pentose and pentitol metabolism in heterofermentative bacteria. I, L-arabinose ketol-isomerase; 2, ATP:Lribulose-5-phospholransferase; 3, L-ribulose-5-phosphate 4-epimerase; 4, D-ribulose-5-phosphate 3-epimerase; 5. Dribose-5-phosphate ketol-isomerase; 6. ATP: D-ribose-5-phospholransferase; 7, ATP: D-ribulose 5-phospholransferase; 8, D-arabinose ketol-isomerase; 9, D-xylose ketol-isomerase; 10, ATP: D-xylulose 5-phospholransferase; and 11, Dxylulose-5-phosphate D-glyceraldehyde 3-phosphate lyase. PTS = phosphotransferase; P = phosphate; and DH = dehydrogenase. The pathways involved have been elucidated in lactobacilli and presumably are similar in *leuconostoc.* Before phosphorylation, xylose and arabinose are isomerized to the respective ketoses (xylulose and ribulose). Xylulose-5-phosphate is metabolized directly, and ribulose-5-phosphate is epimerized at C4 to form xylulose-5-phosphate. In contrast, ribose is phosphorylated to ribose-5-phosphate and isomerized to ribulose-5-phosphate before epimerization to xylulose-5-phosphate. Disaccharides. In lactococci, the initial enzyme of lactose fermentation is phospho-l3galactosidase (p-~-GAL), which hydrolyzes the

lactose-phosphate formed during transport to glucose and galactose-6-P. In contrast, I3-GAL is the enzyme that hydrolyzes lactose to glucose and galactose in *Leuconostocs.*

2.8 Dextran in sugar cane juice

Dextrans are polymeric chains of glucosyl units synthesized by dextransucrase, transferring D-glucosyl units from sucrose to acceptor molecules (Buchholz & Monsan, 2002). This polymerization results in the formation of dextran, while a fructose unit is released free. Dextrans contain a significant number of consecutive α-(1-6) linkages in their main chain, typically making up more than 50% of the total linkages (Neely, 1961). The enzymes synthesizing these glucans from sucrose are known as dextransucrase (1,6-α-D-glucan-6-α-glucosyltransferase) (Khalikova *et al*., 2005). They are glucansucrases produced by various species of *Leuconostoc* and Streptococcus and by the mold Rhizopus spp. Other bacteria that produce dextran, such as Acetobacter capsulatus (renamed Gluconobacter oxydans) and Acetobacter viscous, produce dextrin dextranase, which converts dextrins to dextran (Khalikova *et al.,* 2005*)*. Sucrose is broken down into fructose, while a glucose molecule polymerizes into dextran. However, fructose remains dissolved and can be measured analytically. The structures and properties of bacterial dextrans vary among microbial strains and are influenced by the growth rate and prevailing reaction conditions. The positioning of branch linkages, the degree of branching, the length of branch chains, and the molecular weight distribution all affect the physicochemical properties of dextrans (Khalikova *et al*., 2005). Dextran fractions found in sugar cane are primarily produced by *Leuconostoc mesenteroides*, a widely distributed bacterium that is especially prevalent in the sugar field. *Leuconostoc mesenteroides* is the lactic acid bacterium that primarily affects sugar cane after harvest. Dextran extracted from deteriorated sugarcane revealed a branched polysaccharide composed solely of D-glucose residues linked in consecutive α-(1–6) arrangements in the main chain with α-(1–3) branches.

2.8.1 Dextran levels in sugar factory and refinery

In sugar production, dextran content increases progressively, from the dilute juice to the final molasses (Jiménez, 2009). Statistics of 5 years in Louisiana showed that in 60% of the harvest time, the contents of dextrans did not exceed 250 ppm in the juice of whole stalk sugar cane, while the chopped sugar cane exhibited a faster dextran formation due to a greater exposed stalk area and hence, a higher degree of bacterial infection (Jiménez, 2009). Additionally, a massive infection with *Leuconostoc* and

other bacteria of up to six inches from the extremes of the sugar cane was reported in chopped sugar cane two hours after harvesting. In burnt sugar cane, a rapid increase in the level of dextrans was observed almost ten times from 12 to 48 h, reaching 3200 ppm (Jiménez, 2009). Burnt cane is more prone to dextran formation than unburnt cane. This observation fits the hypothesis that burnt cane is often partially covered externally with juice, supporting extensive bacterial growth if it does not dry out immediately after burning. It was also reported that the dextran content in sugar cane that was burned and left standing in the field increased rapidly from 280 ppm on the third day after burning to 2,900 ppm after one day per week (Bashari, 2023). Any delay of over 14 hours in the arrival of the cut sugar cane to the mill under warm and humid conditions favors the formation of dextrans, which will reach the mills and enter with the juice during the industrial flow process. Therefore, a sanitation technique with steam every eight hours and biocides is recommended. A study in South Africa showed that the normal dextran levels in mixed juice are 200 mg/kg Brix, but can exceed 10,000 mg/kg Brix in deteriorated cane. Levels above 1000 mg/kg in mixed juice are problematic. It has been shown that to meet a specification of 150 mg/kg in raw sugar, the levels in mixed juice must be below.

2.8.2 Dextran associated with processing problems in sugar production and refinery performance

Dextranaffects sugar processing from the moment they are formed because of the irreversible sucrose intake it causes(Bashari, 2023). According to research, during the first six hours at 30ºC, a *Leuconostoc mesenteroides* strain could consume sucrose at 8.46 g/L/h (Bashari, 2023). The dextransucrase enzyme converts one sucrose molecule into one glucose molecule and one fructose molecule, so a significant amount of sucrose molecules must be converted for the dextran chain to be created; therefore, numerous glucose molecules are required for dextran formation (Khalikova *et al*., 2005). Polarization and crystal size distribution are the two key considerations when buying raw sugar, and dextran formation significantly impacts both (Bashari, 2023). Juice, syrup, and sweets containing dextran may lead to false polarization. This is due to dextrans having a dextrorotatory property that causes them to polarize approximately three times more than sucrose, resulting in a high false Pol value(Bashari, 2023). The efficiency of the clarification stage during polarization determinations will decrease when dextran levels are raised, impacting the price of raw sugar. Usually, high dextran

levels harm sugar production during the clarification stage because they act as a protective colloid and prevent coagulation, allowing fine suspended particles to enter the cleared juice. According to Bashari (2023), the carry-over causes the sugar generated from the juice's color and ash to rise due to this behavior. More importantly, the suspended particles may enter the refinery, leading to inadequate melt filtration and decreased throughput. The drop could be significant, forcing affected refiners to seek alternative raw sugar sources. Clarification processes often become less efficient due to the thickness of the solution, which slows the rate at which impurities settle, leads to scale buildup, reduces the heating efficiency of the flow, and contributes to scale formation deposits (Bashari, 2023). More lime was needed to neutralize the more acidic pH values of the comparable juice made from degraded sugar cane, which increased turbidity and produced more sticky mud, clogging press filters. The extra viscosity of the juice further hinders mud filtering. According to Bashari (2023), the high viscosity of the juices, the elevated molecular weight of dextrans, and other insoluble debris present in them lead to filter blockages, causing juice losses from spills that are often overestimated. Recently, Bashari *et al*,(2012) demonstrated through laboratory trials that adding standard dextrans to pure sucrose solutions increased apparent viscosity and dynamic modulus with higher dextran concentrations. They demonstrated a strong dependence on its (Mw), indicating that the presence of dextran during the sucrose manufacturing process will increase the viscosity of juices and syrups. This behavior is expected to result from two factors: first, a reduction in sucrose's molecular mobility due to the high molecular weight (HMw) polymers, and second, the chain end effect, along with the flexibility of the branching point. The dextran chain's 95% α-(l-6) links increase flexibility through a co-monomer impact, which accounts for the HMw dextran's intermediate behavior. Additionally, each branching produces one dangling non-reducing chain end per external chain, increasing the mobility of nearby residues overall. Another study showed that an increase in dextran content increases the viscosity of the juice, which retards the mud-setting rate in clarifiers and results in the suspension of precipitated impurities in the clarified juice. Consequently, the clarified juice is turbid, resulting in a higher mud volume on the rotary screen during filtration, making screen cleaning more difficult. Additionally, the production of HMW dextran during the clarifying processes has a detrimental effect on the crystallization of calcium carbonate. The smaller calcium carbonate particles that result from increasing filtration pressures hinder the second carbonation filtration. It is commonly assumed that the

dextran content and raw sugar filterability correlate. Studies have shown that dextran is not the direct cause of poor filterability. The demonstration shows that a high dextran raw sugar showed no improvement in filterability after enzymatic removal of the dextran (Bashari, 2023). The harmful effects of dextran contamination during the evaporation process raise the viscosities of juice and syrup, which reduces mass transfer rates and slows mass transfer operations. This ultimately decreases the evaporation rate in boilers, lowers the crystallization rate in the vacuum pan, and increases scaling on equipment surfaces, resulting in lower heat transfer efficiency and thus increasing energy consumption loss (Bashari, 2023). Eggleston (2002) reported that the high viscosity associated with the HMW portions (> 1000 KDa) of dextran impacts boiling house operations, often decreasing evaporation rates. From the processing point of view, the crystallization process is expected to have the worst consequences of high dextran concentrations in a technical sucrose solution. Dextrans slow the crystallization rate or even inhibit crystallization (Bashari, 2023). It is estimated that for every 300 ppm dextran in syrup, there is a 1% increase in the molasses purity (the percentage ratio of sucrose) in total solids in a sugar solution.

2.9 Strategies to minimize post-harvest sucrose losses in sugarcane

2.9.1 Field losses

Research efforts to assess the extent of cane deterioration in the field and factory have encountered only partial success (Singh *et al*., 2019). Some of the proper parameters to assess the juice quality of cane arriving at the factory are dextran, gum, oligosaccharides, ethanol, mannitol, reducing sugars, titratable acidity, invertase content, juice viscosity, purity drop, etc. (Roy & Chandra, 2018). Based on these indicators, the quality of cane supplied to the mills could be assessed. There is no substitute for better communication and quick and efficient transport to minimize post-harvest losses. The harvested cane must be brought to the mill and processed quickly. The factory management must ensure that fresh cane is supplied regularly, and all indents should be placed accordingly (Singh *et al*., 2019). Solomon & Singh (2024) have advocated the following milling schedule for harvested cane/billets for Indian sugar factories: Full green cane: Milled within 48 h (early season) and Milled within 24 h (late season). Burnt entire cane milled within 24 hours. Billets (green/burnt) Milled within 12 hours. The soil content of cane is also one of the factors influencing not only cane deterioration but also causes process difficulties, such as cane preparation, milling,

and clarification, and is a source of millions of microbes that can grow in juice (Misra *et al*., 2022). Soil particles are directly responsible for damage to the processing unit's hammers, knives, conveyors, juice screens, pipes, and other parts. It is, therefore, important that the processing of muddy cane should be avoided. It has been observed that topped cane deteriorates faster than cane with the crown of leaves attached. In case of any anticipated delay in crushing, topping should be avoided (Davis *et al*., 2010). Cane maturity is a significant factor in storing sucrose's inversion and subsequent reduction. As the maturation level increases, the extent of sucrose loss is minimized. Harvesting immature or over-mature cane should be avoided to reduce post-harvest sugar losses. It is necessary to implement maturity-wise harvesting, especially in low-recovery areas (Kumar, 2019).

2.9.2 Pre-harvest application of chemicals:

Many preventive methods for post-harvest sugar cane developed earlier could not be scaled up into commercial ventures due to the poor effectiveness of chemicals and operational challenges in field conditions. These methods include treating cane with bactericidal chemicals, gamma irradiation from 60 Co, and a pre-harvest spray of divalent cations such as Zn++, Cu++, Co++, and Ba++.

2.9.3 Use of sodium chloride.

Sodium chloride has shown antimicrobial efficacy against *Leuconostoc mesenteroide* bacteria (Cabezas-Pizarro *et al.*, 2018). It has also demonstrated efficacy in controlling deterioration when sprayed on harvested sugar cane (Misra *et al.*, 2022). However, a suitable concentration for controlling deterioration has not yet been studied. Sodium chloride is a common salt that is easily available and not harmful for human use. If its effective concentration is established, it will greatly assist in increasing the sugar recovered from harvested sugar cane. This study, therefore, seeks to determine the suitable concentration of sodium chloride and its application on harvested cane as potential strategies to mitigate deterioration in harvested sugar cane.

CHAPTER THREE

MATERIALS AND METHODS

3.0 EXPERIMENTAL SITE.

The experiment was conducted in the Pathology Laboratory of Kenya Agricultural & Livestock Research Organization- Sugar Research Institute Kibos, located 1250 meters above sea level, 0° 2'11''S and 34°49'17 '' E. The average temperature in Kibos is 19.7°C, and precipitation is about 1912 mm per year. The soils are heavy black clay classified as vertisols. The study area experiences bimodal rainfall characterized by two rainy seasons per year, known as long and short rains. Long rains occur from March to May, while short rains take place between September and October each year. This bimodal rainfall pattern reflects the lake region in Kenya (Jaetzold *et al*., 2007). The range for maximum and minimum temperatures is 28 – 33 °C and 21 – 24 °C, respectively, while the average temperature is 23 °C.

3.1 Determination of the biological and biochemical characteristics of selected *Leuconostoc mesenteroides* isolates

3.1.1 Collection of isolates

Three *Leuconostoc mesenteroides* isolates were collected from three sugarcane-growing regions in Kenya (Kakamega, Nyando in Kisumu County, and Sony sugar in Migori County) by harvesting three stalks from any sugar cane variety in each of the six fields per region, resulting in a total of eighteen stalks per growing region. These were chopped in half and left overnight in the field to collect the bacteria, modified from (Omayio *et al*., 2018). The coordinates of the sample sites are shown in Table 3.1 below. The chopped cane was then transported to the Sugar Research Institute pathology lab, where the cane was milled, juice collected in 18 different containers, labeled accordingly, and left overnight.

Table 3. 1: Coordinates of the location of isolates collected from Nyando, Sonysugar, and Kakamega.

Location of the Isolates	**NYANDO** Longitude in Decimal Degrees	Latitude in Decimal Degrees
Kajulu_Isolate 1	34.8264	-0.0553511
Miwani_Isolate 2	34.9139	-0.0542807
Ombeyi_Isolate 3	34.939	-0.135309
Chemelil_Isolate 4	35.1052	-0.0893031
Muhoroni_Isolate 5	35.1869	-0.143042
Fort Tenan_Isolate 6	35.3098	-0.186489
Location of the Isolates	**SONY SUGAR** Longitude in Decimal Degrees	Latitude in Decimal Degrees
Opapo _isolate 1	34.5542	-0.699192
Cham Gi Wadu_isolate 2	34.3883	-1.08888
Uriri_ Isolate 3	34.442	-0.952153
Sony _ Isolate 4	34.4754	-1.07116
Raneni _ Isolate 5	34.5654	-0.861203
Awendo_Isolate 6	34.5408	-0.905074
Location of the Isolates	**KAKAMEGA** Longitude in Decimal Degrees	Latitude in Decimal Degrees
Butsotso South_Isolate 1	34.6505	0.26351
Matungu__Isolate 2	34.4703	0.457947
Ingoste_Isolate 3	34.7101	0.35287
Kambili_Isolate 4	34.9123	0.377724
Kipkaren _Isolate 5	34.9111	0.636847
Shinyalu_Isolate 6	34.7944	0.225736

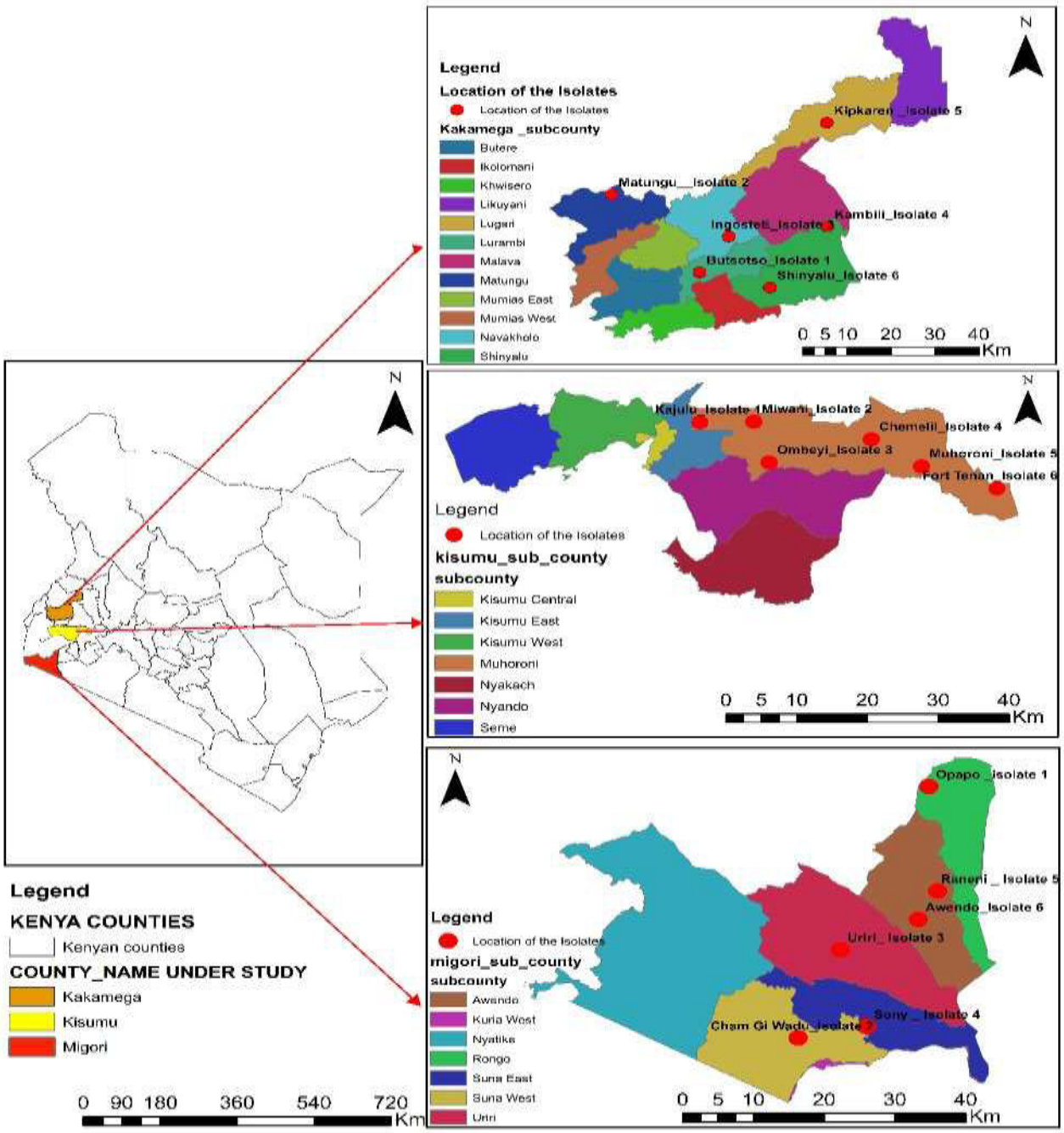

Figure 3. 1: Map of western Kenya showing isolates collection sites.

3.1.2 Isolation and identification of the isolates

The overnight sugar cane juice was homogenized by shaking for two minutes with a mechanical shaker, and 1 mL was drawn and inoculated onto *Leuconostoc* specific media consisting of 5% sucrose and sodium azide broth (5% sucrose (Duchefa), 10% tryptone (Himedia), 0.1% yeast extract (Merck), and 0.25% K2HPO4 (Misra *et al*.,

2020). The pH of the medium was adjusted to 7.0. After autoclaving, 0.005% sodium azide was added to the medium (Sarwat *et al.*, 2008). This was incubated at 37°C for one day. Following one day of incubation, the viscous broth was spread onto solid media containing 2% sucrose (Duchefa), 1.3% nutrient broth (Oxoid), and 1.6% nutrient agar (Oxoid) using a sterile wire loop.

3.1.3 Characterization of biological characteristics of the isolates

The biological characteristics of the isolates were determined by observing the colony traits as follows and recorded as detailed by Gudisa & Yenasew (2022): form, elevation, surface, opacity (for example, transparent, opaque, or translucent), color (pigmentation)—for instance, white, buff, red, purple, etc.—and the morphology of the bacteria, either cocci or bacilli.

3.1.4 Gram Staining

The Gram staining determined the morphological status of isolated bacteria (Kohlerschmidt *et al*., 2021). A colony loopful was picked and placed in a drop of sterile water on a slide, air-dried, and fixed by exposure to a flame. Briefly, staining was performed with a crystal violet solution for one minute as the first step, followed by washing with tap water. Next, the iodine solution was applied to the slide and allowed to sit for one minute, followed by washing under tap water. Then, 95% ethanol was used for 15 seconds until the violet stain was washed away from the slide. After rinsing with tap water to remove the ethanol, the slide was counterstained with safranin for 30 seconds. Finally, the slide was washed again under tap water, dried with blotting paper, and examined under a light microscope with 100x magnification. Gram-positive organisms stained blue-purple, while gram-negative organisms appeared pink-red after Gram staining (Kohlerschmidt *et al*., 2021).

3.1.5.Biochemical tests

3.1.5.1 Catalase test

The catalase enzyme breaks down hydrogen peroxide (H_2O_2) into oxygen and water molecules ($2H_2O + O_2$), with oxygen production observed in the formation of O_2 bubbles (Hadi *et al*., 2024). The presence of these gas bubbles indicates the presence of the enzyme, confirming the bacterium's catalase-positive nature. A sterile loop was

used to transfer a small amount of the colony onto the surface of a clean, dry glass slide. A drop of 3% H_2O_2 was placed directly over the colony and the evolution of oxygen bubbles was observed.

3.1.5.2 Gas production from glucose

Carbon dioxide production from glucose is the primary criterion for determining whether an isolate is homofermentative or heterofermentative (Gudisa & Yenasew, 2022). To assess CO_2 production, MRS broths containing inverted Durham tubes were utilized. A wire loop was used to aseptically transfer a colony of cultured bacteria from 2% sucrose agar and 0.005% sodium azide media into 8 ml of MRS broth with inverted Durham tubes, conducted in triplicate and incubated for 5 days at 37°C (Gudisa & Yenasew, 2022).

3.1.5.3 Sugar fermentation test

The sugar (carbohydrate) fermentation tests were performed by inoculating bacterial culture into individual tubes containing sugars and incubating at 37ºC for 24 hours (Gudisa & Yenasew, 2022). Ten sugars, e.g., glucose, lactose, salicin, trehalose, melibiose, sucrose, maltose, fructose, arabinose, and cellobiose, were used for the sugar fermentation test in this study. Firstly, a 10% solution of each sugar was prepared in distilled water with gentle heat to help it dissolve completely, then filtered through 0.2 µm filters. The stock solution was prepared by using Tryptone 1% (Difco), Yeast Extract 0.1% (Merck), K_2HPO_4 0.25% (Merck), and 0.004% bromocresol purple, sterilized by using an autoclave at 121ºC for 15 minutes. Fifty microlitres of overnight activated culture were transferred into a test tube containing 4ml stock solution with 950 µl specific sugar solution. Acid production was indicated by the change of media color from purple to yellow. After identifying all the isolates from each area, the six isolates were combined to create a composite isolate representing isolates from the three sugarcane-growing regions.

3.1.6 Hierarchical Cluster Analysis

The characteristics of each isolate were analyzed using SPSS for hierarchical cluster analysis (version 21.0) to assess the relationships between the three isolates. A dendrogram was generated to aid in the analysis.

3.2 Determination of bio-degradation levels of selected post-harvested sugar cane varieties by *Leuconostoc mesenteroides* isolates.

3.2.1. Experimental materials

The materials for the investigation included four sugar cane varieties commonly grown in Kenya, based on the Cane Census report by the Sugar Directorate in 2022. These varieties are CO 421, KEN83-737, KEN 82-808, and N14, which account for 36.58%, 30.04%, 15.46%, and 2.51% of the area cultivated per variety, respectively. Three isolates of *Leuconostoc* spp. were previously isolated and identified. Approximately 600 stalks of each variety were harvested and used during storage periods to assess the deterioration levels of each variety by the three isolates.

3.2.2 Experimental design

The experiment utilized a 6 × 4 × 4 factorial design, arranged in a complete randomized design (CRD). The factors included time at six levels: P1: 24 hours, P2: 48 hours, P3: 96 hours, P4: 144 hours, P5: 192 hours, and P6: 240 hours. The second factor consisted of varieties at four levels: CO421, KEN83-737, KEN 82-808, and N14. The third factor involved isolates at four levels: Kakamega isolate, Nyando isolate, Sony sugar Isolate, and a control. Ninety-six treatment combinations were replicated thrice, resulting in two hundred eighty-eight (288) experimental units. Each stalk from each variety was cut 60 cm from the lower end. Each variety contained four bundles of 100 stalks, with an average weight of 10.00 ± 0.85 kg. The inoculations were conducted aseptically using 1000 ml of 104 CFU per ml of each isolate, with one left un-inoculated as a control (Stern & Pretanik, 2006). After inoculation, each bundle was placed in a separate, clean plastic container that was covered and labeled. Approximately 10 sets of cane were randomly picked aseptically from each container and crushed using a cane mill at the Sugar Research Institute (SRI), and the juice was collected for analysis.

3.2.3 Data collection

3.2.3.1 Determination of Brix in Juice.

150 mL of sugarcane juice was added to a 250 mL Erlenmeyer flask and 2 g of filter aid. The mixture was stirred thoroughly and filtered through Whatman No. 91 filter paper, then covered with a watch glass to prevent evaporation. The first 20 mL of the filtrate was discarded. Subsequently, enough filtrate (approximately 100 mL) was

collected in a 150 mL beaker to determine the refractometric Brix as closely as possible to 200 °C using a KRUSS OPTRONIC German refractometer (Analysis, 1964).

3.2.3.2 Determination of Pol in juice.

The polarization of sugar is the resultant optical rotation of sucrose and other optically active substances, primarily glucose and fructose. Only products like raw sugar, bagasse, and filter cake can serve as an approximation of sucrose. A sugar solution must be clarified before determining its polarization. Horne's dry basic acetate is commonly used as a clarifying agent. (Analysis, 1964).

Clarification

A 400 ml sugarcane juice sample was put in a 600 ml beaker. 4.0g of lead acetate was added for clarification. This was mixed vigorously and set aside for about 30 seconds to allow flocculation to occur. The mixture was filtered through a fluted Whatman paper No. 91 filter paper. The first 25 mL of the filtrate was discarded, and about 200 200mL was collected in a 250 mL beaker (Analysis, 1964). The pol reading was taken using an Anton Paar saccharimeter without delay at as close to 20^0 °C as possible.

3.2.3.3 Dextran determination in raw cane juice.

A Collaborative study on CSR methods for dextran determination, which is a modification of Nicholson and Horsley's method, was conducted by (Analysis, 1964) in 1982. The CSR method is the official ICUMSA method for dextran determination. In 50% alcohol, dextran quantitatively forms a haze measured turbidimetrically at 720nm. Other substances forming haze under the same experimental condition were removed, including suspended matter removed by filtration, solubilized starch by enzymes such as Kieseguhr, and protein precipitated by Trichloro-acetic acid solution.

Reagents

Standard dextran solution (1mg/mL)

Determine the moisture content of Pharmacia dextran 110 by weighing 2±0.1000g and drying it in an oven at 105 ^{0} C for 3 hours. Weigh 0.1000g of anhydrous dextran and dissolve to make 100 mL in a volumetric flask. Trichloroacetic acid solution TCA (10% w/). Denature absolute ethanol, Sucrose working solution (20% Bx). AR sucrose (21.6 + 0.5g) is dissolved and diluted to 100 mL. Sucrose standardization solution (50% w/v). Acid-washed filter aid.Ion exchange resin mixture.

Standardization procedure

0.5 mL of TCA was pipetted into eight 25 mL volumetric flasks. 5.0 mL of sucrose solution was then added to each flask. Distilled water was added to the 25 mL mark in one of the flasks. Standard dextran solution volumes of 0, 0.5, 1, 1.5, 2, 3, and 4 mL were added to the remaining seven flasks. Distilled water was added to achieve a total volume of 12.5 mL in each flask, specifically adding 7, 6.5, 6, 5.5, 5, 4, and 3 mL of distilled water to the respective flasks. To the first of the seven flasks, denatured absolute alcohol was added dropwise to reach the 25 mL mark while gently swirling the flask. The stopwatch was immediately started afterward. One cell was filled with the test solution and another with the blank. Twenty minutes after mixing, the absorbance of the test solution was measured against the blank at 720 nm. This process was repeated for each dextran concentration, and afterward, a graph of mg dextran/25 mL versus absorbance was created and developed.

Determination of dextran in raw cane juice.

Sixty milliliters of the juice were transferred to a 100 mL flask containing 1 g of mixed resins, and the flask was shaken for 30 minutes using a flask shaker. The solution was filtered through mesh gauze. Fifty milliliters of the filtrate were transferred into a 100 mL stoppered cylinder, where 10 mL of TCA solution and 10 g of acid-washed filter aid were added. This mixture was shaken well and filtered through a Buchner funnel fitted with Whatman No. 5 filter paper. 12.5 mL of the filtrate was transferred into two 25 mL volumetric flasks. Denatured absolute alcohol was added dropwise to the 25 mL mark of one flask while swirling gently. The contents of the flask were mixed well by gently inverting three times. The stopwatch was immediately started afterward.

Distilled water was added to the 25 mL mark of the second flask, which was the blank solution. One cell was filled with the blank solution and another with the test solution. After 20 minutes, the absorbance of the test solution was read at 720 nm against that of the blank solution.

Calculation

$$\text{Ppm Dextran in juice} = \frac{\text{mg dextran/25mL} \times 1200}{\text{Cell length (cm)} \times \text{conc. Of total solids g/mL} \times \text{aliquot}}$$

3.2.4 Statistical analysis

The data obtained was subjected to an analysis of variance (ANOVA) adapted for Split-plot Design using PASW version 22. The difference between the mean was separated using the Tukey procedure at 5% significance.

3.3 Determination of selected suitable chemical concentration in controlling deterioration in harvested sugar cane.

3.3.1 Experimental materials

Materials for the investigation comprised two sugarcane varieties, CO421 and KEN 83-737. About 1300 stalks of each variety were harvested during storage periods to determine the effective approach to controlling deterioration in harvested sugar cane. Trash from harvested sugar was collected and used for covering the treatment. 2 kg of sodium chloride (AR) was used for the treatment of the trial.

3.3.2 Experimental design

The experimentwas conducted in CRD design with two treatments of varieties, CO 421, KEN83-737 as main plots, eight treatments of 2 levels of cover, 4 levels of Sodium chloride as subplots, and five cane storage periods after harvest of canes as a subplot, P1: 0 h, P2: 48h, P3: 96h, P4:144h and P5:192 with three replications. The treatment combination was ($2 \times 8 \times 5 = 80$) multiplied by three replicates total to ($80 \times 3 = 180$) combinations. Each stalk from each variety was cut 1.5 m from the lower side of the stalk and grouped into about 100 stalks for treatment. Each variety had eight bundles of 100 stalks each.

3.3.3 Growth at Different Sodium Chloride Concentrations

A preliminary study was conducted to find out the optimal concentration of sodium chloride for the growth of *Leuconostoc mesenteroides.* This was done as follows; A 50 µL sample of overnight activated culture was transferred into a tube containing 10 mL of NaCl test media (Gudisa & Yenasew, 2022). The NaCl test media was prepared by adding 0.004% bromocresol purple to the necessary amount of NaCl in MRS broth. Isolates were inoculated to assess growth at 2%, 4%, and 6.5% NaCl solutions (Gudisa & Yenasew, 2022). They were incubated for 7 days at 37°C in an incubator—a color change from purple to yellow indicated evidence of cell growth. After the fifth day, the

cultures were inoculated onto sucrose media containing sodium azide for growth enumeration.

3.3.4 Data collection

3.3.4 .1 Cane weight loss % at storage periods

Cane weight losses (%) for each sample were calculated by subtracting the weight of a sample after storage from the fresh weight and multiplying by 100 as the following equation.

[(Fresh weight of sample − weight at specified storage period of the sample)/Fresh weight of sample] x100 (Urgesa *et al*., 2021).

3.3.4 .2 Brix% juice

T 150 mL juice in a 250 mL Erlenmeyer flask, add 2g filter aid. Mix well.

Filter through Whatman No. 91 filter paper and cover the funnel with a watch glass to avoid evaporation. Reject the first 20 ml of the filtrate. Collect sufficient filtrate (about 100mL) in a 150 beaker to determine refractometric Brix at as close to 20^0 C as possible.

3.3.4 .3 Determination of Pol in juice

The Pol of sugar is the resultant optical rotation of sucrose and other optically active substances, mainly glucose and fructose. Only products such as raw sugar, bagasse, and filter cake can be taken as an approximation of sucrose. A sugar solution has to be clarified before its pol is determined. Horne's dry basic acetate is commonly used as a clarifying agent.

Clarification

To 400ml sample in 600ml beaker, add the minimum amount of lead acetate for clarification, as over-leading will introduce error. 4.0 g is sufficient.

Mix vigorously. Set aside for about 30 seconds to allow flocculation to occur.

Filter through a fluted Whatman paper No. 91 filter paper, using acid-washed kieselguhr if necessary.

Discard the first 25 ml filtrate and collect 200 mL in a 250 mL beaker.

Polarization.

Polarize the filtrate without delay at as close to 20^0 C as possible in a tube using the 26/100mL standard weight.

Brix determination.

Determine the Brix of the sample at as close to 20^0 C as possible, as described above.

Calculation.

The pol % juice is obtained by multiplying the saccharimeter reading by the pol factor obtained from the table using the refractometer Brix of the sample corrected to 20^0 C.

Purity percentage

The juice purity percentage wascalculated according to the following formula described by Satisha *et al*. (1996).

$$Purity\ percentage = \frac{pol\ \%\ juice}{Brix\ \%\ juice} \times 100$$

Pol % juice is the sugar content in the juice, and Brix % juice is the total dissolved solutes.

3.3.4 .4*Leuconostoc mesenteroides* count

The *Leuconostoc mesenteroides* count was determined by culturing the bacteria from each sample on solid media containing 2% sucrose (Duchefa), 1.3% nutrient broth (Oxoid), and 1.6% nutrient agar (Oxoid). The pH of the medium was adjusted to 7.0. After autoclaving, 0.005% sodium azide was added to the medium. Serial dilutions were performed for each sample up to 10-3 before plating using the pour plate inoculation method for three days at 37°C. Only shiny, mucous-like colonies specific to Leuconostoc mesenteroides were counted and recorded.

3.3.5 Statistical analysis

The data obtained were subjected to an analysis of variance (ANOVA) adapted for a split-split-plot design using PASW version 22. The difference between the means was separated using the Tukey procedure at 5% significance.

CHAPTER FOUR

RESULTS

4.1 Determination of the biological and biochemical characteristics of selected *Leuconostoc mesenteroides* isolates

4.1.1 Identification of *Leuconostoc* bacteria

After 24 to 48 hours of incubating the extracted juice on specific *Leuconostoc* spp media, the resulting colonies were mucous-rich and circular, convex with flat edges, smooth, shiny, and semi-transparent, as shown in Figure 4.1.

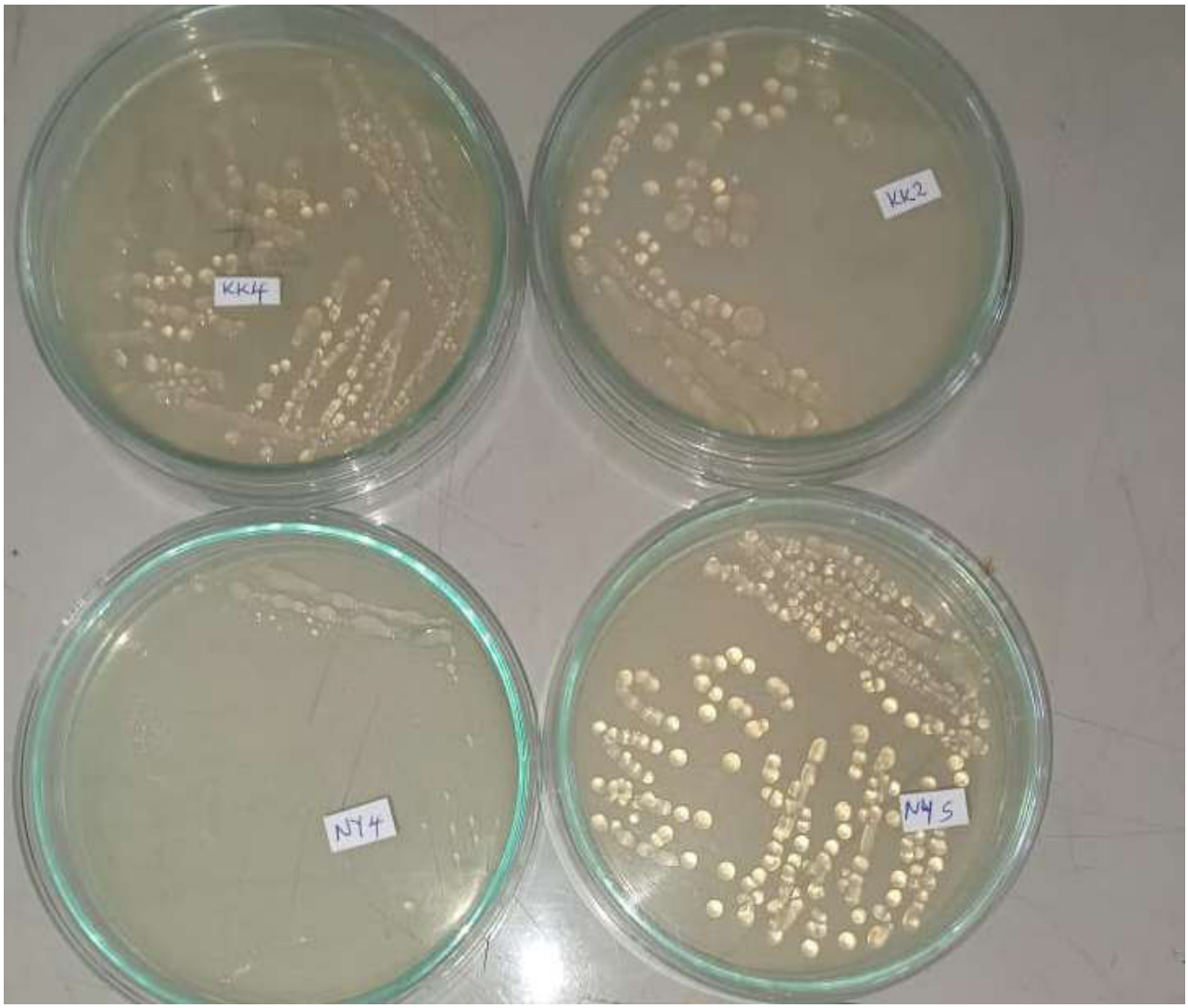

Figure 4. 1: Colonies of *Leuconostoc mesenteroides* spp on sucrose and sodium azide agar

When Gram staining was performed, all the isolates retained crystal violet stains, which appeared blue. All the cells were spherical or oval, occurring singly or in pairs (appendix i)**.** When exposed to 3% hydrogen peroxide, none of the isolates evolved the gas (Appendix ii).All the isolates were used to ferment glucose for 5 days at 37° C and

were all seen to produce a gas trapped in the inverted Durham tube (appendix iii).Under different temperatures, all *Leuconostoc mesenteroides* isolates grew optimally at 37 °C, while the least growth was observed at 45°C. The Kakamega isolate recorded the highest growth at 37°c, at 7.5*104 cfu/ml, while Nyando and Sony produced 6.8*104 cfu/ml and 6.6*104 CFU/ml, respectively (Fig 4.2).

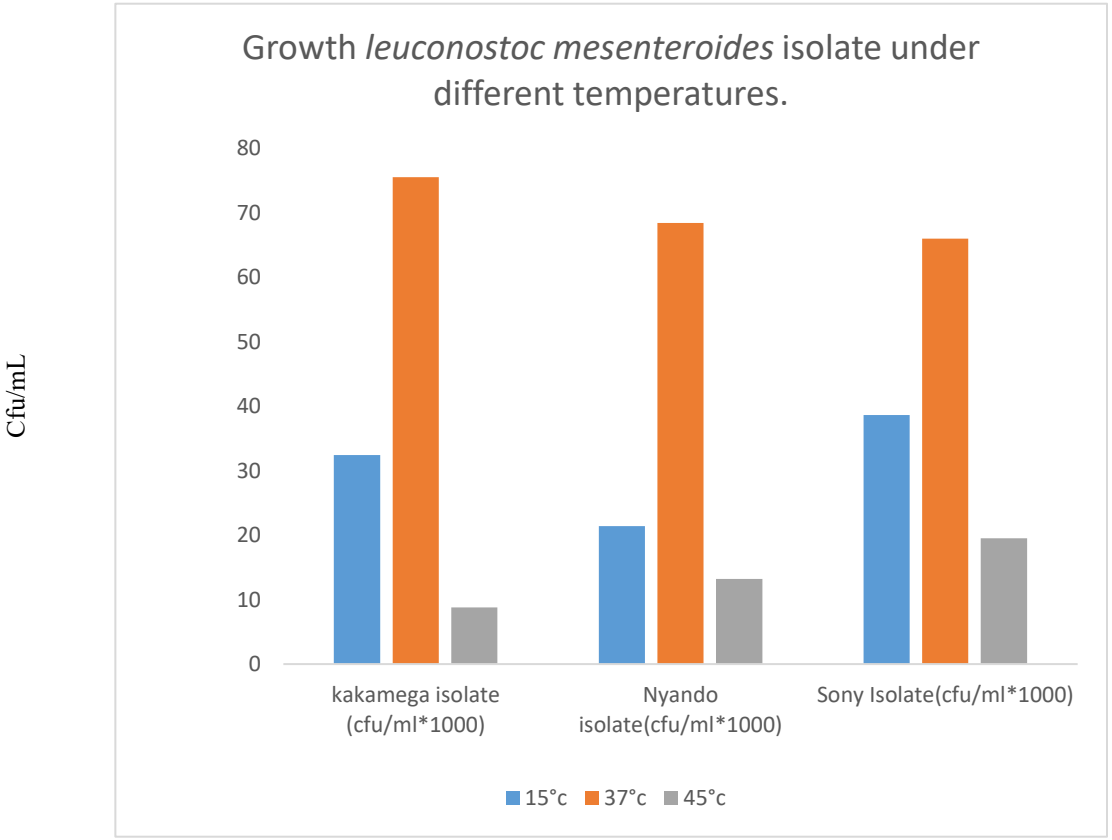

Figure 4. 2: Growth of *Leuconostoc mesenteroides* isolates under different temperatures.

Table 4. 1: Summary of morphological and Biochemical characteristics of the three *Leuconostoc mesenteroides* bacteria isolates

Name of isolate		Kakamega					Nyando						Sony					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Elevation of the colony	convex	convex	convex	convex	convex	convex	convex	convex	convex	convex	Convex	convex	convex	convex	convex	convex	convex	convex
Margin of the colony	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	Smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
The surface of the colony	Shiny	shiny	shiny	shiny	shiny	shiny	shiny	shiny	shiny	shiny	Shiny	shiny	shiny	shiny	shiny	shiny	shiny	shiny
The opacity of the colony	translucent	translucent	translucent	translucent	translucent	translucent	translucent	translucent	translucent	translucent	Translucent	translucent	translucent	translucent	translucent	translucent	translucent	translucent
Color of the colony	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy	creamy
Texture of the colony	Slimy	slimy	slimy	slimy	slimy	slimy	slimy	slimy	slimy	slimy	Slimy	slimy	slimy	slimy	slimy	slimy	slimy	slimy
Gram stain reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cell shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Catalase activity	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gas from glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	FERMENTATION OF DIFFERENT SUGARS																	
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Maltose	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key: ＋Means Positive　－ Means Negative

4.1.2 Hierarchical Cluster Analysis

All the isolates were grouped into two main clusters. Cluster one contained eleven isolates, primarily from Sony and Nyando. These isolates included Son5, Sony6, Sony3, Sony4, Sony1, Sony2, Nyando5, Nyando6, Nayndo2, Nayndo3, and Nyando4. The isolates Sony5, Sony6, Sony3, and Sony4 were closely related, as their Euclidean distance was one. Isolates Sony1, Sony2, Nyando5, and Nyando6 were also closely related, with a Euclidean distance of one, while isolates Nayndo2, Nayndo3, and Nyando4 were similarly related, with a Euclidean distance of one. However, within this cluster, isolates Sony1, Nayndo2, Nayndo3, and Nyando4 are grouped with Sony1, Sony2, Nyando5, and Nyando6 at a Euclidean distance of 4. These two clusters are combined with Sony5, Sony6, Sony3, and Sony4 at an Euclidean distance 10, indicating dissimilarity among the isolates. Cluster two contained most of its isolates from Kakamega, with only one isolate from Nyando1. Kakamega2, Kakamega3, and Kakamega1 are clustered together with a Euclidean distance of 1, while Kakamega6, Nyando1, Kakamega4, and Kakamega5 are grouped together.

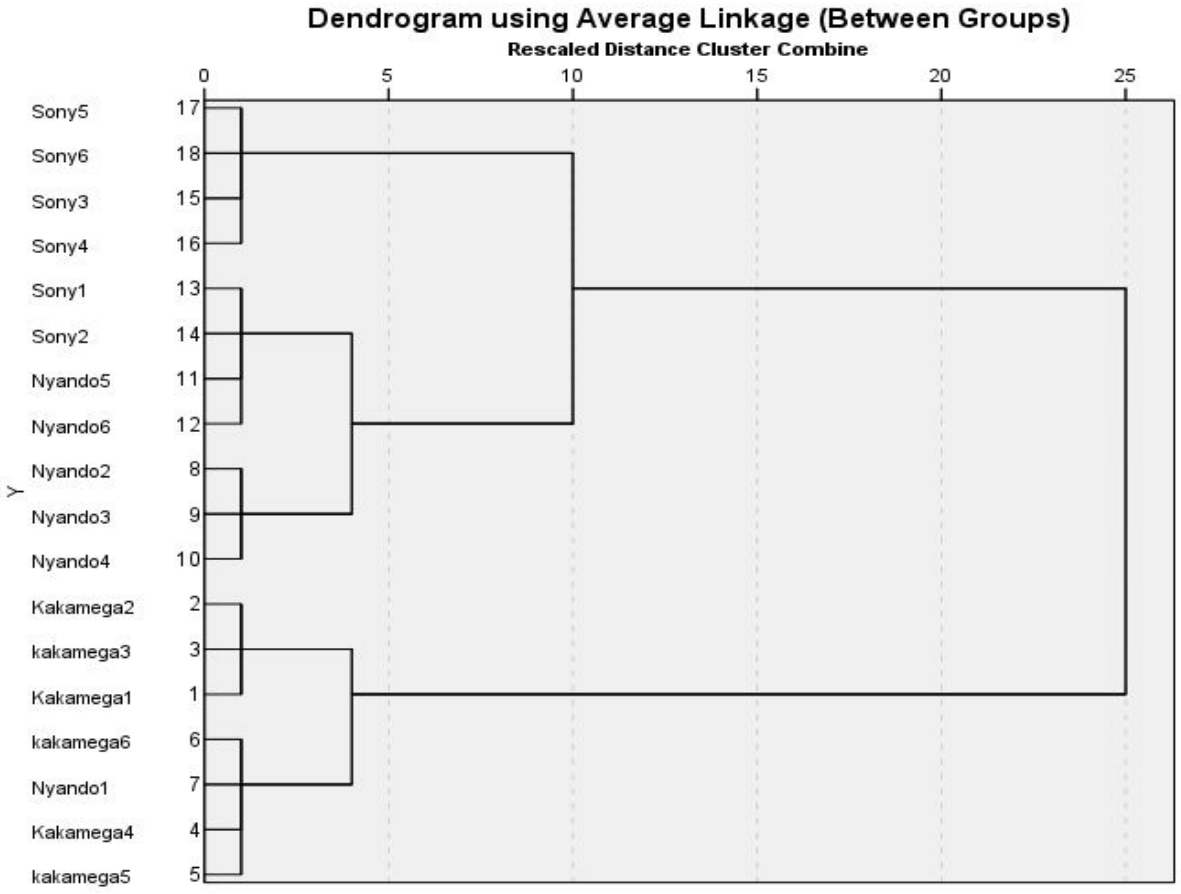

Figure 4. 3: Dendogram showing similarities among the three *Leuconostoc mesenteroides* spp isolates

4.2 Determination of bio-degradation levels of selected post-harvested sugar cane varieties by *Leuconostoc mesenteroides* isolates.

4.2.1 Effect of different *Leuconostoc* spp isolates on dextran, pH, purity, brix%

There was significant variation ($p < 0.05$) in dextran yield among the three isolates (Table 4.2). The Sony isolate produced the highest dextran level at 1654.99 ppm, followed by the Kakamega and Nyando isolates, which yielded 820.95 ppm and 820.39 ppm, respectively. The control produced the least amount of dextran, measuring 56.94 ppm. The Nyando isolate had the lowest pH value of 5.38, followed by Sony at 5.42, Kakamega at 5.43, and the control at 6.13. There was a significant difference in Pol% juice, with the power exhibiting the highest value of 18.28, followed by Kakamega at 17.32, Nyando at 17.27, and Sony at 17.09. The highest Brix% juice was found in the control at 20.04, followed by Kakamega at 19.49, Nyando at 19.35, and Sony at 19.29. However, no significant difference ($p > 0.05$) was noted in purity among the isolates.

Table 4. 2: The mean±SD of Dextran, pH, Brix, and Pol of sugar cane inoculated with three isolates of *Leuconostoc mesenteroides* from Nyando, Kakamega, and Sony Sugar.

Isolate	Dextran(ppm) Mean±S.D	pH Mean±S.D	purity Mean±S.D	Brix % juice Mean±S.D	Pol% Juice Mean±S.D
Control(uninoculated)	56.94±5.38^{c}	6.13±01^{a}	89.71±2.42^{a}	20.04±0.70^{a}	18.28±0.93^{a}
Kakamega Isolate	820.95±12.03^{b}	5.43±0.61^{b}	88.74±3.59^{a}	19.49±1.19^{b}	17.32±1.58^{b}
Nyando Isolate	820.39±12.03^{b}	5.38±0.52^{b}	89.12±3.50^{a}	19.35±1.05^{b}	17.27±1.55^{b}
Sony Isolate	1654.99±23.02^{a}	5.42±0.54^{b}	88.39±3.91^{a}	19.29±1.06^{b}	17.09±1.61^{b}
N	72	72	72	72	72
Df	3	3	3	3	3
F-value	50.20	39.93	2	8.04	9.92
p-Value	p<0.001	p<0.001	P=0.115	p<0.001	p<0.001

Means (column) that were marked with different alphabetic letters (a-c) were considered statistically significant differences ($p \leq 0.05$).

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In KEN83-737, Sony isolate produced the highest dextran recorded at 2549.49 ppm, while Kakamega and Nyando produced intermediate dextran levels at 2016.91 and 1813.49 ppm. (Fig 4.8) after 240 hours of cane storage. When Kakamega isolate and Nyando isolate were plotted, their curves were sandwiched on each other in a way that could not differentiate them. The zero isolates produced dextran, but it could not be significantly raised to be noticed when plotting.

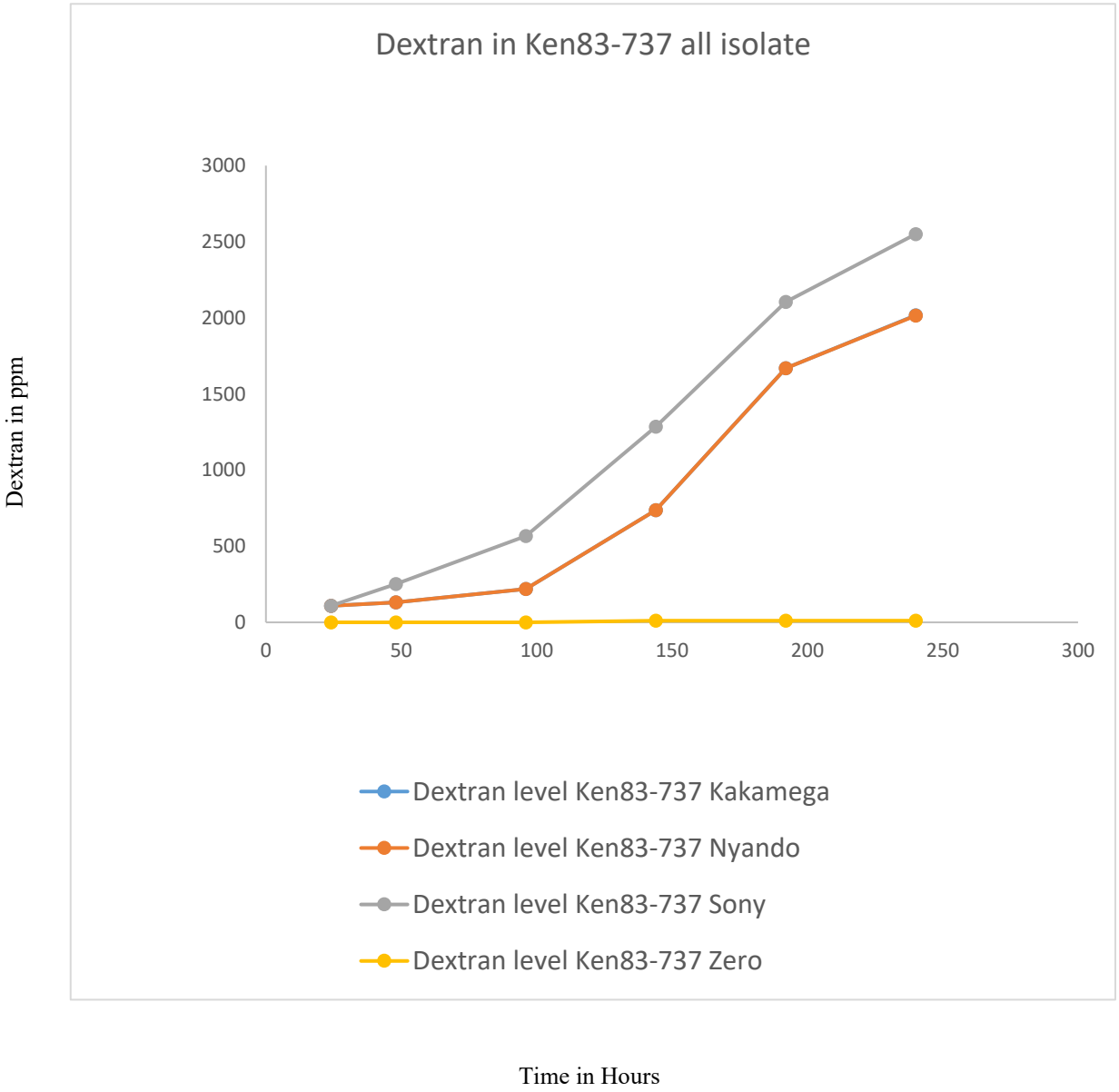

Figure 4. 4: Dextran produced by different isolates in KEN83-737.

In Co421, Kakamega and Nyando isolates produced the highest dextran at 1384.91 and 1390.10 ppm, respectively, compared to 967.60 ppm in the Sony sugar isolate (Fig 4.9). Initially, the Kakamega isolate picked up quickly from 24 hours of cane storage. It maintained a steady rise in dextran as the Nyando isolate from 150 to 240 hours of storage. The Sony sugar isolate gradually increased from 24 hours to 240 hours of cane storage compared to the two isolates.

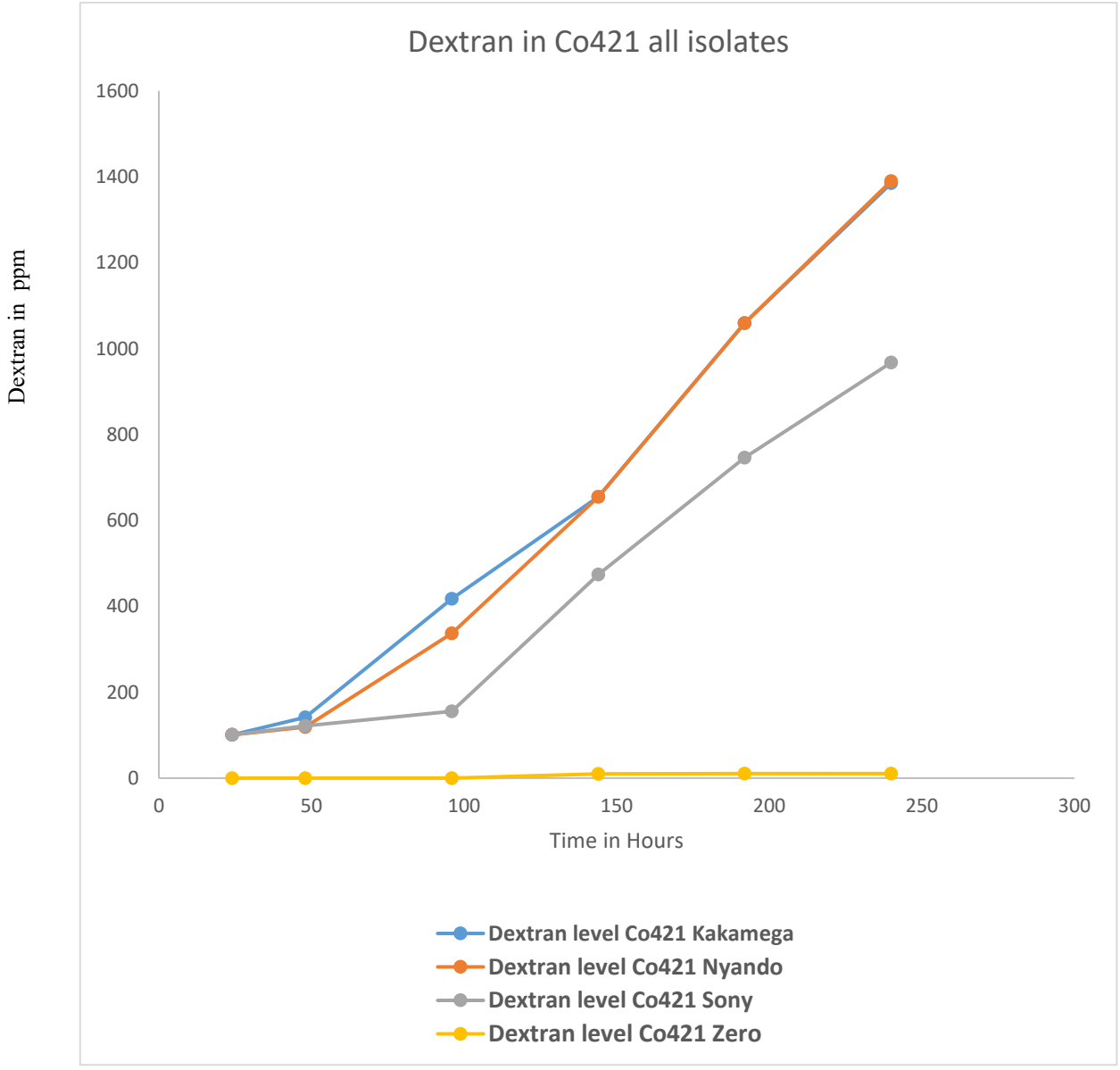

Figure 4. 5: Dextran produced by different isolates in Co421.

In KEN82-808, the highest dextran was recorded in the Sony isolate at 3104.55 ppm, while Kakamega and Nyando isolates produced the least dextran recorded at 1813.41 and 1806.38 ppm, respectively (Fig 4.10) below. The curves for the Kakamega and the Nyando isolates are sandwiched on each other.

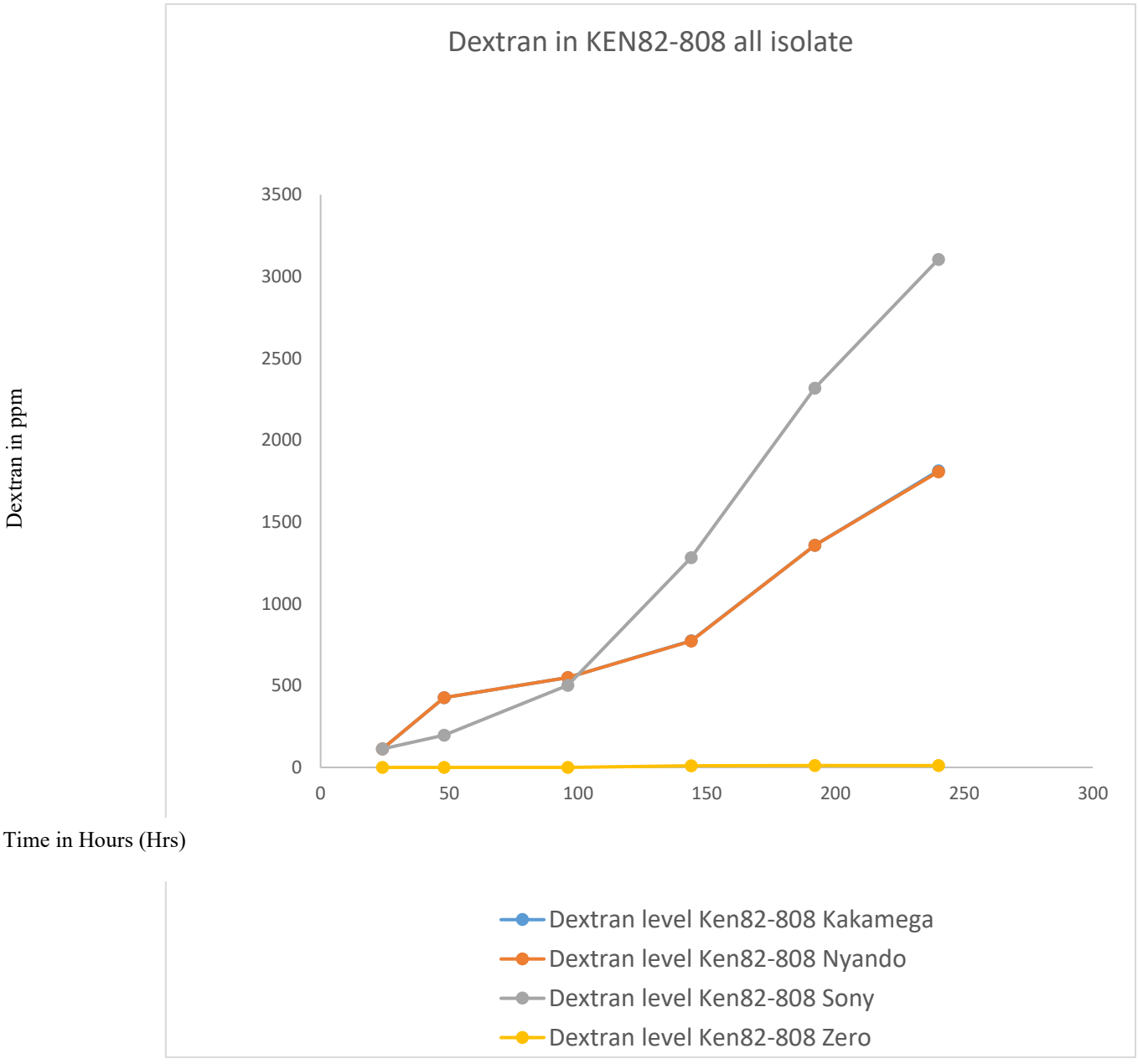

Figure 4. 6: Dextran produced by different isolates in KEN82-808.

N14 recorded the highest dextran from Sony at 6925.45 ppm. The Kakamega and Nyando isolates in this variety produced the same dextran at 2007.98 and 2006.18 ppm, respectively (Fig 4.11) below. The trend of dextran rise in Kakamega and Nyando followed the same trend and were all sandwiched on each other.

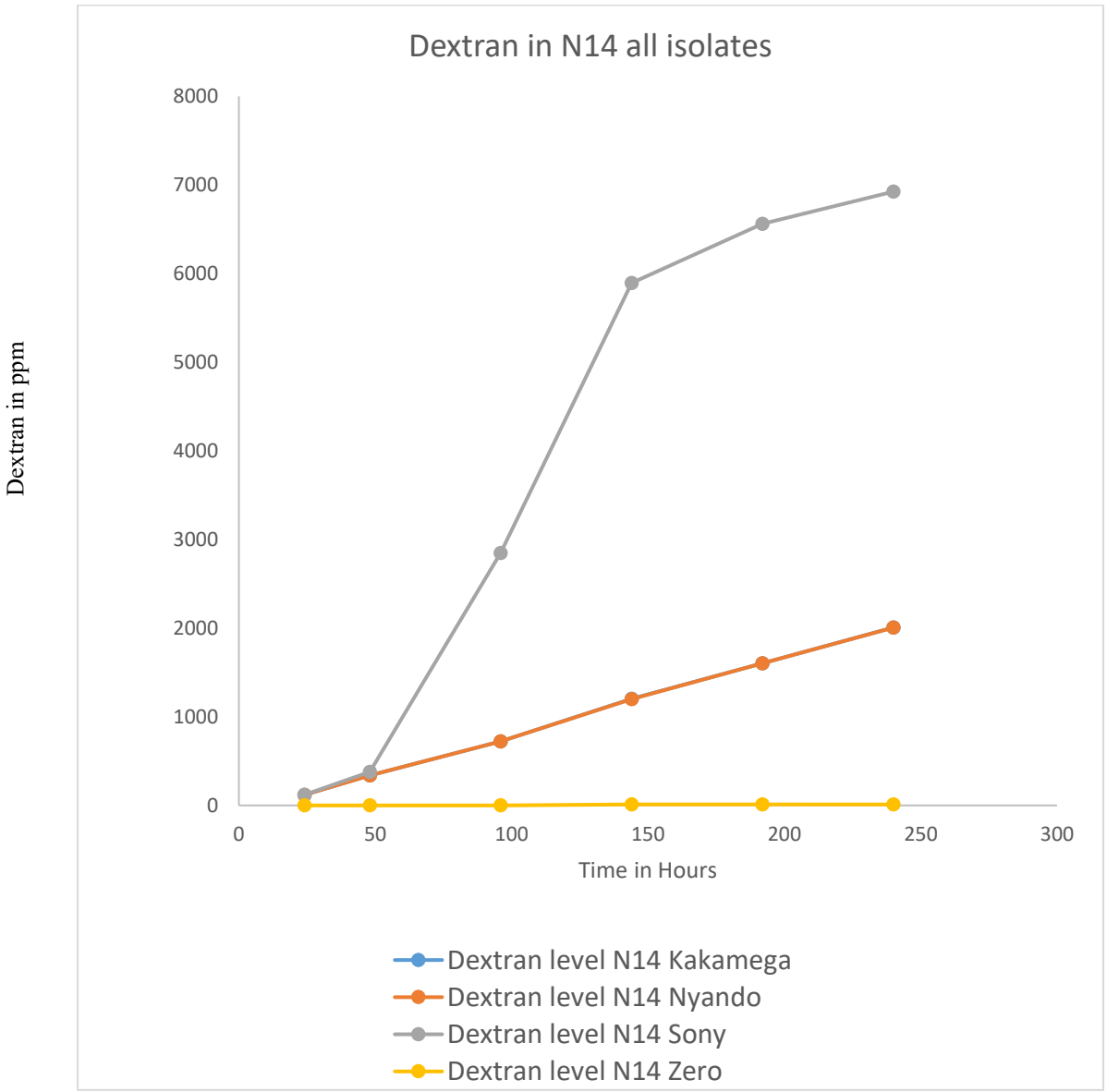

Figure 4. 7: Dextran produced by different isolates in N14.

4.2.2 Effect of selected sugar cane variety on dextran, pH, purity, Brix% % juice, and Pol% % juice.

There was significant variation ($p < 0.05$) across all varieties (Table 4.3). N14 produced the highest dextran level at 871.59 ppm, followed by KEN82-808, KEN83-737, and Co421 at 512.37, 457.37, and 306.27 ppm, respectively. N14 had the lowest pH value at 5.52, while Co421 had the highest at 5.62. The variety had a significant impact on purity, with KEN82-808 showing the lowest purity percentage at 86.81%. This was followed by N14, Co421, and KEN83-837 with percentages of 87.06%, 90.91%, and 91.16%, respectively. The effect of varieties on Brix % juice and Pol % juice also varied significantly, as indicated in Table 4.3.

Table 4. 3: The mean±SD of Dextran, pH, Brix, and Pol produced by different sugar cane varieties.

Variety	Dextran(ppm) Mean±S.D	pH Mean±S.D	Purity Mean±S.D	Brix % juice Mean±S.D	Pol% Juice Mean±S.D
CO421	306.27±11.36^{b}	5.62±0.54^{a}	90.91±1.83^{a}	20.47±0.59^{a}	18.7±0.83^{a}
KEN 82-808	512.37±15.51^{ab}	5.61±0.54^{a}	86.81±2.51^{b}	18.59±0.78^{d}	16.23±1.11^{d}
KEN83-737	457.82±15.90^{b}	5.61±0.58^{a}	91.16±3.4^{a}	19.76±0.72^{b}	18.11±1.18^{b}
N14	871.59±24.48^{a}	5.52±0.63^{a}	87.06±3.0^{b}	19.33±5.1.05^{c}	16.93±1.47^{c}
N	72	72	72	72	72
Df	3	3	3	3	3
F-value	5.91	0.43	53.69	69.08	65.87
p-Value	P=0.001	0.73	p<0.001	p<0.001	p<0.001

Means within a column that are marked with different alphabetic letters were considered statistically different ($p \leq 0.05$).

4.2.3 Effect of Time on dextran, pH, purity, brix % juice, and Pol % juice

There was a significant change ($p < 0.05$) in all parameters from 24 to 240 hours of storage time. The dextran concentration rose from 62.02 ppm at 24 hours to 1421.44 ppm at 240 hours of storage (Table 4.4). The pH of the sugar cane juice decreased from 6.16 at 24 hours to 5.07 at 240 hours of storage. The purity of the sugar cane juice fell from 89.48% at 24 hours to 85.12% at 240 hours of storage. There was a slight decline in Brix % juice from 19.38 at 24 hours to 18.57. Similarly, the Pol% juice, or sucrose %, decreased from 17.37 at 24 hours to 16.09 at 240 hours of storage.

Table 4. 4: The mean±SD of the Dextran, pH, Brix, and Pol produced between 24 hours and 240 hours of sugar cane storage.

Time(Hrs)	Dextran(ppm) Mean±S.D	pH Mean±S.D	Purity Mean±S.D	Brix % juice Mean±S.D	Pol% Juice Mean±S.D
24	62.02±4.6d	6.16±0.10a	89.48±3.38ab	19.38±0.978b	17.37±1.46b
48	133.91±7.52cd	6.14±0.10a	90.98±2.4a	20.12±0.77a	18.32±1.10a
96	313.08±13.63c	5.58±0.36b	90.99±2.5a	19.93±0.82ab	18.19±1.16a
144	764.01±15.74b	5.33±0.47c	89.48±2.19ab	19.78±0.87ab	17.81±1.17ab
192	1162.78±17.83ab	5.26±0.47cd	87.87±3.05b	19.38±1.00b	17.178±1.34b
240	1421.44±19.19a	5.07±0.57d	85.12±2.93c	18.66±1.18c	16.09±1.64c
N	6	6	6	6	6
Df	5	5	5	5	5
F-value	35.98	66.79	30.85	14.66	18.34
p-Value	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

Means within a column that are marked with different alphabetic letters were considered statistically different ($p \leq 0.05$).

4.2.4 Effect of interaction between Varieties and Time on Sugar cane juice Quality.

Significant differences were observed across all parameters, as shown in Table 4.5. For Co421, the dextran concentration rose from 56.64 ppm at 24 hours to 843.09 ppm at 240 hours. Conversely, the pH decreased from 6.16 to 5.11. Similar trends were noted in brix% juice and pol% juice, which fell from 20.28 to 19.65 and 18.58 to 17.43, respectively. In KEN 82-808, the dextran concentration at 24 hours was 63.62 ppm and increased to 1443.39 ppm at 240 hours. The pH dropped from 6.16 to 5.14. Brix % and pol% juice also declined from 18.91 to 17.31 and 16.59 to 14.46, respectively. KEN 83-737 produced 61.07 ppm of dextran at 24 hours, rising to 1429.44 ppm at 240 hours. The pH fell from 6.16 to 5.06. Brix % and pol% juice also decreased from 19.73 to 19.14 and 18.36 to 16.7, respectively. N14's dextran concentration at 24 hours was 66.98 ppm, increasing to 2113.43 ppm at 240 hours. The pH decreased from 6.17 to 4.97, while brix % and pol% juice fell from 18.58 to 18.54 and 15.94 to 15.76, respectively.

Table 4. 5: The mean±SD of the Dextran, pH, Purity, Brix, and Pol produced by different varieties between 24 hours to 240 hours of sugar cane storage.

Variety	Time(Hrs)	Dextran Mean±S.D	pH Mean±S.D	purity Mean±S.D	Brix % juice Mean±S.D	Pol% Juice Mean±S.D
CO421	24	56.64±4.53e	6.16±0.09a	91.61±0.45a	20.28±0.41a	18.58±0.45a
	48	75.57±5.27d	6.14±0.1a	92.25±1.2a	20.79±0.44a	19.18±0.58a
	96	177.56±8.72cd	5.59±0.35b	92.2±1.17a	20.83±0.42a	19.26±0.55a
	144	430.6±6.67c	5.4±0.44c	91.5±0.86a	20.8±0.33a	19.12±0.34a
	192	659±9.55b	5.33±0.44c	90.17±0.9ab	20.51±0.43a	18.64±0.48a
	240	843.09±11.47a	5.11±0.55d	87.74±0.82dc	19.65±0.54ab	17.43±0.84c
KEN 82-808	24	63.62±4.81e	6.16±0.1d	87.7±0.63dc	18.91±0.28bc	16.59±0.37de
	48	191.6±8.84d	6.14±0.11a	88.31±0.51	19.13±0.39bc	16.89±0.44e
	96	299.57±10.46cd	5.52±0.4a	89.19±1.6a	19.09±0.4bc	17.07±0.61de
	144	650.769.6c	5.37±0.45b	87.31±0.75d	18.72±0.28bc	16.43±0.41de
	192	1101.24±14.43b	5.3±0.45c	85.87±1.3e	18.4±0.43c	15.93±0.69e
	240	1443.39±17.44a	5.14±0.52c	82.49±1.99e	17.31±0.86c	14.46±1.3e
KEN83-737	24	61.07±4.71e	6.16±0.1d	93.02±0.79a	19.73±0.46ab	18.36±0.56a
	48	93.93±6.14d	6.13±0.1a	93.68±1.1a	20.33±0.33a	19.05±0.51a
	96	178.57±8.91cd	5.73±0.28ab	93.73±1.02a	20.19±0.28a	18.97±0.42a
	144	631.92±9.64c	5.34±0.46bc	91.04±1.91a	19.66±0.68b	18±1.04bc
	192	1194.67±14.57b	5.24±0.5c	89.24±3.62b	19.53±0.87b	17.56±1.1c
	240	1429.44±16.38a	5.06±0.65d	86.27±2.6dc	19.14±0.86bc	16.7±1.23de
N14	24	66.98±4.94e	6.17±0.1a	85.56±2.96d	18.58±1.29bc	17.68±1.69e
	48	197.66±8.52d	6.14±0.11a	89.67±1.58b	20.25±0.67a	18.17±0.89ab
	96	717.17±19.94cd	5.5±0.42ab	88.86±1.89bc	19.61±0.76bc	17.48±1.03bc
	144	1533.82±24.91c	5.19±0.57d	88.11±1.11bc	19.94±0.45a	17.66±0.69cd
	192	1847.108±26.12b	5.17±0.54d	86.2±2.99cd	19.07±0.76b	16.58±1.13de
	240	2113.43±26.7a	4.97±0.62d	83.97±2.69e	18.54±0.92c	15.76±1.47e
	N-Value Variety	4	4	4	4	4
	df Variety	3	3	3	3	3
	F-value Variety	9.78	0.9	132.43	119.15	11.99
	p-Value Variety	p<0.001	P=0.443	p<0.001	p<0.001	p<0.001

Means within a column that are marked with different alphabetic letters were considered statistically different ($p \leq 0.05$).

4.2.5 The rate of incline or decline in dextran, pH, purity, brix% juice, and pol% juice produced by Co421, KEN82-808, KEN83-737, and N14 varieties

All four sugarcane varieties showed an increase in dextran over time. Co421 exhibited the smallest increase in dextran after 240 hours of storage, measuring at +7.85, while N14 had the highest rate, recorded at +16.5. The other two varieties, KEN 82-808 and KEN 83-737, were intermediate at +13.82 and +14.55, respectively (Table 4.6).

Table 4. 6: The rate of incline or decline in Dextran, Brix, pH, and Pol produced by Co421, KEN82-808, KEN83-737, and N14 varieties.

Variety	Dextran	pH	purity	Brix % juice	Pol% Juice
CO421	+7.85	-0.009	-0.032	-0.006	-0.01
KEN82-808	+13.82	-0.009	-0.044	-0.014	-0.017
KEN83-737	+14.55	-0.01	-0.063	-0.01	-0.016
N14	+16.5	-0.011	-0.026	-0.005	-0.017

+ Means incline, - means decline

4.3 Determination of a suitable sodium chloride concentration for controlling deterioration in harvested sugar cane.

Based on the preliminary study of *Leuconostoc mesenteroides* bacteria under different concentrations of sodium chloride, all the isolates grew optimally under 2% sodium chloride. The least growth was witnessed in 6.5% of sodium chloride. The Kakamega isolate recorded the highest increase at 2.0 NaCl at 1.0*105 cfu/ml, while Nyando and Sony produced 8.9*104 cfu/ml and 8.6*104 cfu/ml, respectively.

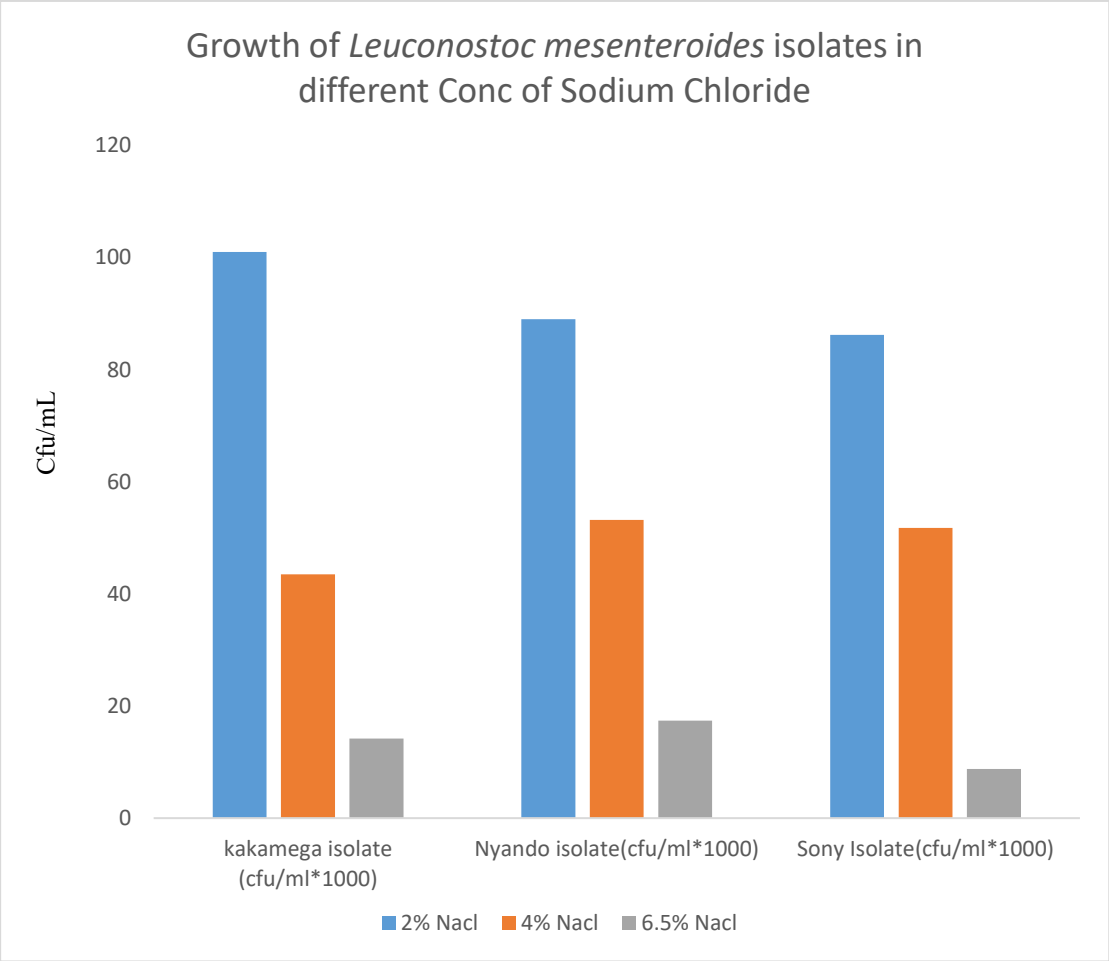

Figure 4. 8: Growth of *Leuconostoc mesenteroides*isolates under different concentrations of sodium chloride.

4.3.1 Effect of different sodium chloride concentrations, covering and uncovering of harvested sugar cane on sugar cane juice quality.

The results on the effect of different NaCl concentrations, covering and uncovering on the quality of sugar cane juice sugar cane (Brix % juice, Pol% juice, weight loss, and cfu of *Leuconostoc* spp) are presented in Table 4.7. There was a significant ($p < 0.05$) variation in all the parameters tested. 13.0% Nacl with uncovering produced the highest Pol% juice at 19.51, while 13.0% NaCl with covering produced the lowest Pol% juice at 17.57. 13.0% NaCl with uncovering produced the highest brix% juice at 22.87, while 13.0% Nacl with covering produced the lowest Pol% juice at 20.46. The highest weight loss was observed in control with uncovering of sugar cane, while the least was observed in water treatment with covering. Control produced the highest number of Cfu/ml at 36.63 while the least cfu/ml was observed in 13.0% NaCl with covering.

Treatment	Pol% juice Mean±S.D	Brix% % juice Mean±S.D	Weight loss(kg) Mean±S.D	CFU/ml Mean±S.D	Purity Mean±S.D
Control* C	18.18±1.19b	21.01±0.89bc	2.04±0.19bc	25.07±14.98ab	86.72±6.95
Control*UC	18.22±0.82b	21.78±1.74ab	1.92±0.27c	36.63±21.60a	84.21±8.04
Water* C	18.24±0.66b	20.76±0.88bc	2.53±0.41a	20.10±12.56ab	88.07±5.72
Water *UC	18.21±0.84b	21.39±1.78bc	2.08±0.34bc	27.97±16.06ab	85.64±7.56
6.5% Nacl*C	18.04±1.03b	20.68±0.74bc	2.19±0.19b	19.307±12.58ab	87.31±5.84
6.5%Nacl UC	18.31±0.96b	21.60±1.53bc	2.14±0.19bc	28.90±17.30ab	85.22±7.93
13.0% Nacl* C	17.58±1.21b	20.46±1.01c	2.11±0.09bc	18.60±18.87b	86.05±6.48
13.0%Nacl* UC	19.52±0.54a	22.87±1.56a	2.13±0.22bc	31.80±21.22ab	85.78±7.37
N	240	240	240	240	240
Df	9	9	9	9	9
F-value	4.352	5.093	12.107	4.182	0.899
p-Value	p<0.001	p<0.001	p<0.001	p<0.001	P=0.518

Table 4. 7: The mean ±SD of the Pol, Brix, Weight loss, and purity produced by different treatment

Means within a column that are marked with different alphabetic letters were considered statistically different ($p \leq 0.05$).

Key: C covering UC Uncovering

4.3.2 Effect of covering and uncovering of harvested sugar cane on sugar cane juice quality over time.

The results on covering and uncovering the quality of sugar cane juice (Brix% % juice, Pol% juice, weight loss, and CFU of *Leuconostoc* spp) are presented in Table 4.8. There was a significant ($p < 0.05$) variation in all the parameters tested except pol% juice. Brix % juice increased progressively from 19.68 and 19.68 on 0 hours of cane storage to 21.66 and 24.09 on 192 hours of storage for Covering and uncovering, respectively. Weight loss decreased from 2.35 and 2.28 on o hours of storage to 2.12 and 1.86 on 192 hours of storage for covering and uncovering, respectively. The purity of the sugar cane juice was also seen to decrease from 95.59 to 78.91 and 96.32 to 73.93 for covering and uncovering, respectively.

Table 4. 8: The mean±SD of the Pol, Brix, weight loss, cfu/ml, and purity produced by covering and uncovering harvested sugar cane over time.

Time (Hrs)	Treatments	Brix% juice	Pol% Juice	purity	Weight (kgs)	Cfu/ mL
0	Covering	19.68±1.00e	18.82±0.76a	95.59±2.32a	2.35±0.33a	2.62±0.71e
	Uncovering	19.68±0.63de	18.95±0.76a	96.32±1.63b	2.28±0.31ab	2.08±4.02e
48	Covering	20.39±1.45de	18.41±0.89a	90.27±1.98c	2.22±0.34bc	11.08±8.62de
	Uncovering	20.63±0.51d	18.47±0.99a	89.52±2.71c	2.09±0.33bc	19.58±10.16cd
96	Covering	20.66±0.76cd	17.87±1.45a	86.48±4.07d	2.21±0.33bcd	25.79±10.16c
	Uncovering	21.59±0.88bc	18.16±0.76a	84.09±1.05d	2.00± bc	40.75±9.97ab
144	Covering	21.24±0.51b	17.82±1.45a	83.91±1.66b	2.18± bcd	32.50±1.05bc
	Uncovering	22.21±0.78a	17.98±1.45a	80.97±2.57c	1.95±0.23cd	48.41±11.17a
192	Covering	21.66±0.99abc	17.10±0.61a	78.91±2.57c	2.12±0.23d	31.91±11.17b
	Uncovering	24.09±0.77a	17.80±1.26a	73.93±4.69e	1.86±0.24d	45.58±8.87a
S.E varieties		0.097	0.126	0.386	0.03	0.776
S.E Mean		0.031	0.04	0.122	0.01	0.245
M.S.E		0.224	0.378	3.574	0.022	14.446
p-value		p<0.001	P=0.088	p<0.001	P=0.015	p<0.001

Means within a column that are marked with different alphabetic letters were considered statistically different ($p \leq 0.05$)

CHAPTER FIVE

DISCUSSION

5.1 Determination of the biological and biochemical characteristics of selected *Leuconostoc mesenteroides* isolates

5.1.1 Identification of *Leuconostoc mesenteroides* bacteria.

The method used for isolation and identification revealed that all eighteen isolates were *Leuconostoc mesenteroides.* This aligns with the findings of Misra *et al*. (2020), who reported *Leuconostoc mesenteroides* bacteria in post-harvest stored sugar cane. In this study, the Gram stain showed that all the isolates retained crystal violet, appearing blue and thus indicating they were gram-positive. All cells were either spherical or oval, occurring singly or in pairs. The initial identification test revealed that the isolates did not produce gas when exposed to 3% hydrogen peroxide. The catalase enzyme breaks down hydrogen peroxide (H_2O_2) into oxygen and water molecules ($2H_2O + O_2$), with oxygen production observed as the formation of O_2 bubbles. The absence of gas bubbles indicated the catalase-negative nature of *Leuconostoc mesenteroides* bacteria. All isolates produced gas trapped in an inverted Durham tube. *Leuconostocs*, like other LABs, lack a tricarboxylic acid cycle or a cytochrome system and cannot obtain energy from oxidative phosphorylation (Cogan & Jordan, 1994; Folch et al., 2021). Instead, they generate energy through substrate-level phosphorylation during the fermentation of sugars into lactic acid, ethanol, acetate, and CO2 (Folch *et al*., 2021). This indicates the heterofermentative nature of *Leuconostoc mesenteroides* bacteria. For confirmatory identification, the carbohydrate fermentation profile revealed that the bacterium obtained from 24–48 hours of incubation utilizes glucose, melibiose, maltose, sucrose, fructose, dextrose, lactose, and trehalose as carbon sources and evolves oxygen. All these characteristics established the identity of Leuconostoc spp. in sugarcane juice (Bergey, 1994).

From the hierarchical cluster analysis, it was found that all the isolates were grouped into two major clusters. This was in agreement with Chentouf *et al*., (2023) and Chun *et al*., (2017) who discovered two clusters of *Leuconostoc mesenteroides* using 16S rRNA analysis.

5.2 Bio-degradation levels of selected post-harvested sugar cane varieties by Leuconostoc mesenteroides isolates.

There are very limited studies done on the effect of different isolates of *Leuconostoc mesenteroides* bacteria on the deterioration of sugar cane. This study clearly showed significant differences in dextran, pH, brix% juice, and pol % juice produced by three isolates (Kakamega, Nyando, and Sony) (Table 4.2). This difference could be associated with the adaptation of the isolates to the different ecological niches of collection (Chase *et al*., 2021). These differences in microorganisms have been

reported to develop due to the ecological pressure of survival, which ends up introducing heritable characteristics in the genome of a microbe, leading to differences in competition for resources (Nguyen *et al*., 2021). Kakamega and Nyando isolates were not significantly different in dextran, pH, brix% juice, pol% % juice, and purity coefficient (Table 4.2). This scenario can be attributed to the proximity of the regions to each other, leading to a high exchange of sugarcane germplasm between the two areas, leading to a relative spread of a homogenous population of the bacteria (Saini *et al*., 2020). Moreover, in recent years, factories in Kakamega County have been encroaching on the Nyando sugar belt for milling sugar cane due to a shortage of raw materials (Wanga-Odhiambo, 2016). As a result, this poses another likely route forthe spread of the isolates in the two regions. However, the Sony isolate produced pH, brix%, juice, and pol% % juice that were significantly different from the two. Sony isolate produced the highest dextran of 1654.99ppm, followed by Kakamega and Nyando isolates that produced 820.95 ppm and 820.39 ppm of dextran, respectively (Table 4.2). The control produced the least dextran of 56.94 ppm. The dextran in the control began accumulating from 144 hours of storage, a case that could be attributed to contamination of the sugar cane by *Leuconostoc* through handling during the study period. This scenario supports *Leuconostoc*spp.producing dextran in harvested sugar cane as published by Misra*et al*., (2020). Nyando isolate produced the lowest pH value of 5.38, followed by Sony, Kakamega, and control with values of 5.42, 5.43, and 6.13, respectively. There was a significant difference in Pol% juice, with the power having the highest value of 18.28, followed by Kakamega, Nyando, and Sony (Table 4.2). Brix% juice was highest in the control at 20.04, followed by Kakamega at 19.49, Nyando at 19.35, and Sony at 19.29. However, there was no significant ($p > 0.05$) difference in purity among the isolates.

Based on the effect of the selected sugar cane variety on dextran, pH, purity, Brix% % juice, and Pol% % juice, there was significant ($p < 0.05$) variation in all the varieties (Table 4.3). N14 produced the highest ppm of dextran, while Co421 produced the lowest (Table 4.3). N14 produced the lowest pH value at 5.52, while Co421 produced the highest at 5.62. The effect of varieties on Brix% % and Pol% % juice also differed significantly (Table 4.3). This can be attributed to the differences in the genomes of the varieties (Misra *etal*., 2022a; Patil *et al.*, 2022). A trait that has been reported to be polygenic, where multiple genes are involved in the trait's expression, leading to variations in post-harvest sucrose losses across different environments (Misra *etal.*, 2020). Varieties are a major component that directly influences sugar recovery, and their impact is further influenced by factors such as environmental conditions and management approaches (Shanthi *et al.*, 2023). In this study, the problem of variety variations in the expression of the trait was managed by conducting the study under controlled conditions.

There was a significant ($p < 0.05$) change in all the parameters from 24 hours to 240 hours of storage time. The concentration of dextran increased from 62.02ppmin 24 hours to 1421.44ppm in 240hours of storage (Table 4). The pH of the sugar cane juice dropped from 6.16 in 24 hours to 5.07 in 240 hours of storage time. There was a slight drop in Brix % juice from 19.38 in 24 hours to 18.57. Similarly, the Pol% juice or the sucrose % dropped from 17.37 on the 24 hours to 16.09 on 240 hours of storage. These results are in agreement with (Kouzi & Kontro, 2024; Misra *et al., 2022a)* who reported that an increase in time after sugar cane harvesting harms the quality of sugar cane juice. This is because *Leuconostocspp* bacteria breaks down sucrose into fructose and glucose using dextransucrase enzyme to produce dextran that accumulates in the sugar cane juice with an increase in storage time (Misra, Solomon, Mall, *et al*., 2020). At the same time, it produces lactic acid that lowers the pH of the medium.

Manifold dextran increase observed in all the sugar cane varieties over time. The highest rate of dextran formation was observed in N14, which showed an ultimate dextran quantity o after 240 hours. KEN 83-737 and KEN 82-808 showed a relatively lower rise in dextran contents, 1429.44 and 1443.39 ppm, respectively, after 240 hours of storage. However, the lowest rate of dextran formation was observed in Co421 at 843.09 ppm after 240 hours of storage (Table 4.5). Juice from N14 showed the highest rate of incline in dextran (m= +16.50), KEN83-737(m=+14.55), KEN82-808(m=+13.82) and Co421 (m=+7.85) (Table 4.6). Juice from Co421 cane represented the lowest rate of increase of dextran with time. It is known that for every one ppm of dextran produced, there is a loss of 0.0025 pounds of raw sugar (Misra *et al*., 2016; Misra, Solomon, Mall, *et al*., 2020). From this result, it is estimated that N14 lost 4.84 pounds of sucrose by conversion to dextran, KEN82-808 lost 4.31 pounds of sucrose to dextran, KEN83-737 lost 4.19 of sucrose to dextran, and lastly, Co421 lost 2.41 of sucrose to dextran after 240 hours of sugar cane storage. The results agreed with previous work done by (Kouzi & Kontro, 2024), (Bashari, 2023a), and (Khan *et al*., 2020), which showed an increase in dextran among different varieties over time.

Leuconostoc is a lactic acid bacteria(LAB) and prefers an initial medium pH of 6.5 for its growth (Schillinger & Holzapfel, 2011). *Leuconostocs*, like other LABs, do not contain a tricarboxylic acid cycle or a cytochrome system and cannot derive energy from oxidative phosphorylation (Cogan & Jordan, 1994; Folch et al., 2021). Instead, they obtain energy through substrate-level phosphorylation during the fermentation of sugars to lactic acid, ethanol or acetate, and CO2 (Folch et al., 2021). Production of these acids lowers the pH of the sugar cane juice. An increase in acidity of the sugarcane juice was observed over time, as seen in (Table. 4.5) in all the sugar cane varieties. The pH values slightly decreased between 24 hours to 48 hours. The *Leuconostoc* bacteria were acclimatizing and adjusting to the new environment before they could pick an exponential phase. The pH value

decreased rapidly from 48 hours to 144 hours of cane storage (Table 4.5). This is because the *Leuconostoc* bacteria had acclimatized to the environment, and enough sucrose was available as a substrate for utilization. Very few secondary metabolites were also released, eventually becoming toxic to the bacteria in the media. The rate of pH value maintained a slow, gradual decrease, signifying the plateau phase where the reproduction rate equals the death rate from 192 hours to 240 hours of storage. N14 recorded the widest margin of change in pH from 6.2 to 5.0, while the rest of the varieties maintained a similar change trend from 6.16 to 4.97(Table 5) for the entire storage time. It was, however, evident from the regression analysis that N14 had the highest rate of change in pH (m=-0.011), followed by KEN83-737 with a rate of (m=-0.01). The remaining two varieties were recorded (m=-0.009) (Table 4.6). These results agreed with the study of (Pandraju & Rao, 2020), (Zaidan et al., 2021), and (Panigrahi et al., 2021), where the pH of sugar cane juice drops with an increase in several storage hours.

Sucrose percent, or pol% juice, is the most important parameter of sugarcane crops from the perspective of sugar mills. This study observed the gradual decrement in sucrose of the evaluated genotypes over the whole staling period. Co421 showed an excellent response against sucrose deterioration. The sucrose% age values were not only highest for this clone during 240 hours of storage but also showed the minimal reduction rate for this parameter of only 1.15 change with a deterioration rate of (m=-0.01). KEN82-808 showed the widest change in pol % juice of 2.13 and a deterioration rate of (m=-0.017). Although N14 recorded a 1.92 change in Pol% juice from 24 hours and 240 hours of storage, it had the same deterioration rate with KEN82-808 of (m=-0.017) (Table 4.6). KEN83-737 showed a moderate change in the sucrose from 24 hours to 144 hours of cane storage with a difference of 1.66 and a deterioration rate of (m=-0.016). The decrease in sucrose resulted from the inversion of sucrose into fructose and glucose in the *Leuconostoc* bacteria. The glucose moiety was used for metabolism, and part was used to produce dextran by dextransucrase enzyme, while the fructose moiety was used to produce mannitol. It was also noted that some roots sprouted on day 4, which used sucrose as an energy source. The results of our study agreed with previous studies, which unanimously reported that the cane degradation over storage time led to loss of sugar content (M. T. Khan *et al.*, 2020) 11)

Brix percentage juice represents total dissolved solids, including all sugars and non-sugars in the juice. Generally, a decrease in brix% was observed for all the genotypes under evaluation (Table 4.5). N14 showed the lowest rate of decline in brix% over 240 hours of sugar cane storage, from 18.58 to 18.54. The highest rate of decrease in brix % juice was witnessed in KEN82-808 from 18.91 to 17.73. Juices from KEN 82-808 showed the highest rate of decline in brix% juice (m= -0.014), KEN83-

737(m=-0.01), Co421 (m=-0.006) and N14 (m=-0.005) (Table 4.6). The decline in total dissolved solute indicates that solids were being utilized by the *Leuconostoc* as a biomass source and converted into a liquid and/or gas state. The observations of a decrease in brix% were reported earlier (Eggleston, 2002). This study, however, differs from other studies done by (M. T. Khan et al., 2020) and (Misra, Solomon, Mall, et al., 2020), who reported an increase in Brix% juice over time. The difference could be because our experiment was done under a controlled environment where evaporation was restricted, while the other experiments were performed in the open where evaporation was so high. If the water evaporates from harvested sugar cane, the total dissolved solute will increase because of evaporation.

5.3 Determination of selected suitable chemical concentration in controlling deterioration in harvested sugar cane

There was a significant ($p < 0.05$) change in all the parameters produced by different concentrations of NaCl, covering, and uncovering except the purity. The highest pol% juice was observed in 13.0% NaCl with covering, while the least followed in control without covering at 19.52 and 18.18, respectively. Brix % juice was recorded highest in 13.0 with uncovering while least in 13.0% NaCl with covering. Weight loss was observed highest in control with uncovering while least in water with covering. Weight loss in harvested sugar cane is significant because sugar cane payment in Kenya is still based on cane weight (Mandela *et al.*, 2024). This study shows that covering sugar cane after harvesting preserves weight loss (Misra *et al*., 2022b). There was a significant difference in CFU/ml with control and uncovering, producing the highest cfu of Leuconostoc spp while the least observed in 13.0% NaCl with covering. This could be attributed to the fact that Sodium chloride has been used as a preservative in some food products as it inhibits and kills food-borne pathogens like bacteria by withdrawing water from the bacterial cells and causing them to dehydrate and die (V.a. *et al*., 2021). Sodium chloride also reduces microbial growth as it causes hindrance with cellular enzymes and even compels cells to expel sodium ions, thereby resulting in a lessening in microbial growth (Misra, Solomon, Mall, *et al*., 2020). Furthermore, microbial cells, on the application of salts, suffer from osmotic shock, which in turn loses water from cells, resulting in cell death and, in this way, also minimizes the growth of microbes in stored food. Covering harvested sugar cane contributes to reducing degradation as it reduces the rate of evaporation of water from sugar cane conservation moisture. Covering sugar cane also provides a lower temperature that is not conducive for *Leuconostoc* spp as the bacteria is mesophilic.

CHAPTER SIX

CONCLUSION, RECOMMENDATION, AND SUGGESTION FOR FURTHER RESEARCH

6.0 Conclusion

- It is concluded from the study that the post-harvest deterioration of sugarcane mainly depends on the sugarcane variety. In this study, Co421 showed a minimum sucrose loss of 2.41 pounds compared to 4.19, 4.31, and 4.84 pounds for KEN83-737, KEN82-808, and N14, respectively.

- Different isolates of *Leuconostoc mesenteroides* bacteria have varying abilities to degrade sucrose in harvested sugar cane. The Sony isolate is more aggressive in degrading sucrose in harvested sugar cane than the Kakamega and Nyando isolates.

- The study concludes that covering sugar cane after harvesting reduces the degradation of sucrose by *Leuconostoc mesenteroides* bacteria. However, the use of different sodium chloride is ineffective unless paired with covering.

6.1 Recommendations

- Since deterioration of harvested sugar cane is variety-based, it's recommended that varieties susceptible to deterioration are given immediate priority for milling over those that are resistant.

- Based on this study, it is recommended that sugar cane milling after harvesting should be managed depending on the region of harvest. Proper management of harvested sugar cane should be put in place to help immediate milling in Sony. This is because Sony isolates were very aggressive in degrading sucrose.

- It is further recommended that harvested sugar cane be covered after harvesting with trash to help control deterioration, which should be supplemented with 6.5% sodium chloride.

6.2 Suggestion for further research

- It is recommended from this study that the *leuconostoc mesenteroides* bacteria can be characterized at the molecular level and eventually used to study the deterioration at the molecular level to control staleness.

- The present study did not address some of the important parameters like titratable acidity, Manitol, color, and others. This could be a basis for future investigations involving the deterioration of sugar cane varieties

- A future study should profile the deterioration of sugar cane in the field. The present study was confined to the lab; therefore, it should be upscaled to the field to gain a clear understanding of how each variety deteriorates in the open field.

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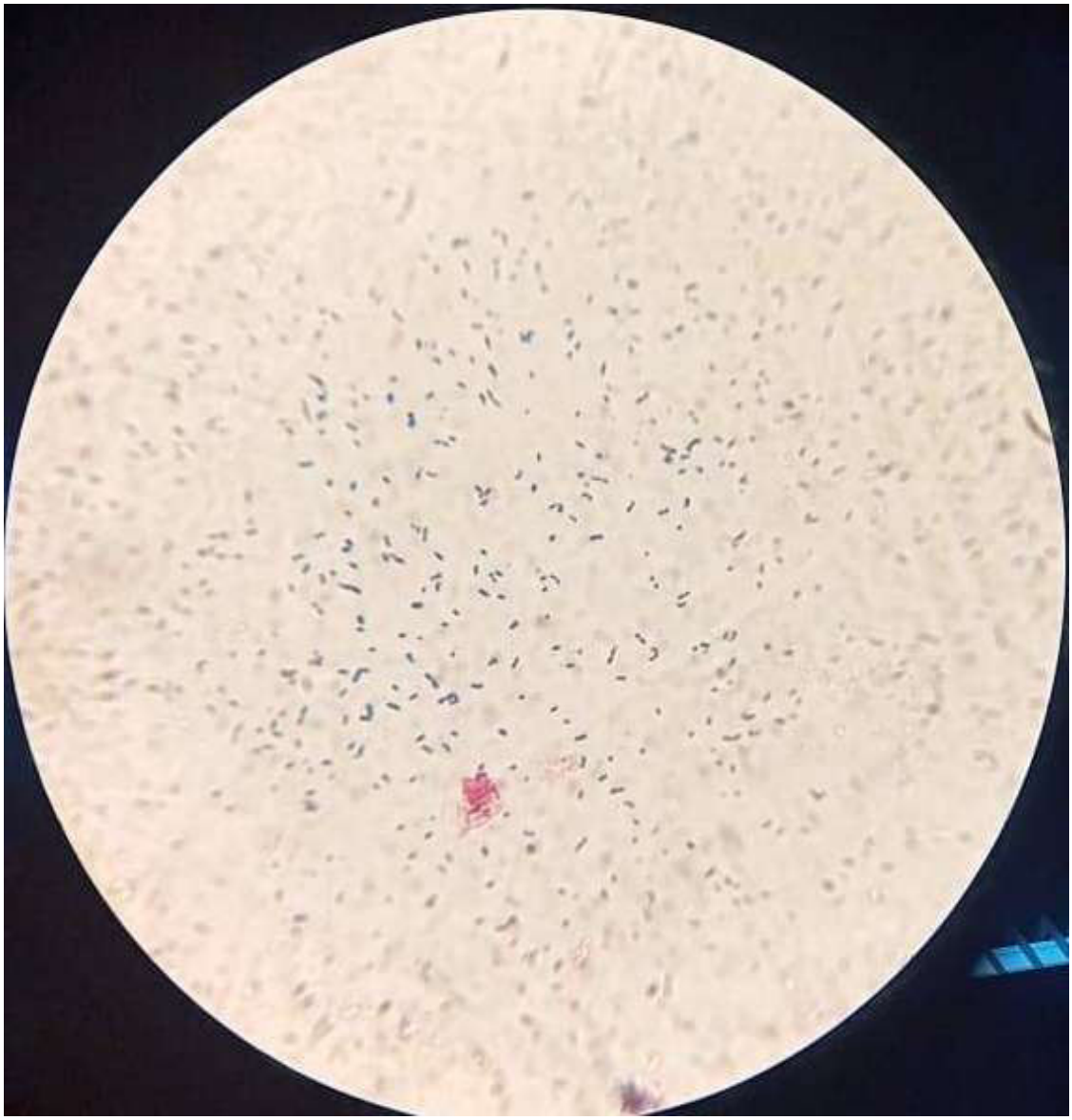
APPENDICES

Appendix I: *Leuconostoc mesenteroides* seen under a microscope; Gram-positive cocci.



Magnification x100 under oil immersion.

Appendix ii: Catalase test of *Leuconostoc mesenteroides* on the slide; catalase negative.

Appendix iii: Gas trapped in an inverted Durham tube from the glucose metabolism by the *Leuconostoc mesenteroide* isolate

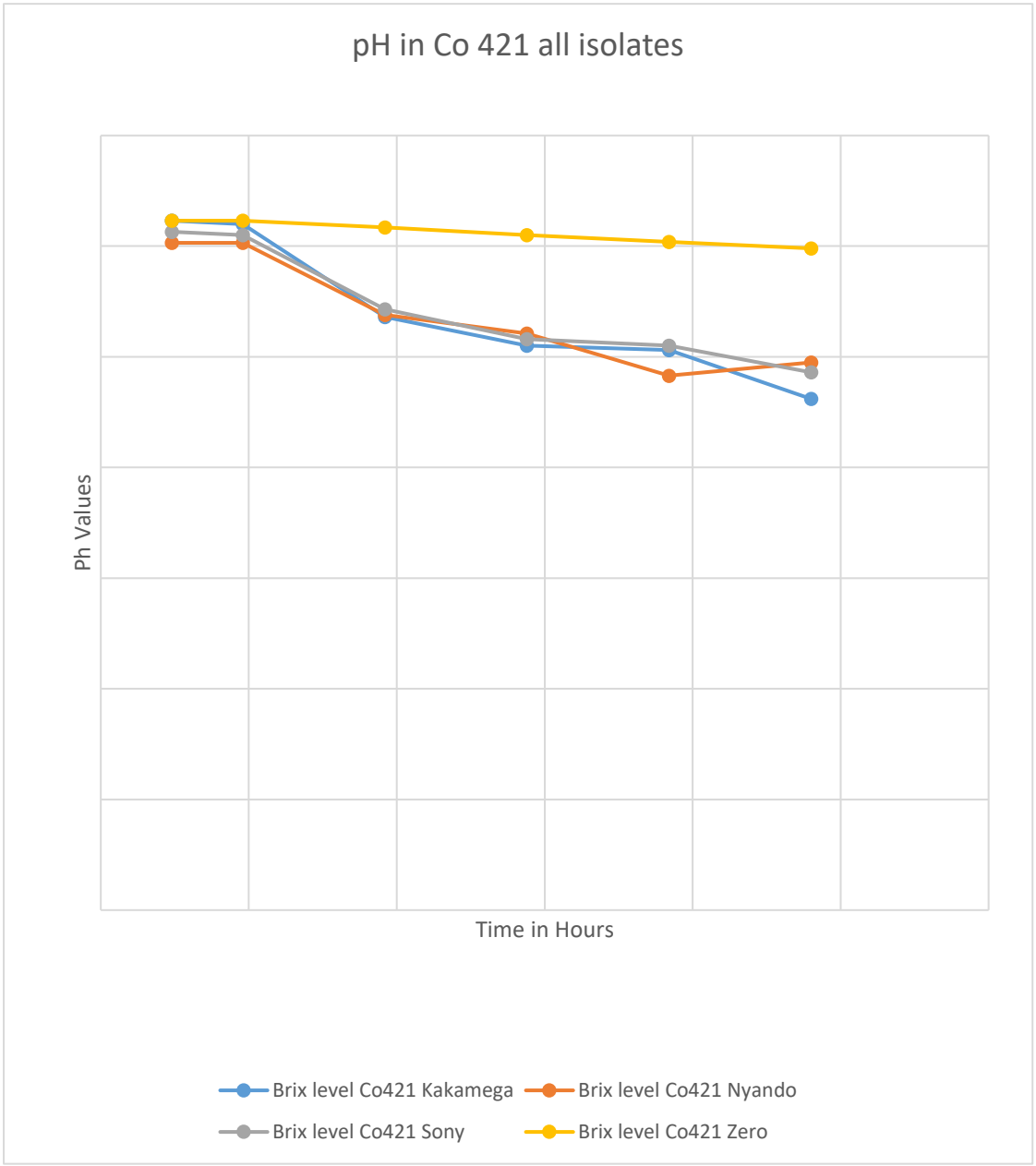
Appendix iv: The mean±SD of the dextran, pH, Purity, Brix, and Pol produced by interaction varieties and isolates between 24 hours to 240 hours of sugar cane storage

Isolate	Time	Cane_Variety	Dextran(ppm) Mean±S.D	Pol% Juice Mean±S.D	pH Mean±S.D	Brix % juice Mean±S.D
Control (Uninoculated)	24	CO421	0	19.27±0.35	6.23±0.05	20.93±0.26
		KEN 82-808	0	17.18±0.11	6.23±0.05	19.36±0.10
		KEN83-737	0	19.16±0.67	6.23±0.05	20.45±0.36
		N14	0	18.73±0.29	6.23±0.05	20.72±0.04
	48	CO421	0	19.27±0.35	6.23±0.05	20.93±0.26
		KEN 82-808	0	17.18±0.11	6.23±0.05	19.36±0.10
		KEN83-737	0	19.16±0.67	6.23±0.05	20.45±0.36
		N14	0	18.73±0.29	6.23±0.05	20.72±0.04
	96	CO421	0	19.08±0.35	6.17±0.05	20.72±0.26
		KEN 82-808	0	17.01±0.11	6.17±0.05	19.17±0.10
		KEN83-737	0	18.97±0.66	6.17±0.05	20.25±0.36
		N14	0	18.54±0.29	6.17±0.05	20.51±0.04
	144	CO421	10.03±0.01	18.89±0.34	6.10±0.05	20.52±0.25
		KEN 82-808	10.63±0.04	16.84±0.11	6.10±0.05	18.98±0.10
		KEN83-737	10.42±0.00	18.70±0.65	6.10±0.05	20.04±0.35
		N14	10.53±0.04	18.36±0.28	6.10±0.05	20.31±0.04
	192	CO421	10.28±0.01	18.70±0.34	6.04±0.05	20.31±0.25
		KEN 82-808	10.89±0.04	16.67±0.11	6.04±0.05	18.79±0.10
		KEN83-737	10.67±0.00	18.59±0.65	6.04±0.05	19.84±0.35
		N14	10.79±0.04	18.17±0.28	6.04±0.05	20.11±0.04
	240	CO421	10.54±0.01	18.51±0.34	5.98±0.05	20.11±0.25
		KEN 82-808	11.16±0.04	16.5±0.11	5.98±0.05	18.60±0.10
		KEN83-737	10.94±0.00	18.41±0.64	5.98±0.05	19.65±0.35
		N14	11.06±0.04	17.99±0.28	5.98±0.05	19.91±0.04
KAKAMEGA	24	CO421	100.69±0.01	18.31±0.01	6.23±0.05	20.08±0.01
		KEN 82-808	113.08±0.00	16.37±0.01	6.23±0.05	18.75±0.01
		KEN83-737	108.57±0.00	18.09±0.01	6.23±0.05	19.46±0.01
		N14	119.06±0.00	17.75±0.00	6.23±0.05	17.86±0.01
	48	CO421	141.63±0.66	19.27±0.35	6.20±0.10	20.93±0.26
		KEN 82-808	426.81±1.03	17.18±0.11	6.20±0.10	19.36±0.10
		KEN83-737	131.02±0.12	19.16±0.67	6.13±0.05	20.45±0.36
		N14	338.67±0.39	18.73±0.29	6.20±0.10	20.72±0.04
	96	CO421	417.55±0.34	19.22±0.19	5.36±0.05	20.95±0.01
		KEN 82-808	548.38±0.89	16.53±0.41	5.36±0.05	18.76±0.35
		KEN83-737	220.01±0.00	18.89±0.03	5.66±0.05	20.13±0.01
		N14	722.4±0.25	18.05±0.59	5.27±0.06	19.83±0.47
	144	CO421	655.17±0.21	19.18±0.41	5.10±0.00	20.86±0.32
		KEN 82-808	773.73±0.11	16.75±0.11	5.07±0.06	18.93±0.20

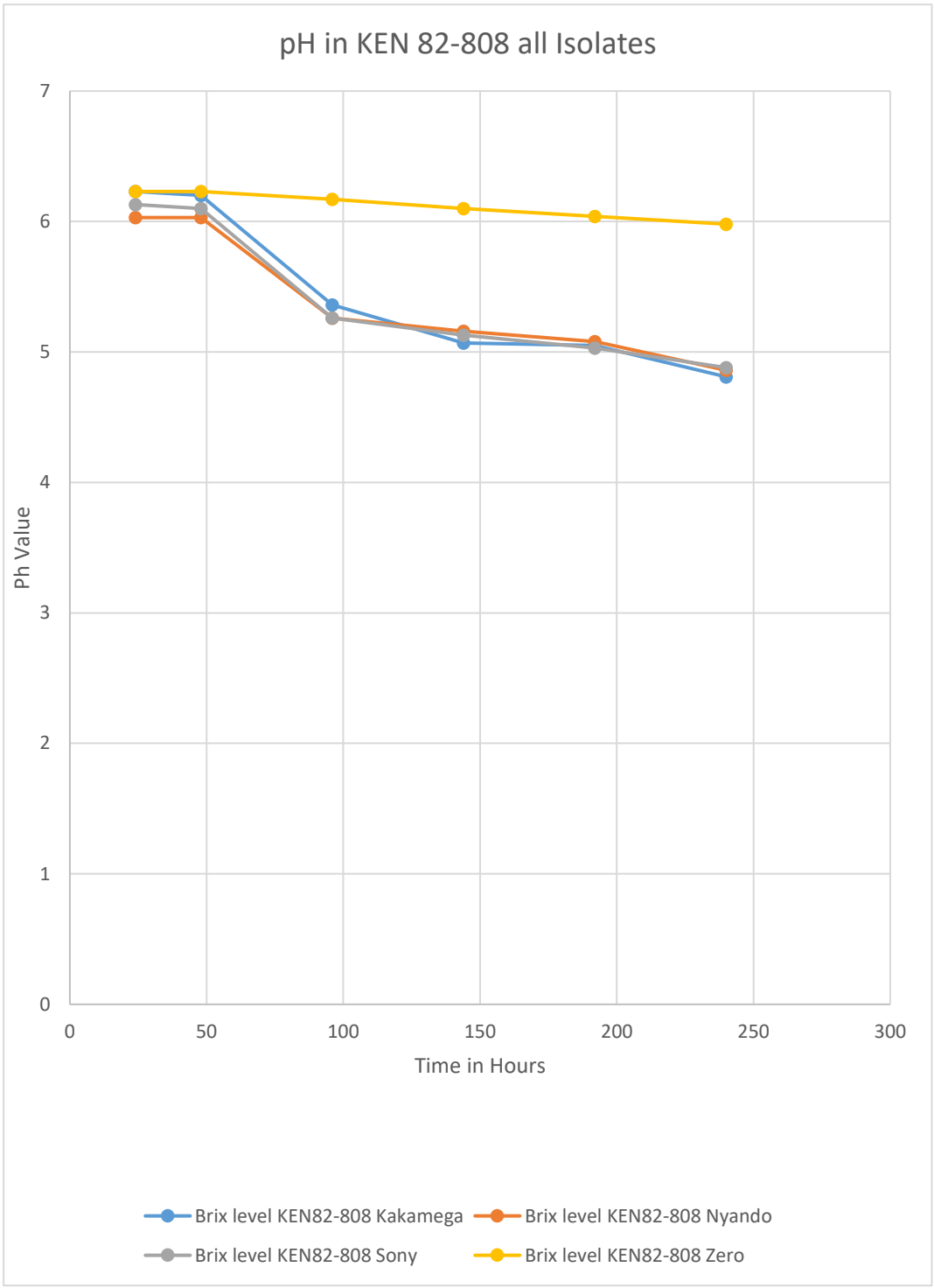
		KEN83-737	736.70±0.70	16.54±0.87	5.13±0.05	18.68±0.57
		N14	1202.67±0.31	17.50±0.76	5.03±0.05	19.87±0.56
		CO421	1059.17±0.29	19.19±0.44	5.06±0.05	21.08±0.35
	192	KEN 82-808	1357.72±3.49	15.54±0.47	5.05±0.08	18.19±0.40
		KEN83-737	1668.64±0.42	17.07±0.17	4.83±0.05	20.04±0.92
		N14	1604.41±1.01	15.84±1.25	5.03±0.05	18.14±0.50
		CO421	1384.91±0.45	17.25±0.45	4.62±0.012	19.7±0.27
	240	KEN 82-808	1813.41±5.40	13.70±0.72	4.81±0.01	16.66±0.58
		KEN83-737	2016.91±0.66	16.90±0.28	4.51±0.07	20.13±0.42
		N14	2007.98±1.93	15.27±0.01	4.64±0.09	18.03±0.30
		CO421	100.69±0.01	18.35±0.01	6.03±0.05	20.02±0.01
	24	KEN 82-808	113.08±0.00	16.37±0.01	6.03±0.05	18.75±0.01
		KEN83-737	108.57±0.00	18.09±0.00	6.03±0.05	19.5±0.01
		N14	119.06±0.00	17.35±0.0	6.03±0.05	17.86±0.01
		CO421	140.09±0.67	18.91±1.19	6.03±0.05	20.57±0.85
	48	KEN 82-808	426.81±1.03	16.19±0.09	6.03±0.05	18.48±0.06
		KEN83-737	131.02±0.12	18.95±0.19	6.03±0.05	20.26±0.11
		N14	337.12±0.40	18.21±0.36	6.03±0.05	20.22±0.29
		CO421	416.01±0.35	19.79±0.25	5.38±0.01	21.2±0.14
	96	KEN 82-808	548.38±0.89	17.44±0.94	5.26±0.05	19.21±0.64
NYANDO		KEN83-737	220.01±0.00	19.34±0.38	5.53±0.05	20.43±0.29
		N14	722.40±0.25	17.08±0.23	5.30±0.09	19.35±0.14
		CO421	655.17±0.21	19.02±0.31	5.21±0.10	20.66±0.29
	144	KEN 82-808	772.02±0.12	15.96±0.22	5.16±0.05	18.43±0.21
		KEN83-737	736.70±0.70	18.22±0.05	5.10±0.00	19.85±0.03
		N14	1201.04±0.32	16.88±0.28	4.83±0.15	19.40±0.16
		CO421	1059.17±0.29	18.33±0.05	5.10±0.10	20.20±0.03
	192	KEN 82-808	1357.72±3.49	16.02±0.65	5.08±0.07	18.41±0.40
		KEN83-737	1668.64±0.04	18.15±0.50	5.03±0.05	19.67±0.43
		N14	1604.41±1.01	16.32±0.04	4.76±0.5	19.11±0.01
	240	CO421	1390.10±0.60	16.67±0.64	4.95±0.12	19.11±0.56
		KEN 82-808	1806.38±5.44	13.82±0.31	4.86±0.11	16.85±0.36
NYANDO	240	KEN83-737	2014.43±0.66	15.91±0.34	4.70±0.10	18.43±0.24
		N14	2006.18±2.00	15.43±0.55	4.59±0.11	18.34±0.75
		CO421	100.69±0.01	18.37±0.09	6.13±0.05	20.08±0.01
	24	KEN 82-808	113.08±0.00	16.40±-0.00	6.13±0.05	18.77±0.02
		KEN83-737	108.576±0.00	18.08±0.00	6.15±0.05	19.5±0.01
		N14	119.06±0.00	15.00±0.00	6.13±0.05	17.88±0.01
		CO421	121.79±0.17	19.24±0.21	6.10±0.10	20.74±0.13
SONY	48	KEN 82-808	197.42±1.17	17.00±0.13	6.10±0.10	19.29±0.09
		KEN83-737	251.99±0.36	18.90±0.66	6.10±0.10	20.13±0.47
		N14	379.22±1.41	16.97±0.94	6.10±0.10	19.32±0.67
		CO421	155.46±0.00	18.91±0.89	5.43±0.04	20.44±0.65
	96	KEN 82-808	501.63±0.12	17.28±0.52	5.26±0.05	19.21±0.37

		KEN83-737	565.86±0.00	18.67±0.11	5.53±0.05	19.94±0.14
		N14	2847.97±7.01	16.22±0.71	5.26±0.05	18.72±0.57
		CO421	474.13±0.75	19.38±0.15	5.16±0.05	21.13±0.09
	144	KEN 82-808	1281.87±0.64	16.17±0.04	5.13±0.05	18.54±0.04
		KEN83-737	1285.09±0.81	18.45±0.55	5.03±0.05	20.05±0.44
		N14	5895.19±0.60	17.89±0.34	4.80±0.10	20.16±0.26
		CO421	745.8±1.07	18.32±0.44	5.10±0.10	20.45±0.36
	192	KEN 82-808	2318.37±0.40	15.46±0.77	5.03±0.05	18.2±0.57
		KEN83-737	2105.15±0.82	16.4±1.21	5.03±0.05	18.54±1.00
	192	N14	6561.21±1.79	15.95±0.15	4.83±0.06	18.91±0.01
		CO421	967.60±1.17	17.26±0.68	4.86±0.14	19.68±0.63
SONY	240	KEN 82-808	3104.55±1.23	13.8±0.52	4.88±0.08	17.14±0.35
		KEN83-737	2549.97±0.22	15.57±0.61	5.04±0.59	18.35±0.38
		N14	6925.45±0.66	14.34±0.23	4.66±0.05	17.87±0.27

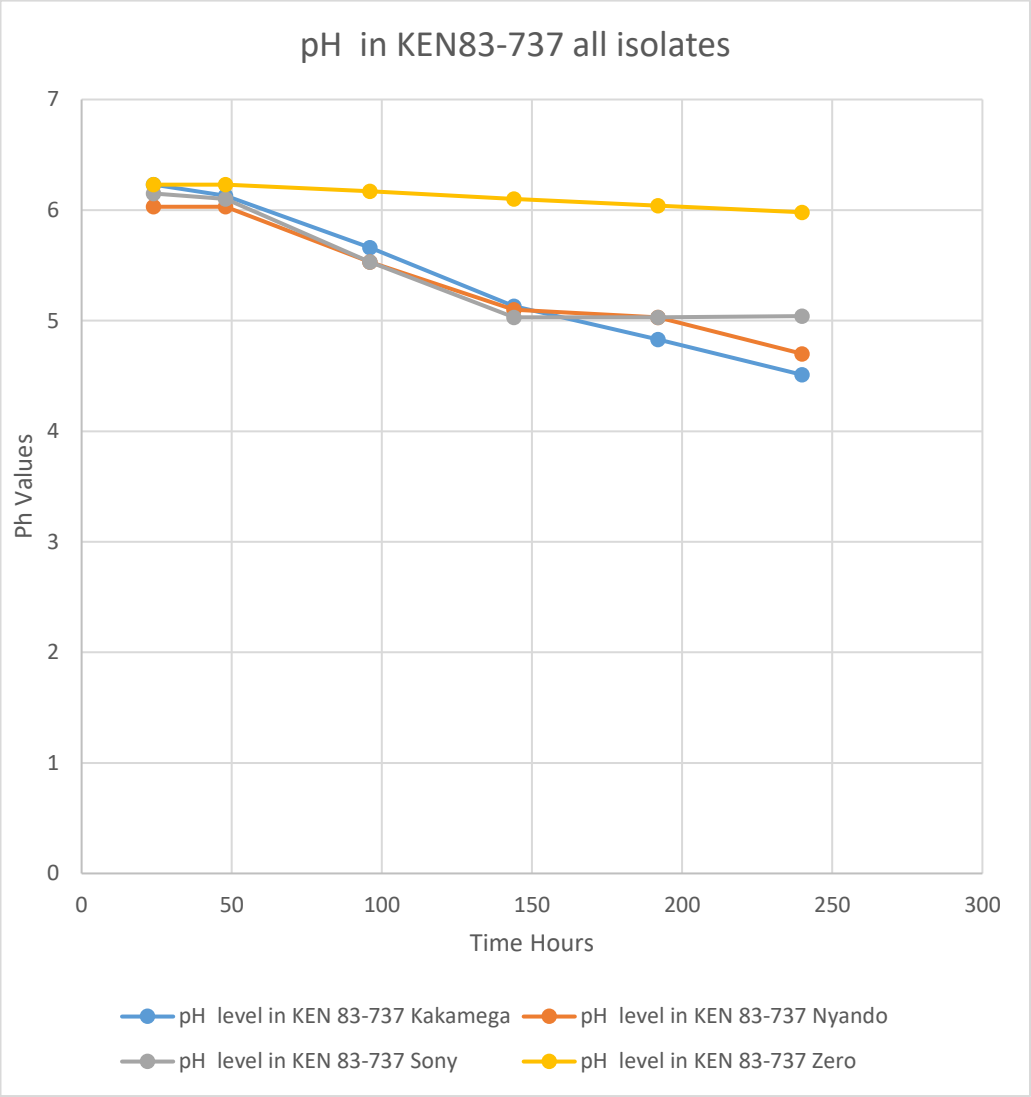
Appendix v: pH produced by all isolates in Co421



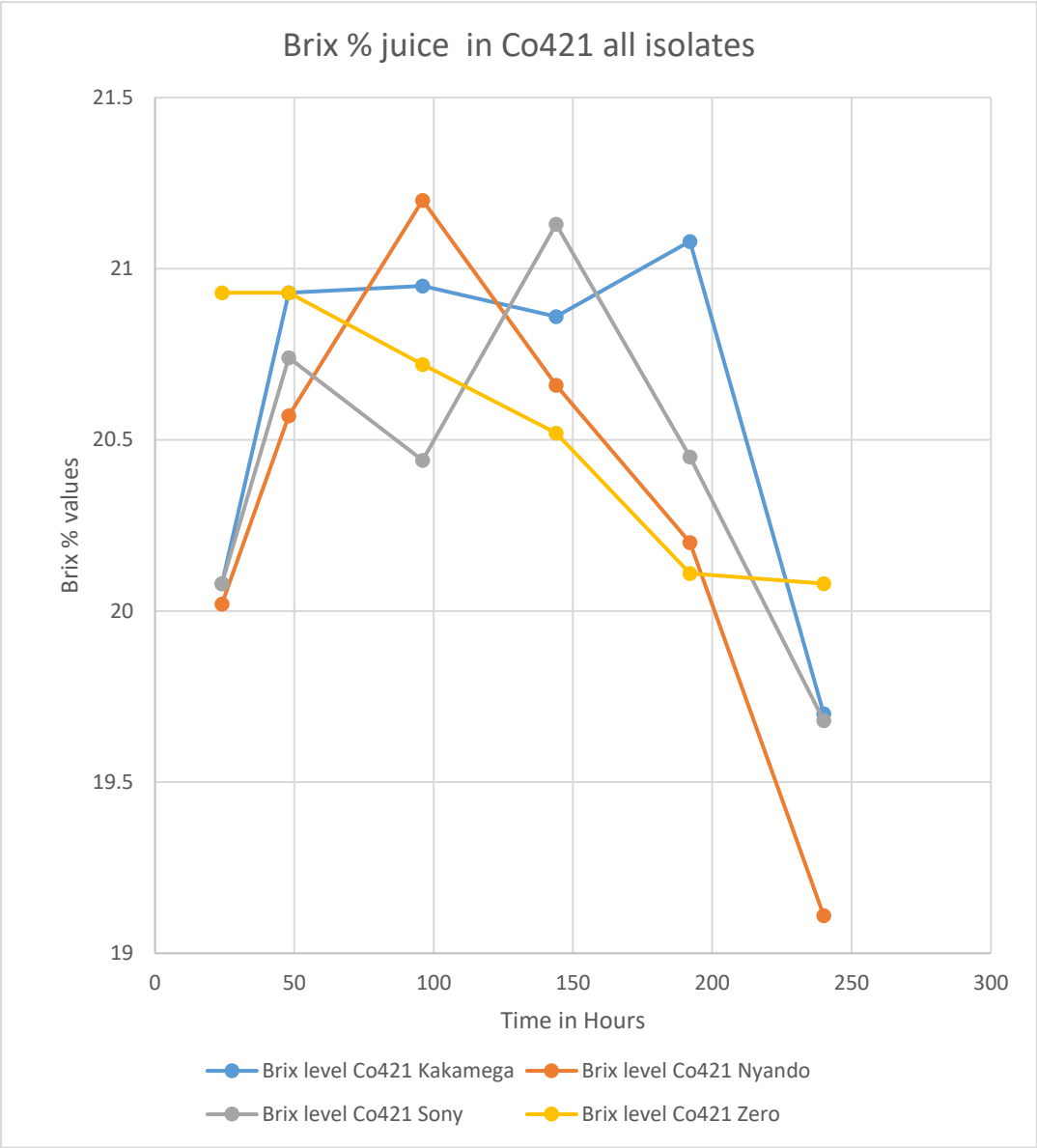
Appendix vi: pH produced by all isolates in KEN82-808



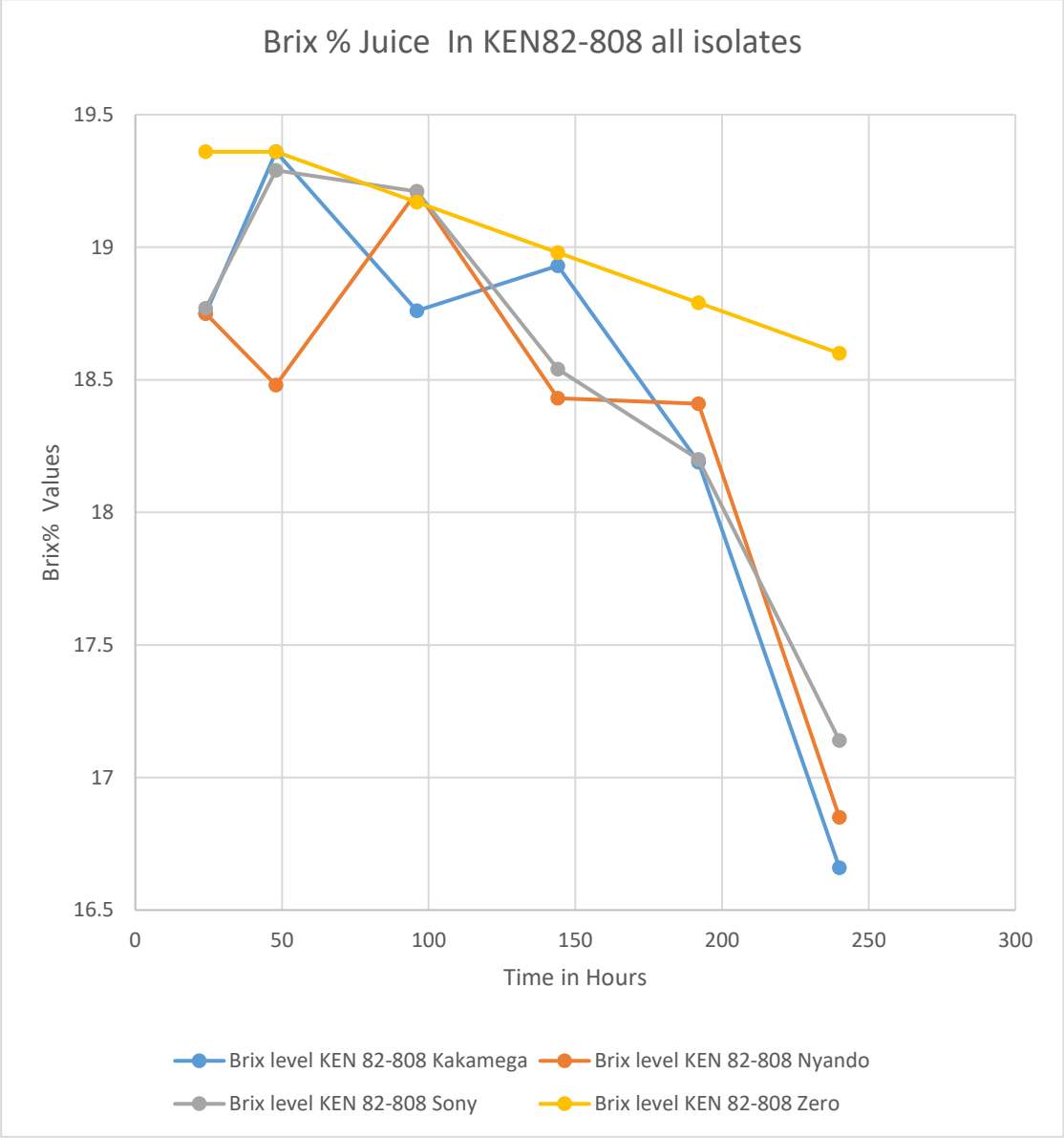
Appendixvii: pH produced by all isolates in KEN83-737



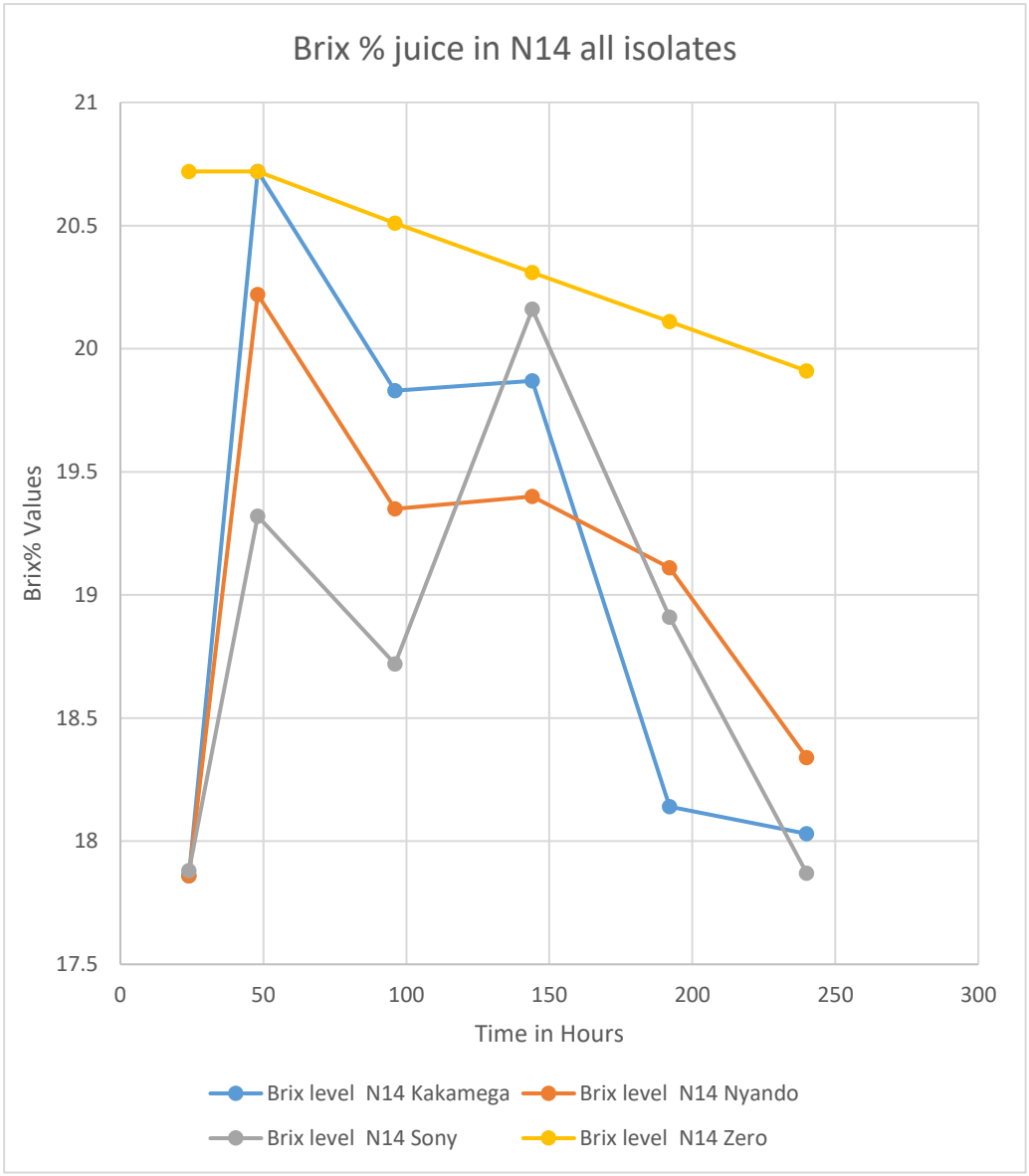
Appendix viii: Brix % juice in CO421 by all isolates



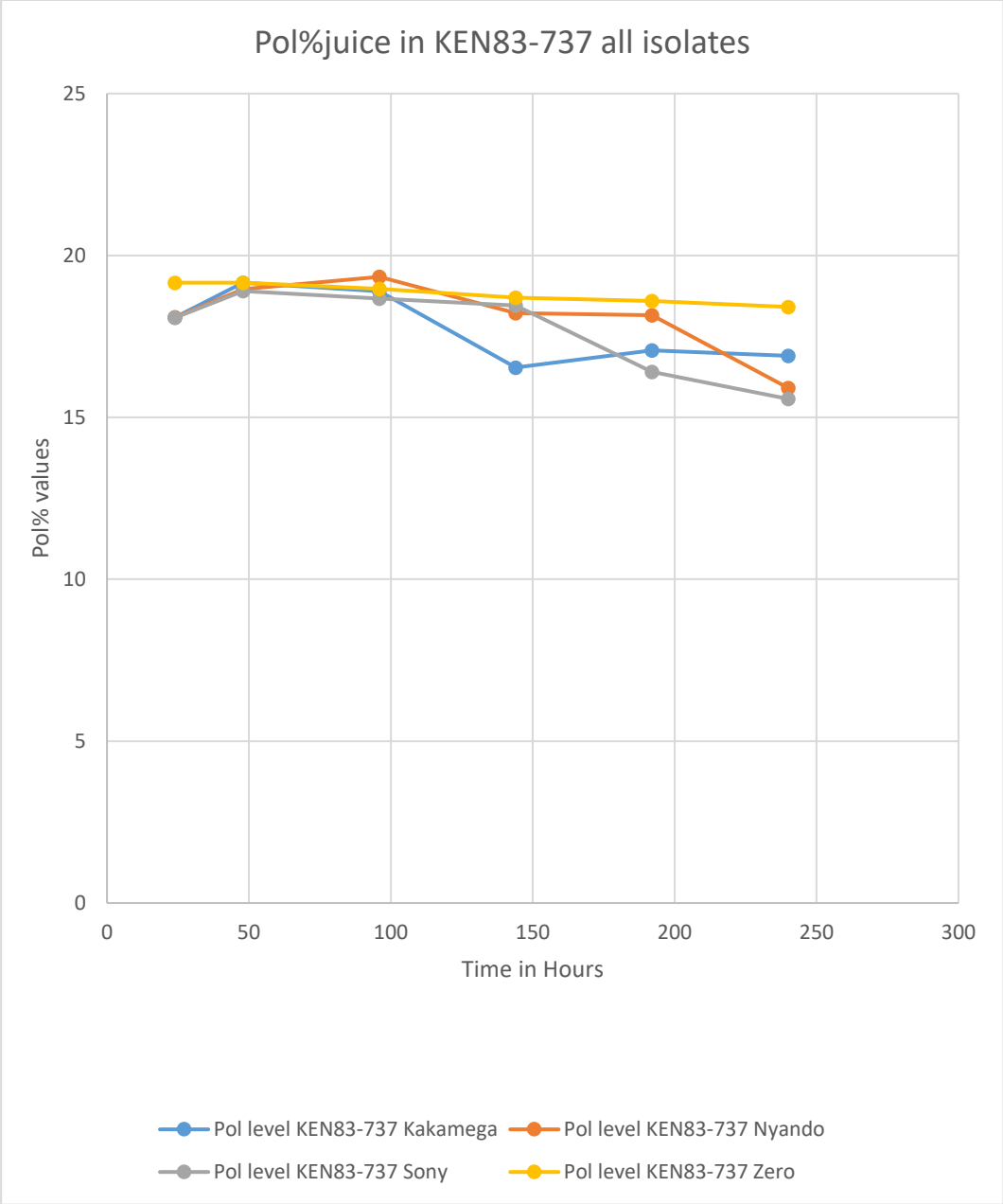
Appendix ix: Brix % juice in KEN82-808 by all isolates



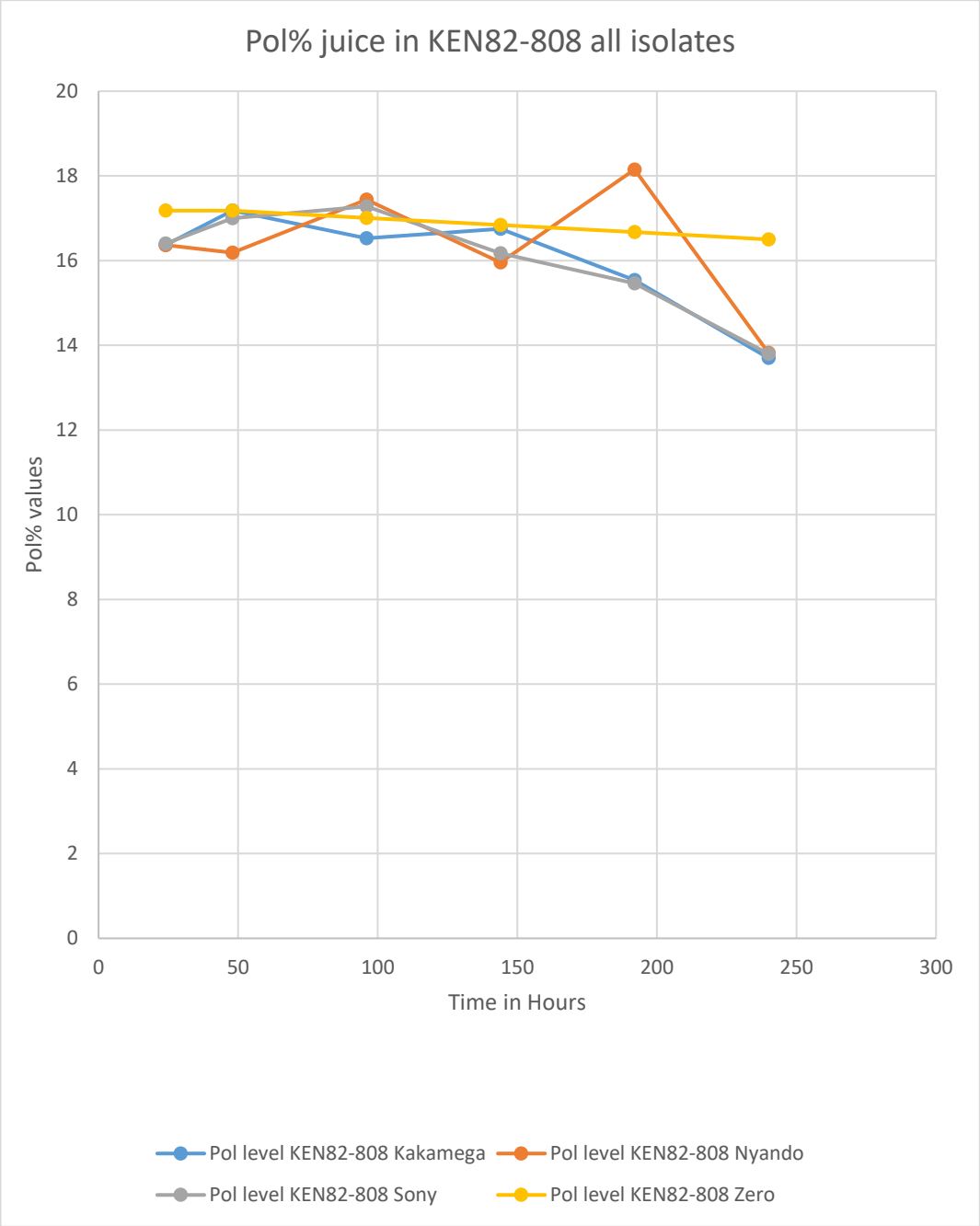
Appendix x: Brix % juice in N14 by all isolates



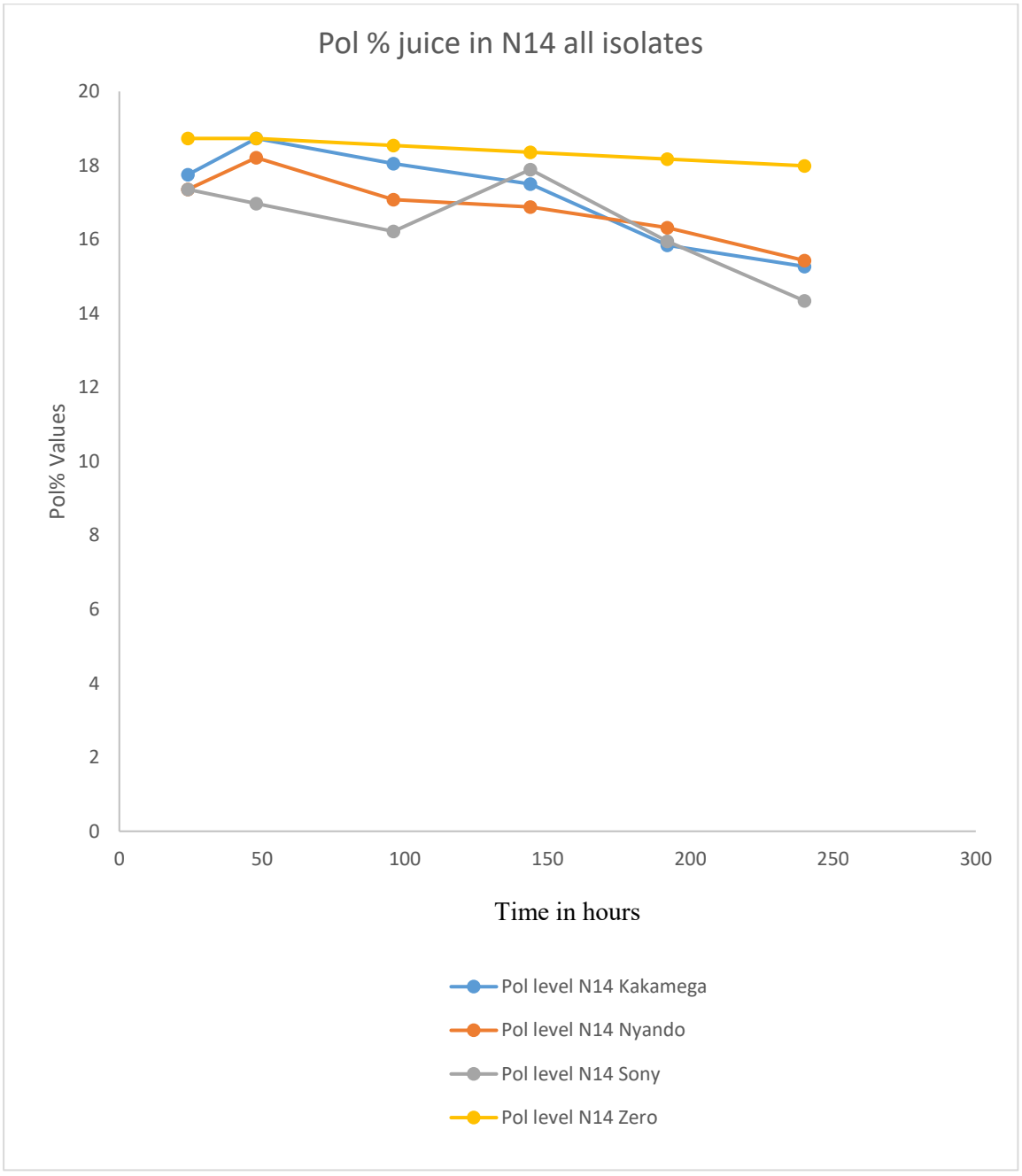
Appendixxi: Pol % juice in KEN-737 by all isolates



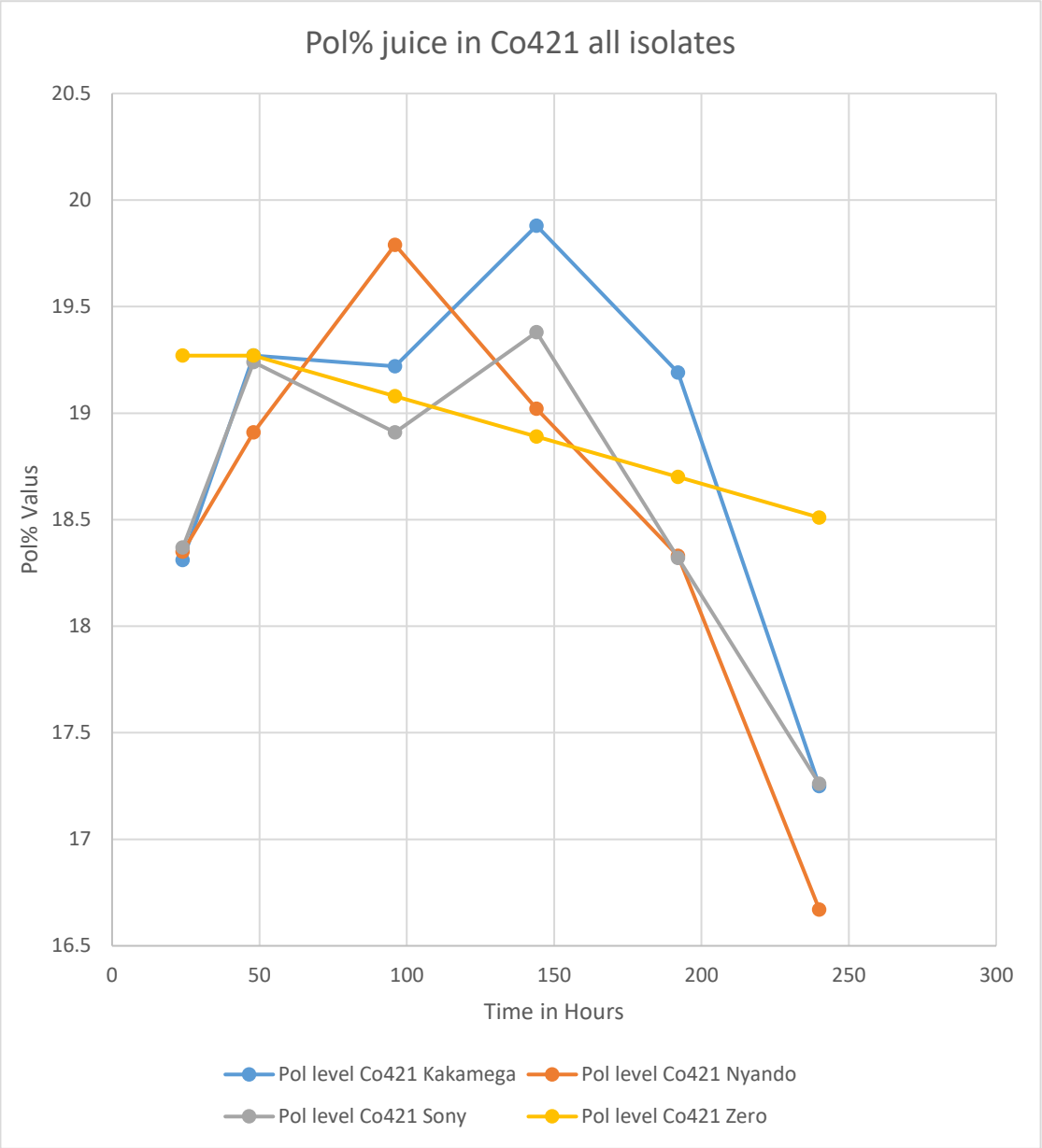
Appendix xii: Sucrose lose by different isolate on KEN82-808



Appendix xiii: Sucrose lose by different isolate on N14



Appendix xiv: Sucrose loss by different isolates on CO421



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Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

13th February 2025

Mark Asamba Luvutse
SMB/G/01-70503/2022
P.O. Box 190-50100
KAKAMEGA

Dear Mr. Luvutse

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Masters proposal entitled: "***Biological and Biochemical Characterization of Leuconostoc Mesenteroides Isolates and the Bio-degradation Levels of Selected Post-harvested Sugarcane Varieties***" and appointed the following as supervisors:

1. Dr. Dennis Omayio - MMUST
2. Dr. Mario Kollenberg - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director of Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Natural Sciences Graduate Studies Committee; Chairman, Department of Biological Sciences Graduate Studies Committee. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of Two years from the date of registration to complete your Master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

Dr. Jane Situma
DEPUTY DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 471348

Date of Issue: 01/April/2025

RESEARCH LICENSE

This is to Certify that Mr.. Mark Asumba Luvutse of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kisumu on the topic: BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF Leuconostoc mesenteroides ISOLATES AND THEIR BIO-DEGRADATION LEVELS OF SELECTED POST HARVESTED SUGAR CANE VARIETIES for the period ending : 01/April/2026.

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