Chapter One

Introduction

1.1 Background to the Study

Soursop fruit (*Annona muricata L.*), commonly known as Graviola, belongs to the Anonaceae family and is native to Central and South America, Africa, Asia, and Australia. Recently, soursop has gained attention in both national and international markets due to research highlighting its biological effects and the potential impact of its consumption on human health[1]. The scientificcommunity has extensively studied the pulp and leaves of the soursop for their anti-carcinogenic properties[2].

The fruit is characterised by its conical shape and consists of pulp, peel, and seeds. The pulp is commonly used in juices, jams, ice creams, and sweets, exhibiting a sweet or sub-acidic flavour profile. It is estimated that 30–50% of fruits suffer damage and are deemed non-standard due to falling and crushing. Additionally, 3–8.5% of seeds, 7–20% of peels, and 7–20% of leaves are classified as byproducts, even though they were initially considered discarded waste[3].

The exploration of these fruits has primarily been conducted in an extractive manner, drawing attention to their bioactive compounds, particularly polyphenols. However, there is a notable lack of research regarding their potential as raw materials for biotechnological applications and the development of new products.Considering the waste generated by the soursop production chain, identifying and proposing applications for this byproduct reduces improper disposal as solid waste presents a financial opportunity for the regions involved in the production[4,5].

Soursop pulp contains important phytochemicals, with variations in phenolic compounds depending on the extraction method employed, whether conventional[6] or ultrasound-assisted[7]. Notable biological properties observed in both in vitro and in vivo studies

include antidiarrheal[8], antidiabetic, antifungal, antihypertensive, anticancer, and antimicrobial effects, in addition to antioxidant properties[9].

The production of fermented alcoholic beverages, often referred to as wine by some authors, from pure or blended fruits is a widely practiced process. The creation of fermented alcoholic drinks from other fruits other than grapes presents a promising opportunity for the value addition of these raw materials[10]. A Study done in 2019 by researchers has highlighted the potential of soursop in production of fermented alcoholic beverages, yielding products with acceptable sensory attributes such as color, flavor, and aroma. This provides a way of optimizing the use of this fruit to enhance better usage and prevent wastage of this fruit[10].

Kombucha, a fermented beverage made from tea leaf and sugar have successfully been enhanced in terms of its flavour by including juice of soursop during its preparation. It is gaining popularity among consumers and it is traditionally made through the fermentation of tea and sugar with symbiotic cultures. To innovate and enhance flavours the inclusion of fruit juice like soursop has been used in kombucha preparation. The development of kombucha using pasteurised soursop juice, black tea and sugar fermented with a SCOBY (symbiotic community of bacteria and yeast). Despite these advancements, further studies on soursop pulp in kombucha production are needed to broaden evaluations and compare it with kombucha made from commonly consumed fruits. Additionally, the potential for using soursop leaves in place of tea for kombucha production warrants further investigation[11].

Further exploring acetic fermentation, two groups of researchers successfully produced vinegar from soursop.This vinegar exhibited a less acidic and sweeter profile than commercial apple vinegar, which typically has a pH of 4.4, contains 3% sugar, 1.7%

reducing sugar, 7.0% ethanol, and 3.5% acetic acid[12, 13]. Therefore, the use of soursop in vinegar production not only reduces waste but also opens the door to additional techniques that can enhance its utilization.Similar to the process in which the pulp of selected fruits is dehydrated and sold as soursop powder, the pulp of non-standard fruits can also be transformed into powder. Consequently, the dehydration of discarded leaves and fruits may present a promising opportunity for recovering these residues. Additionally, lyophilizing the pulp with 18% maltodextrin produced soursop powder with enhanced brightness compared to the control[14].

A study was conducted by using pulp dried in a fluidized bed using the same quantity of maltodextrin[15]. This indicates that it is feasible to produce soursop powder through various drying methods. However, it is crucial to select the method that best preserves the nutritional, aromatic, and microstructural qualities of soursop, as well as one that aligns with the producer's capabilities. This choice is essential for delivering a high-quality product through re-use, allowing the powder to find applications in various sectors of the industry. By properly sorting and following necessary sanitary measures, dehydrated fruits can be transformed into powdered soursop, preventing waste and reducing composting.

Research has been conducted on fruits with lower added value as potential sources of essential oils. Extraction of essential oil from fresh soursop pulp, was achieved with a yield of 0.11%[16]. A variety of compounds were identified, the most abundant of which included: 2-hexenoic acid methyl ester, β-caryophyllene, 1,8-cineole, linalool, Ç-sitosterol, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 2-propenoic acid, 3-phenyl-, and its methyl ester.

Exploring the potential applications of crushed soursop fruits reveals promising opportunities for starch production from these residues. In 2009, starch was successfully extractedfrom soursop pulp, achieving a yield of 27.30%. This finding underscores the potential for future research focused on utilizing this novel starch source[17].

Fermented foods, defined as "foods produced through controlled microbial growth and enzymatic transformations of food components", are vital means for humans to encounter live microbes that may offer health benefits[18]. The origins of fermented foods (FFs), which boast a variety of microbial populations, can be traced back to the shift from hunter-gatherer societies to more settled, agriculture-based communities approximately 15,000–20,000 years ago[19].

Fermented pulps can enhance the value of these fruits, offering appealing beverages that cater to a public eager for innovative functional fruit-based products with health benefits[20]. Fermentation processes may enhance sensory properties and aromatic profiles, while the application of specific starter cultures can alter phenolic compounds, thereby increasing their bioactivity[21]. Among the earliest living microbes on the planet are bacteria[21]. They contribute to the spread of dead materials or the movement of elements in nature, while having a simpler structure than eukaryotic creatures. Food manufacturing has also made use of these organisms. LAB species are the primary food-fermenting microbes, regardless of the type of food. According to the complete genome sequence data, which was retrieved on October 10, 2023, 261 LAB species are classified into 26 genera (http://lactotax.embl.de/wuyts/lactotax/)[22].

The food sector uses lactic acid bacteriathe most. Probiotic cultures are used in the food sector to enhance food quality, and LAB is used to create newfood. Certain LAB species are beneficial probiotic strains. Applications for LAB in milk processing and preservation

date back many years[23]. Dairy products become acidic as a result of lactose being converted to lactic acid during the natural fermentation of milk with LAB. Thislowers pH and creates an environment that is inhospitable to bacteria, lactic acid generation during fermentation is crucial for preventing spoilage[23]. LAB breaks down lipids into free fatty acids and breaks down milk proteins into peptides and amino acids. Dairy fermented products' unique texture and rheological characteristics, as well as their flavor, shelf life, and safety, are all influenced by fermentation, which also affects their physical and chemical characteristics[24]. Back-slopping, which involves adding the fermented product to the new batch to speed up the subsequent step. Spontaneous or native LAB fermentation are examples of traditional, natural milk fermentation procedures. The back-slopping approach helps choose the finest starting cultures for the quality of fermented products by reusing a successful batch[25].

However, using artisanal and empirical methods frequently results in inconsistent food products[26]. Raw milk is utilized to contain a range of autochthonous microorganisms, including several strains of LAB, in traditional dairy products. Bacteriocins are antimicrobial peptides produced by LAB that provide the natural and safe preservatives of traditional dairy products[24]. A starter culture can be classified as either "defined strain starters," which include commercial single strain starters, or "mixed strain starters," which include, among other things, "artisanal or natural mixed starter cultures"[27]. Although LAB species predominate, a diverse spectrum of microorganisms can be found in the microflora of mixed starters[28].

In order to guarantee the safety and predictability of fermented products, the dairy sector now replaces the aforementioned methods with the use of particular starter cultures for inoculation. Starter cultures are chosen based on characteristics like hetero- and homofermentation, probiotic qualities, acid tolerance, organic acid production,

temperature range, salt tolerance, oleuropein separation ability, flavor development, and bacteriocin production. The novel fermented products that are produced as a result of fermentation are categorized as second-generation beverages when conventional starter cultures are used in conjunction with gut bacteria. The novel fermented product is identified as a third-generation beverage when intestinal strains are the only ones utilized[29]. Utilising yeasts as starter cultures can lead to superior quality products and health benefits, especially when these yeasts are indigenous to the fruits[30].

1.2 Statement of the Problem

The majority of the public are always eager for innovative functional fruit-based beverages of Sub-Saharan Africa origin with health benefits and capable of satisfying their taste buds, the use of starter culture fermentation process is not common and so most fermented beverages have within them other associated by products that are not necessarily needed and some that are relatively toxic to the body. These by products may reduce the overall keep-ability or preservation of nutrients within these fermentedbeverages. The consumption of such toxic products on a regular basis has been reported to have adverse effect on the body in the long run.

1.3 Justification of the Study

This study is significant because it seeks to discover and develop a value-added fermentation product from Soursop fruit which may serve to eventually reduce wastage of the fruit.It also addresses limitations that can be encountered in the design of this biochemical process at laboratory scale and improve the commercial potential of the Soursop fruit.

1.4 The Aim and Objectives of the Study

This study aim to develop a relatively acceptable and nutritionally viable fermented beverage from Soursop pulp using identified starter culture(s).

The Specific Objectives are to:

i. extract soursop juice, spontaneously ferment it and determine its proximate composition.

ii. select starters from spontaneously fermenting soursop juice and identify them using molecular biology tools

iii. ferment soursop juice using the selected starter(s) singly and in combination 3under controlled environmental conditions

iv. determine the proximate, mineral and other physicochemical composition of the starter fermented Soursop samples

v. To determine the acceptability of the starter fermented soursop juice using sensory evaluation by a panel.

1.5 Significance of the Study

There is potential for creating a functional and probiotic-rich beverage by fermenting soursop with yeast (*Kurtzmaniella quercitrusa*) and *Leuconostoc mesenteroides*. Therefore, this work may help fruit-based probiotic drinks become more widely available while tackling issues of sustainability and food security.

1.6 Scope of the Study

1. The study investigates the substrate suitability and fermentation efficiency of Lactobacillus and Yeast in Soursop obtained in Lagos and Ibadan, Nigeria.

2. The study aims to analyze the product quality and sensory evaluation of starter fermented Soursop.

3. The study focuses on *Lactobacillus* and Yeast as sole microorganism on a laboratory-based fermentation.

1.7 Limitation of the Study

1. The sample size for this project was limited to samples taken from Lagos and Ibadan

2. Use of metagenomics techniques for this study would have given a better picture of the dynamics of the microbial interactions of the microbes of interest and other microbes within the fermentation process however, this was limited by resources, thus the work was done only to identifying the microbe of interest by targeting their 16SrRNA gene.

3. Determining acceptability, the last stage, depends on human perception (sensory evaluation). The objective assessment of the juice's quality is limited because this is subjective and results may be impacted by panellist bias, cultural preferences, and the inherent diversity of human taste.

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Chapter Two

2.1 Soursop as a Fruit

Soursop is grown in most regions of the world. Owing to its possible health advantages. It is a member of the *Annonaceae* family and is technically known as *Annona muricata L*. The ovoid, dark green, prickly fruit has a flesh that is delicious, acidic, white, and fragrant. Soursop's medicinal, nutritional, taste, and color qualities have made it more and more significant on a worldwide scale. The antioxidant capacity of *Annona muricata L*. has been related to a number of health benefits. Flavonoids and phenols are said to be abundant in the fruit[1].

2.1.1 Soursop's Attributes

By weight, the soursop fruit is composed of 67.5 percent edible pulp, 20 percent skin, 8.5 percent seeds, and 4 percent core. Eighty to eighty-one percent water, one percent protein, eighteen percent carbohydrates, 3.43 percent titratable acidity, twenty-five percent non-reducing sugar, and vitamins B1, B2, and C are all present in the white edible pulp[2].

2.1.2 Challenges of Preserving Soursop

Owing to its high moisture content, soursop deteriorates within a few days of harvest. The fruit softens quickly during ripening and is extremely prone to spoiling. Physical and metabolic alterations reduce the fruit's organoleptic attractiveness and economic value since it is perishable[1].

2.2 Fermented Beverages

People have been eating fermented foods for a very long time because of their health advantages, organoleptic properties, and long shelf life[3]. According to estimates, one-third of the human diet worldwide consists of fermented foods, and many consumers'

dietary habits are still influenced by their consumption of these foods. The popularity of fermented foods has been greatly influenced by ancestral dietary patterns, such as the traditional Mediterranean and Japanese diets, from the standpoint of human health. All foods or drinks made by microbial activity under regulated enzymatic conditions that convert dietary ingredients into products with added value are considered fermented foods and beverages[4].

A fast-growing worldwide population is the direct cause of the growing worry regarding the availability of sustainable and safe natural food supply. Fermentation stands out among other methods, such as cellular or acellular products, edible biomass, and edible insects, for the formation of the next generation of food components and products. Fermented product production uses less water, produces fewer greenhouse gas emissions, and requires less land than normal farming. Furthermore, there are health benefits associated with fermented foods, including decreased cholesterol, increased immunity, protection against infections, cancer, osteoporosis, obesity, diabetes, allergies, atherosclerosis, and decreased lactose sensitivity[5].

2.2.1 Fermented Beverages from Fruits

Probiotic-containing juices can be created by fermenting the juice with the probiotic bacteria or by adding the probiotic strain straight to the juice. The latter is preferable to the former over direct addition because it produces a low-sugar product and a better-adapted microbial strain, which may boost its survival rates[6]. Additionally, as the juice ferments with the probiotic bacteria, microbial metabolites such as bacteriocins may be produced. These might help improve the product's quality and extend its shelf life when kept[6].

In probiotics, fermentation involves the transformation of carbohydrates into organic acids. When lactose is fermented by dairy-based probiotics, LA (Lactic acid) is created. Lactose fermentation occurs through a mechanism known as glycolysis or the glycolytic pathway. Lactose is first converted to glucose and galactose, and then galactose is further converted to glucose. After a few conversions and interconversions, it gains one phosphate and is converted to galactose-1-P and then to glucose-1-P, which results in the formation of pyruvate through the process of glycolysis. The reducing energy that was previously produced in the cycle in the form of NADH is then used to convert this pyruvate to LA[7].

Pyruvate and LA are produced from fruit sugars such fructose, sucrose, and glucose. The metabolic route that transforms 1 molecule of glucose into 2 molecules of LA and 2 molecules of ATP includes the d- or l-lactate dehydrogenase enzyme (aldolases)[8]. When lactic acid is mostly made from glucose up to 85% of the time, this is known as homo-fermentation. This means that for every glucose molecule that is fermented, up to two molecules of LA are produced, resulting in the production of two molecules of adenosine triphosphate[8]. Hetero-fermentation occurs when the production of LA is about 50% of the fermentation process. Throughout the fermentation process, one glucose molecule is transformed into one adenosine triphosphate, one LA, one ethanol, and one carbon dioxide molecule[9]. Glucose and other C6 carbohydrates are catabolized into LA via the Embdenn-Meyerhof pathway.

Table 2.1: Lists the Several Fruit Probiotics that are on the Market[10].

Serial Number	Commodity	Microorganism	Product
1.	Beverage (Mango)	*Lactobacillus plantarum* 299V	Juice drink
2.	Beverage (purified water, lemon, cayenne, maqui berry, vegan probiotics and stevia)	*Bacillus coagulans* GBI-30 6086	Juice drink
3.	Beverage (orange guava)	*Bacillus coagulans* GBI-30 6086	Juice drink
4.	Beverage	*Lactobacillus acidophilus, Lactobacillus delbreukii*	Cocobiotic
5.	Gummies	*Lactobacillus*	Probiotic amla gummies
6.	Beverage (blueberry)	*Bifidobacterium*	Juice smoothie
7.	Beverage (strawberry, passion fruit, lemon, rose water)	*Bacillus coagulans* GBI-30 6086	Juice drink
8.	Beverage (pomegranate)	*Lactobacillus casie*	Juice drink
9.	Beverage	*Bacillus*	Juice

	(mango mangue)	*coagulans*	smoothie drink
10.	Beverage (Pineapple, mango)	*Bifidobacterium lactis* HN019	Juice drink
11.	Beverage (raspberry, lemon, ginger)	*Bacillus Coagulans* GBI-30 6086	Kombucha

Table 2.1 Cont'd

Serial Number	**Commodity**	**Microorganism**	**Product**
12.	Beverage (Peach, mint)		Juice drink
13.	Beverage (orange, pineapple, ginger)	*Bacillus coagulans*	Juice drink
14.	Beverage (blackberry hops)	*Bacillus coagulans* MTCC 5856	Master brew combucha drink
15.	Beverage (mixed fruit)	*Lactobacillus sporogens*	Probiotic health drink

2.3 Fermented Soursop

It is standard practice to turn pure or mixed fruit into a fermented alcoholic beverage. A viable substitute for the value-adding of these raw materials is the creation of a fermented alcoholic beverage made from tropical fruits. By producing products with acceptable color, flavor, and scent, some researchers have demonstrated the potential of soursop for the creation of fermented alcoholic beverages, suggesting the utilization of these leftover fruits from the soursop manufacturing chain[5, 11]. Customers are becoming more interested in kombucha drinks. Traditionally, it is made by using symbiotic bacteria to ferment tea and sugar[12]. The use of fruit juice, including soursop, in the making of kombucha was investigated as a way to experiment and add tastes. In one study, pasteurized soursop juice, black tea, and sugar were fermented (SCOBY—symbiotic community of bacteria and yeast) to create kombucha. Notwithstanding the aforementioned research, new investigations using soursop pulp in kombucha production are needed in order to broaden the evaluations and contrast it with kombucha made from typically eaten fruits. . Soursop leaves can be further studied in future works, replacing tea in the production of kombucha[13].From acetic fermentation, some researchers developed vinegar from soursop, resulting in a vinegar with a less acidic taste and a sweeter characteristic compared to commercial (apple) vinegar, which has 4.4 pH, 3% sugar, 1.7% reducing sugar, 7.0% ethanol, and 3.5% acetic acid. Thus, in addition to the use of soursop in the production of vinegar in order to reduce waste, it is also possible to use techniques favoring the improvement of this production and generation of other fermentation products[14, 15].

2.4 Starters and their Importance

Starter cultures are preparations that include a high number of cells, either of one species or a combination of two or more microorganisms. They are introduced to food to exploit the substances or products that are produced by their metabolism or enzymatic activity[16].

Starter cultures are widely employed in the food business across the world since they are needed to carry out fermentation processes in food manufacturing. As a result, a number of products, including probiotics, bioprotective cultures, and starters, have been commercialized with the goal of ensuring food safety, giving meals particular nutritional and sensory qualities, and possibly even improving health[17]. To make cheese, yogurt, and other fermented dairy products, several food industries, including the dairy industry, use starter cultures[18]. Mostly for the manufacturing of sausages from the meat and alcoholic beverages, fermented fruits, vegetables, fermented cereals, baking, vinegar, rice and soy-based oriental dishes[19, 20]. Starter cultures enable us to manage the fermentation process and get consistent outcomes since they are tailored to the substrates.

Since they are well suited to the environmental conditions of food and can regulate food deterioration and pathogenic microbiota, the most promising microorganisms chosen as starting cultures are those isolated from the natural microbiota of traditional goods[21].

Yeast, mold, and bacteria are microorganisms that are utilized as starting cultures. Lactic acid bacteria (LAB), which are employed in the fermentation processes of meat and dairy products, are the most representative category of bacteria. Other bacterial groupings are also utilized, including *Micrococcaceae*, primarily coagulase-negative *staphylococci* (CNS), Gram-positive, and catalase-positive cocci. The primary function of yeasts is the fermentation of alcoholic drinks, the most common of which is the manufacture of wine and beer[22]. In terms of starter molds, they are employed to produce fermented meat, cheese, and vegetable products[23]. In order to regulate the microbial populations during fermentation, standardize product quality, and lower the possibility of fermentation failure, industrial production of fermented foods frequently depends on starter cultures. The following procedures have served as the foundation for the development of starter

cultures: (a) microbial strains from high-quality reference products were isolated and taxonomically characterized; (b) desirable and undesirable physiological properties were determined in vitro; (c) ecological fitness in the fermentation substrate and its beneficial impact on product quality were determined in situ; and (d) the strain was adapted for large-scale biomass production. Genome sequencing has been a standard technique for characterizing starting cultures throughout the last ten years[24]. Fermentation is not the only purpose for starter cultures. Food is altered by their metabolic processes to enhance flavor, safety, and nutrition.

a) Food Safety and Preservation

Food preservation is one of starting cultures' most crucial roles. The environment created by fermentation's production of acids, alcohols, and bacteriocins (antimicrobial peptides) is inhospitable to pathogenic and spoiling bacteria. Food safety is maintained while shelf life is increased by this natural bio preservation. For instance, lactic acid bacteria swiftly lower the pH of pickled vegetables and sauerkraut to levels that stop dangerous infections like *Clostridium botulinum* from growing. Similarly, fermented beverages' alcohol, acids, and lower pH prevent harmful microorganisms from developing.

b) Enhancement of Nutritional Value

Through a number of ways, fermentation may greatly enhance a food's nutritional profile:

i. Enhanced bioavailability: Microbial enzymes increase the bioavailability of nutrients by breaking down anti-nutritional substances like phytates, which typically bind minerals.

ii. Vitamin synthesis: During fermentation, a variety of starter cultures create B vitamins, which naturally enhance food.

iii. Digestibility of proteins: Proteins become more digestible and occasionally less allergenic when they undergo partial breakdown during fermentation.

iv. Probiotics: When taken alive, some starter cultures themselves offer probiotic advantages that promote intestinal health.

For example, fermentation of idli and dosa batter increases the amount of vitamins B and decreases phytates, increasing the availability of minerals. In a similar vein, fermentation of kimchi produces important antioxidants and boosts the bioavailability of minerals.

c) Development of Sensory Characteristics

The effects of starting cultures on flavor, fragrance, texture, and appearance are arguably the most obvious contributions:

i. Compounds with flavor: Complex taste profiles are created during fermentation by the production of acids, alcohols, esters, aldehydes, and ketones.

ii. Modification of texture: The enzymatic breakdown of proteins and carbohydrates changes the texture of food, giving it the soft crumb of sourdough bread, the stretchiness of mozzarella, or the smoothness of yogurt.

iii. Appealing appearance: While some molds give blue cheeses their unique looks, gas production gives bread its open structure or Swiss cheese its eyes[25].

2.5 Fermented Beverages Improved by the Use of Starter.

2.5.1 Fermented Beverages Development

The beverages category, which includes a vast array of fermented products, is a rapidly expanding segment of the food industry due to the fact that contemporary health-

conscious consumers around the world view them as convenient, healthful, and refreshing products that also serve as probiotic vehicles that may enhance well-being and lower the risk of degenerative and chronic illnesses. Marsh. Cereals, fruits, vegetables, tea leaves fermented by a range of microbes, and milk from various sources are the major sources of fermented drinks. They are divided into three categories based on their alcoholic strength by volume (ABV) content: non-alcoholic fermented beverages (NA-FB) with less than 0.5 percent ABV, low-alcoholic fermented beverages (LA-FB) with less than 1.2 percent ABV, and alcoholic fermented beverages (A-FB) with more than 1.2 percent ABV[26]. A team of experts has carried out a thorough and fascinating analysis of the many kinds of traditional fermented drinks, including their history, cultural issues surrounding their usage, fermentation technique, and product qualities[26].

It's interesting to note that the prevalence of lactose, gluten, and cow's milk protein allergies, along with the widespread popularity of vegan and vegetarian diets, have accelerated the development of A-FB, LA-FB, and NA-FB with unconventional ingredients as a substitute to meet the aforementioned needs. Therefore, during the last ten years, there has been a surge in scientific interest in designing fermented drinks with unconventional ingredients and health-related qualities as well as choosing the best processing conditions[27, 28, 29, 30, 31, 32, 33].

Since *Saccharomyces cerevisiae* (yeast) is the primary microbe in charge of alcoholic fermentation, it typically produces A-FBs by its activity on various substrates. Beer is one of the most popular A-FB beverages. It is typically made by removing the raw ingredients from the malt using boiling water, followed by a 7–14 day fermentation with *Saccharomyces cerevisiae* (yeast)[27]. In conventional beer manufacturing, barley is the most common raw material. However, in order to meet the current customer demand for gluten-free goods, a variety of cereals, such as rice, corn, sorghum, or millet, are utilized

to manufacture different types of beers[27]. Similar to this, wine is traditionally manufactured from fermented grapes by various yeast species and strains that interact to produce wines with varying quality characteristics. The most common yeast used as a starter in winemaking is *S. cerevisiae*. Due to their high phenolic chemical concentration and promise as antioxidants, various fruits such berries, apricots, plums, and cherries are now employed to create new fruit wines. In a recent study, tamarind (*Tamarindus indica L.*) was utilized as a substrate for the fermentation of tamarind wine using *S. cerevisiae*. According to these writers, tamarind might be used as a substitute for winemaking, increasing the fruit's usefulness[34]. Another well-known A-FB is cider, which is made from concentrated apple juice fermented with S. cerevisiae for 10–15 days. The apple juice extracted from freshly pressed apples of various types and ripening stages[29]. The use of non-*Saccharomyces* yeasts to improve cider fermentation conditions and generate volatile molecules with a honey and rose aroma was assessed in a recent research[30]. This led to the development of a cider with a distinct scent profile that is very appealing to customers in terms of sensory qualities.

Otherwise, bacteria, yeasts, fungi, or their combination, inoculated in various raw materials of plant or animal origin, are typically responsible for the production of LA-FB and NA-FB. For instance, milk fermentation, especially with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, produces drinkable yogurt, which is regarded as a traditionally popular fermented beverage globally. Being an excellent source of highly digestible proteins, vitamins A and B, and minerals including calcium, magnesium, zinc, and phosphorus, among other essential substances, it has played a significant part in human diets[31]. One of the earliest NA-FBs, kefir has become quite popular recently because of its distinct sensory qualities, nutritional worth, and practical qualities.

Any kind of milk is used to make it, along with a variety of homo- and heterofermentative LAB species, including *Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus acidophilus,* and *Bifidobacterium bifidum,* as well as yeasts like *Kluyveromyces, Saccharomyces, Candida,* and *Torulopsis* that are found in "kefir grains"[26]. As previously stated, the creation of plant-based yogurt and kefir-like products has been extensively studied in recent years due to the rising demand for dairy-alternative goods. In order to produce products that are comparable to traditional yogurt or kefir in terms of nutrition, functionality, texture, and sensory appeal, as well as having the capacity to host LAB for an extended period of time, scientists and technologists have concentrated on identifying plant-derived ingredients, such as cereals, pseudocereals, and legumes, to be utilized as a substrate for fermentation with LAB[32]. According to a study in this area, pulses such beans, peas, chickpeas, cowpeas, and lentils have the potential to be used as yogurt substitutes due to their higher protein content, amino acid composition, and gelling behavior when fermented with LAB[33]. In another study, *Streptococcus thermophilus, Lactobacillus delbruckii sp. Bulgaricus, Lactobacillus acidophilus*, and *Bifidobacterium lactis* were combined with almond beverage (38.88 percent) and Jerusalem artichoke beverage (61.12 percent) and fermented to create a plant-based yogurt that is safe for consumption by consumers looking for dairy-free products with good sensory acceptability and physicochemical properties[34, 35]. However, kombucha, which is presently widely drank globally, is regarded as a refreshing LA-FB with positive health effects[36]. It is often made by fermenting sweetened black tea with a symbiotic consortia of yeasts and acetic acid bacteria for seven to ten days at room temperature (20 to 30 °C). The end product has probiotic qualities and a sour, slightly sweet flavor[35]. Nevertheless, kombucha fermentation has also been carried out on various substrates, including green tea, oolong tea, and medicinal plants[37]. In order to better understand the

microbial diversity of this LA-FB and its potential probiotic effects, the majority of current scientific activities pertaining to kombucha processing concentrate on the isolation, enumeration, biochemical characterization, and identification of the bacteria present[37].

Overall, the potential for fermented drinks to promote health and well-being is one of its most alluring features[38]. In this regard, the food business has faced a significant challenge and a broad field for research: the creation of new products with enticing tastes, natural components, and useful qualities. In order to meet the demands of present customers, it is also crucial to employ sustainable technology to lower processing costs and environmental effect. In this sense, the use of new techniques to support fermentation treatments has shown promise and might be expanded to an industrial scale for the production of fermented beverages[38].

2.6 Review of Researches with LAB and Yeasts Used as Starters for Fermented Beverages

It is thought that fermentation is a good way to preserve plant materials without sacrificing their nutritional value. Depending on where the raw material comes from, different microorganisms might have an impact on the fermentation of fruit and vegetables.

Generally, LAB and yeasts are utilized in European and American nations, however in Asiatic locations, a considerable number of items (particularly vegetables) are fermented by molds. LAB, however, is the method that is most frequently utilized. Fruit juice fermentation using protective cultures or probiotic bacteria is becoming a highly fascinating way to combat the nutritional value loss of pasteurized or sterilized juices. On the other hand, both wealthy and developing nations routinely ferment raw vegetable material.

A number of vegetable by-products can be utilized to create novel useful vegetable beverages, as has been previously noted for fermented milk. Among fruits and vegetables, bananas, plantains, and pineapples, for instance, have among of the greatest rates of loss during storage and transit. To improve digestibility, eliminate naturally occurring toxins, enhance sensory end textural qualities, and enrich fermented foods with desirable metabolites such lactic acid, amino acids, and phenolic compounds, fermentation is crucial for enhancing the nutritional value of these meals. Complex microbial interactions take happen during fermentation. Generally speaking, the quality of the end fermented product is greatly influenced by the quality of the raw material. The species of *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus xylosus, Lactobacillus brevis, Lactobacillus reuteri, Bifidobacterium lactis,* and *Bifidobacterium bifidum* are the most commonly used commercial strains in fruit juice fermentation[39]. In contrast, strains of *Leuconostoc mesenteroides* are used in the fermentation of sauerkraut. While *Lactobacillus plantarum* is used for cassava fermentation, *Lactobacillus acidophilus, Lactobacillus buchneri*, and *Lactobacillus fermenti* are utilized for lupins, lentils, and other legumes. *Pediococcus species, Aspergillus oryzae*, a number of *lactobacilli*, and yeasts are used to ferment soy products.

In fact, the global market for fruit juices and drinks is showing signs of a vibrant expansion. Specifically, probiotic-containing functional vegetable drinks are seen as a desirable alternative for those who avoid dairy. Furthermore, because fruit juices include vitamins, minerals, and antioxidant chemicals that provide microorganisms the right development substrate and have a high health appeal for consumers, they have been regarded as a unique acceptable carrier for the proper intake of probiotic bacteria. Fruit juice fermentation by probiotic bacteria can enhance the functional characteristics of the juice and boost cell viability[40]. Numerous recent studies have examined the generation of

fermented fruit juice with different probiotic strains, emphasizing the enriching properties of the juices that are produced during the fermentation process[40]. In a particular research, a new fermented pomegranate beverage was produced using *L. plantarum ATCC 14917*, which is endowed with good qualities. Fruit juice fermentation by probiotic bacteria can enhance the functional characteristics of the juice and boost cell viability[40]. Numerous recent studies have examined the generation of fermented fruit juice with different probiotic strains, emphasizing the enriching properties of the juices that are produced during the fermentation process. The scientists treated the juice with multiple HPH (High pressure homogenization) treatments and added varying quantities of trehalose to promote the strain's availability in the juice. This showed that the functional features of CECT 4063 were impacted by both the food matrix and processing. Trehalose enhanced the hydrophobicity of cells and impacted the effects of HPH, ensuring more protection against later applied stressors. In a follow-up study, it was suggested that the same strain might be protected in mandarin juice by HPH microencapsulation[41]. A potential method for safeguarding *L. salivarius spp. salivarius* during the gastrointestinal digestion process and storage was microencapsulation by homogenization with alginate at 70 MPa. Using *L. plantarum* ATCC 14917 immobilized in a delignified wheat bran carrier, a new functional cherry juice was created. In comparison to fermented juice with free cells (165e199 mg GAE/100 mL) and non-fermented juice (135e169 mg GAE/100 mL), the fermentation, which was carried out at 30°C for 24 hours, produced a fermented cherry juice with a greater total phenolic content (214e264 mg GAE/100 mL). Without negatively affecting the sensory qualities of the juice, immobilized cells maintained their viability at greater levels (9.95 log CFU/mL at the fourth week) than free cells (7.36 log CFU /mL at the fourth week). *Meyerozyma caribbica* was isolated from pineapple in 2018 in order to produce a fermented pineapple-based beverage. The juice from the

commercial probiotic *Saccharomyces cerevisiae var. boulardii* was used as a control[42]. In comparison to the control, the juice made with *M. caribbica* had greater levels of total phenolic compounds, catechin, chlorogenic acid, vanillin, and ferulic acid, lower levels of acetic acid, and higher levels of residual glucose and fructose. Over the course of 21 days, *M. caribbica* maintained cell loads more than 7.0 log CFU/mL while being stored in juice refrigeration. The diversity of the gene ATF, which is in charge of ester production during fermentation, was also examined in ten strains of *S. cerevisiae* that were isolated from fermented foods in the Western Himalayas. The findings revealed a number of noteworthy variances in ATF1 gene sequences, which may indicate variations in the flavor and fragrance of the brewing products made with the various strains.

Additionally, it is quite tempting to make fermented beneficial vegetable drinks and beverages. Since vegetable drinks are low in fat, cholesterol, and salt and high in vitamin C, polyphenols, and flavonoids, which enhance their beneficial antioxidant qualities, people actually see them as "healthy" meals[42]. Likewise, using vegetable by-products might be a crucial strategy to enhance process sustainability and the circular economy model. These elements have contributed to the growth of their market in recent years. Even if almond rice milk is popular, soymilk and soy products are by far the most extensively eaten and well-liked among Western customers. Soymilk, an easily digested meal, often has a high protein content, a high sugar content, and a fair ratio of b-conglycinin to glycinin. Soybeans' high protein and fat content includes all of the necessary amino acids in quantities that are quite similar to what humans need. Additionally, a significant amount of unsaturated fatty acids, such as oleic, linoleic, and linolenic acids, as well as trace amounts of phospholipids, phytosterols, and tocopherols, which are known to have health benefits, are present in soybean lipids. Furthermore, because soy-based meals have been shown to have positive effects on metabolic illnesses

and menopausal symptoms, they have drawn more attention in health-conscious communities, particularly in Western nations[43]. In recent years, there has also been an upsurge in the use of carrot juice, which is the most popular nonalcoholic beverage in Germany and northern European nations. In actuality, it is a significant natural source of antioxidants with anticancer and physiological benefits, including a- and b-carotene, as well as precursors of vitamin A and polyacetylene. Additionally, there is proof of its antianemic properties and advantages for wound healing. However, because of their high pH and high sugar content, carrot and soy drinks might encourage the growth of harmful microbes and spoiling agents[43]. Recent foodborne illness outbreaks have also been linked to unpasteurized juices with lower pH values, such as orange and apple juices tainted with pathogenic organisms like *Salmonella spp., Escherichia coli O157: H7*, and *Listeria monocytogenes*[44].

Lately, there has been an increasing interest in mild heat treatments in conjunction with refrigeration or nonthermal biotechnologies like HHP (High Hydrostatic Pressure), HPH, and lactic fermentation of vegetable matrices due to the need to extend the shelf life of such products without negatively affecting their sensory, functional, and nutritional qualities[44]. Since more and more raw materials are being treated in this manner in the food sector, lactic acid fermentation is regarded as a significant technology. This interest stems primarily from the process's nutritional, physiological, and sanitary features as well as the associated manufacturing and implementation costs.

In published research, the number of fermented vegetable juices made mostly from tomatoes, celery, carrots, red beets, and cabbage has increased. According to reports, fermentation has been used to change the flavor, stabilize soy, and even produce new food items[45]. It has also been used to boost the bioavailability of vitamins, minerals, and isoflavones in soy. *L. lactis* strains that generate nisin have recently been suggested as

prospective bio preservatives to achieve safer, more regulated, and repeatable fermentations of soymilk and carrot beverages[39]. In the European Union, nisin was the first bacteriocin to be identified and approved for use as a food preservative. Nisin, especially the Z form, is efficient against gram-positive bacteria and has a broad antibacterial range. It is also highly soluble and stable in a variety of dietary systems. Nevertheless, there are not many investigations on nisin generation *in situ*. Three strains of *Lactobacillus lactis* that produce nisin have been shown to have the ability to microbiologically stabilize fermented carrot juice and soymilk[39]. A similar strategy was employed to optimize a creative process for the creation of a novel probiotic beverage made from red beet juice and enhanced with leftover sucrose syrup. The level of functional strains in the juice rose as their presence increased. For impoverished individuals worldwide, it was suggested that a probiotic beverage based on beetroot and Moringa leaves, which are well-known for their nutritional and physiological advantages. *L. plantarum* and *Enterococcus hirae* supervised the fermentation for 48 hours at 37°C. Strong antibacterial action against pathogenic microorganisms was demonstrated by the fermented beverage that resulted. Along with having a phenolic content of 5 mg/mL and minerals (mg/mL) such 11.8 calcium and 0.2 iron, it also demonstrated radical scavenging activity (20.79 percent). The use of cereals in the creation of a nondairy probiotic beverage based on the utilization of multigrains was also emphasized [46]. Generally speaking, cereal-based drinks are high in fiber, minerals, phenolic compounds, sterols, and vitamins B and E. They may, however, include phytate that inhibits the absorption of minerals by creating insoluble complexes with cations at normal pH levels. This characteristic is taken into account while choosing strains to produce functional or fermented vegetables. Several strategies were put forth to increase the mineral

bioavailability, taking into account yeasts or LAB that are either naturally present in flour or supplied as starting cultures for mono- or co-cultivation [47].

Table 2.2: Non-alcoholic Fermented Cereal Beverages and their Microorganisms, Nutritional Composition, and Health Benefits[48]

Beverage/Place of Origin	Cereals	Microorganisms	Functional Compounds	Health Benefits
Amazake–Japan	rice *koji*	*Lactobacillus sakei*, *Aspergillus oryzae;*	amino acids; vitamins B1, B2, B6; pantothenic acid, vitamin E, flavonoids, dietary fibre, polysaccharides, sterols;	improves digestion; mitigates hypertension, skin-enhancing action, alleviates liver cirrhosis (200 kcal/150 mL/day/ 12 weeks);
Borş/*Borsht*–Central and Eastern Europe, Romania	wheat bran, corn flour	*Lactobacillus delbrueckii* ssp. *Delbrueckii*;	lipophilic and hydrophilic antioxidants (tocopherols, tocotrienols), phenolic compounds, *group B vitamins*, vitamin E, alkylresorcinols; lignans;	alleviates respiratory and digestive diseases (indigestion, vomiting), effective management of hepatic and bile diseases, potentially beneficial in cancer treatment;
Boza–Turkey, Greece, Bulgaria, Albania, Romania, Bosnia Herzegovina; South	barley, oats, rye, millet, maize,	*Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus pentosus*, *Lactobacillus paracasei*, *Lactobacillus fermentum*,	vitamin A, vitamins B1, B2, B6, nicotinamide; Ca, Fe, P, Zn, Na, β-glucan, dietary	improves gastrointestinal health, stimulates the immune

Africa	wheat, rice	*Lactobacillus brevis*, *C. inconspicua*, *C. pararugosa*;	fibres;	system, decreases cholesterol level;

Table 2.2 Cont'd

Beverage/Place of Origin	Cereals	Microorganisms	Functional Compounds	Health Benefits
Busa–Syria, Egypt, Kenya, Turkistan;	rice or millet	*Lactobacillus* sp. *Saccharomyces* spp.	dietary fibre, amino acids, fatty acids, vitamins B1, B2;	lowers cholesterol and reduces risk of cancer and obesity, lowers blood pressure, beneficial in diabetes;
Gowé–West Africa, Benin	malted and non-malted sorghum, maize	*Lb. fermentum*, *Weissella confusa, Weissella kimchii, Lactobacillus mucosae, Pediococcus acidilactici, Pediococcus pentosaceus*;	amino acids (glutamic acid and leucine), minerals (Fe, Ca, Zn, P);	certain lactic acid bacteria strains can help in preventing infections by urogenital pathogens; antimicrobial efficacy;
Kunun-zaki–Nigeria	wheat and sorghum /millet, wheat, malted rice	*Lb. plantarum, Lb. fermentum, Lactococcus lactis; Saccharomysces cerevisiae;*	minerals (Fe, Ca, Mg, K);	provides micro- and macronutrients, improves nutritional status;
Kvass–Lithuania, Russia, Eastern Poland	extruded rye, malted barley	*Lactobacillus casei, Lb. sakei, P. pentosaceus, S. cerevisiae;*	vitamins B1, B3, B2, B6; dietary fibres, Zn, Cu, maltose, maltotriose, glucose, fructose;	modulates metabolism, reduces flatulence, alleviates

Mahewu/*Amahewu*–Africa (Botswana, South Africa, Zimbabwe)	maize, sorghum, millet malt or wheat flour	*Lb. brevis, L. casei, L. lactis, Lb. plantarum, S. cerevisiae, S. pombe;*	Na, K, Ca, Fe, Zn, Mn; dietary fibre, carbohydrates, *group B vitamins*;	hyperacidity; bacteriostatic and bactericidal properties against enteric pathogens;

Table 2.2 Cont'd

Beverage/Place of Origin	Cereals	Microorganisms	Functional Compounds	Health Benefits
Munkoyo–Zambia, Democratic Republic of Congo	maize	*Lb. plantarum,Weissella confusa, L. lactis Enterococcus italicus;*	fibre, vitamins B1, B2, B3, B6, B12, Ca, Fe, Zn, proteins, crude fat;	suppresses diarrhoea; anti-allergen, antimicrobial properties;
Oshikundu/*Ontaku*–Namibia	pearl millet meal, sorghum, or pearl millet malt	*Lb. plantarum, L. lactis, Lb. delbrueckii ssp. delbrueckii, Lb. fermentum, Lb. pentosus, Lactobacillus curvatus;*	shikimic acid, maleic acid, phytic acid, succinic acid; vitamins B1, B2; Ca, Cu, Fe, K, Mg, Mn, Na, S, Zn, P;	NA
Pozol–South Eastern Mexico, Central America	maize	*Lb. plantarum, Lb. fermentum, Lb. casei, Lb.*	group B vitamins; dietary	reduces cholesterol levels;

		delbrueckii, Leuconostoc sp., Bifidobacterium sp., Streptococcus sp., Saccharomyces sp.;	fibre;	improves gastrointestinal health; bactericidal, bacteriolytic, bacteriostatic activities;

Table 2.2 Cont'd

Beverage/Place of Origin	Cereals	Microorganisms	Functional Compounds	Health Benefits
Shalgam–Turkey	bulgur flour (wheat)	*Lb. plantarum, Lb. paracasei, Lb. brevis Lb. fermentum, S. cerevisiae;*	β-carotene, group B vitamins, Ca, Na, Fe;	antiseptic agent; probiotic food; regulates the digestive system's pH; diuretic action;
Tobwa (without malt, only LAB)/*Togwa*–East Africa, Tanzania, Zimbabwe	maize, finger millet (togwa)	*Lb. plantarum, Lb. brevis, Lb. fermentum, Lactobacillus cellobiosus, P. pentosaceus, W. confusa;*	amino acids; dietary fibre;; vitamin B2, B9, B12;	antimicrobial activity; enteropathogenic inhibition of *Campylobacter jejuni* and *Escherichia coli*; eases diarrhoea and prevents malnutrition;

2.7 Main Types of Microorganisms in Global Food Fermentation

The following categories (with genera) are the primary kinds of bacteria linked to fermented foods and drinks worldwide:

Acetobacter, Arthrobacter, Bacillus, Bifidobacterium, Bacillus, Brevibacterium, Carnobacterium, Corynebacterium, Enterobacter, Enterococcus, Gluconacetobacter, Hafnia, Halomonas, Klebsiella, Kocuria, Lactobacillus, Lactococcus, Leuconostoc, Macrococcus, Microbacterium, Micrococcus, Oenococcus, Pediococcus, Propionibacterium, Staphylococcus, Streptococcus, Streptomyces, Tetragenococcus, Weisella, and *Zymomonas* are among the bacteria[49].

Aspergillus, Fusarium, Lecanicillium, Mucor, Neurospora, Penicillium, Rhizopus, Scopulariopsis, Sperendonema, and *Actinomucor* are examples of fungi[50].

Yeasts: *Guehomuces, Kluyveromyces, Lachancea, Metschnikowia, Pichia, Candida, Cyberlindnera, Cystofilobasidium, Debaryomyces, Dekkera, Hanseniaspora, Kazachstania, Galactomyces, Geotrichum, Starmerella, Torulaspora, Trigonopsis, Wickerhamomyces, Yarrowia, Zygosaccharomyces, Zygotorulaspora, Saccharomyces, Schizosaccharomyces,* and *Schwanniomyces*[51].

2.7.1 Bacteria

The most prevalent microorganisms in foods that are naturally fermented or that are fermented with the use of starter cultures are bacteria. While non-LAB bacteria including *Bacillus, micrococcaceae, Bifidobacterium, Brachybacterium, Brevibacterium,* and *Propionibacterium*, among others, are also engaged in food fermentation, usually as minor or secondary groups, lactic acid bacteria are often linked to acidic fermented foods.

2.7.1.1 Lactic Acid Bacteria

Gram-positive, catalase-negative bacteria that generate a lot of lactic acid are known as lactic acid bacteria. Because of their connections to the human environment and a variety of naturally fermented dairy products, cereal crops, vegetables, and other foods, the bacterial groups that comprise the LAB are among the most well-known to humans. With over 380 species spread across 40 genera and 6 families, the LAB is a sizable bacterial group that is phylogenetically related to the order *Lactobacillales* within the phylum *Firmicutes*. *Alkalibacterium, Carnobacterium, Enterococcus, Lactococcus, Lactobacillus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus*, and *Weissella* are common genera of LAB that have been isolated from a variety of fermented foods worldwide[52].

2.7.1.2 Non-Lactic Acid Bacteria

Alkaline-fermented foods from Asia and Africa have been found to contain Bacillus. *Bacillus amyloliquefaciens, circulans, coagulans, firmus, licheniformis, megaterium, pumilus, subtilis, subtilis* variety natto, and *thuringiensis* are among the species of Bacillus found in fermented foods, primarily soybean-based foods[53]. In Nigeria, strains of Bacillus cereus have been isolated from the fermentation of *Prosopis africana* seeds used to make okpehe[54]. Certain *B. subtilis* strains generate λ-polyglutamic acid (PGA), an amino acid polymer frequently found in Asian fermented soybean dishes that gives the

product its distinctively sticky texture[55]. Cheese and other fermented milks have been shown to include species of *Bifidobacterium, Brachybacterium, Brevibacterium,* and *Propionibacterium51 Bourdichon*. Fermented milk products, fermented sausages, and meat and fish related products have been found to include a number of *Kocuria, Micrococcus*, and *Staphylococcus species*[56].Numerous fermented foods also include *Enterobacter cloacae, Klebsiella pneumoniae, K. pneumoniae subsp. ozaenae, Haloanaerobium, Halobacterium, Halococcus, Propionibacterium, Pseudomonas*, and others. Meat fermentation involves *Arthrobacter* and *Hafnia species*[50,51].

2.7.2 Yeasts

In food fermentation, yeasts are responsible for the fermentation of sugar, the production of secondary metabolites, the inhibition of mycotoxin-producing mold development, and a variety of enzymatic activities, including glycosidasic, urease, lipolytic, proteolytic, and pectinolytic activities[57].*Brettanomyces, Candida, Cryptococcus, Debaryomyces, Dekkera, Galactomyces, Geotrichum, Hansenula, Hanseniaspora, Hyphopichia, Issatchenkia, Kazachstania, Kluyveromyces, Metschnikowia, Pichia, Rhodotorula, Rhodosporidium, Saccharomyces, Saccharomycodes, Saccharomyces, Schizosaccharomyces, Sporobolomyces, Torulaspora, Torulopsis, Trichosporon, Yarrowia, and Zygosaccharomyces* are among the genera of yeasts found in fermented foods, alcoholic beverages, and non food mixed amylolytic starters[49].

2.7.3 Fungi

Fungi, primarily filamentous molds, play important roles in fermented foods and alcoholic beverages by producing intracellular and extracellular lipolytic and proteolytic enzymes that greatly affect the product's flavor and texture. They also help to improve the bioavailability of minerals by breaking down antinutritive factors. Numerous fermented

foods, Asian nonfood amylolytic starters, and alcoholic drinks have been found to include species of *Actinomucor, Amylomyces, Aspergillus, Monascus, Mucor, Neurospora, Penicillium, Rhizopus, and Ustilago* respectively[58].

2.8 Advantages of Using Starter in Fermentation

There are a number of advantages to using starter cultures in your sausage production:

i. **Faster Fermentation**

Fermented beverage made the old-fashioned way might take three months or longer. The fermentation process is started by more recent and sophisticated starter cultures, which enable you to get the identical outcomes in a matter of hours.

ii. **Food Safety**

Starter cultures increase the safety of your product because they compete for food with undesirable bacteria. Some also produce lactic acid which lowers pH and creates an environment that inhibits the growth of bad bacteria which can lead to contaminated products.

iii. **Health Benefits**

There are several health advantages to starter cultures as well. Probiotics found in them aid in enhancing immunity, lowering inflammation, and improving intestinal health. This implies that they not only improve the flavor and consistency of your product-soursop but also advance your clients' general health and wellbeing[59].

2.9 LAB Starters

Commercially manufactured starting cultures are now widely utilized in the food fermentation sector. Fermentation is used to create commercial starter cultures on a

massive scale, with quantities exceeding 40,000 l[60]. It is possible to inoculate tens of thousands of tons of raw material with several tons of collected cells from a single production cycle. Because of the ensuing economies of scale, the starting culture's cost is negligible in comparison to the cost of the fermentable raw material. But the value that comes from using premium commercial starting cultures keeps becoming better. The manufacturing methods are becoming close to pharmaceutical standards, and commercial starting cultures are subjected to high demands. In order to get a high output without using elements that might cause allergies or conflict with the religious views of possible consumers of the fermented food product, the production medium's composition is carefully chosen. This satisfies vegetarians' demands that their food sources be devoid of animal derivatives and enables the starting cultures to have both Halal and Kosher labeling.

The material to be fermented can be directly inoculated using commercial starter cultures, which come in frozen and freeze-dried forms. Compared to conventional bulk starters made on-site, the utilization of these cultures, also known as direct vat set (DVS) cultures, offers several benefits. To guarantee constant quality, performance, and the lack of unwanted microbes, commercial starting cultures undergo a rigorous quality control procedure. There is less chance of bacteriophage buildup in the fermentation factory since they are made apart from the food that will be fermented. Because the proportions of the standardized inoculation material used may be tailored to the quantity and quality of the raw material to be fermented, direct vat inoculation offers flexibility in the manufacture of fermented foods. A good beginning culture can always be available since the cultures are supplied in forms that have a long shelf life. Additionally, starter cultures may be tailored to produce fermented meals with certain, predefined qualities by combining particular strains. Globally, consumer preferences differ greatly and are

always evolving. Furthermore, there is a great deal of curiosity in trying new foods, which may be met by either industrializing foods that are typically made by small-scale spontaneous fermentations or by fermenting new raw materials. As a result, new starting cultures that increase product performance must be continuously developed. New microorganisms for starter cultures can come from two possible sources: either they are found in nature, or they can be generated by using contemporary microbiological techniques to enhance strains that are currently in use[60].

2.10 Yeast Starter Cultures

One or more yeast strains are used in the production of almost all alcoholic drinks[61]. The majority of wine producers have been using starter culture yeasts, mostly strains of Saccharomyces cerevisiae, even though many wines produced worldwide are formed by natural fermentations that rely on yeasts often found on the grape surface or on processing equipment. Similar to LAB, the main criteria for choosing strains are the desired qualities of the finished product. Additionally, characteristics like the capacity to grow at high sugar concentrations, generate sufficient amounts of ethanol, and flocculate (and, thus, be readily removed from the wine) are also taken into account. The brewing business also uses yeast starter cultures, and while many big brewers have their own. Once more, the selection of strains is based on traits that are pertinent to the specific requirements of the brewer, such as rates of ethanol production, taste development, and flocculation. Another significant usage of yeast starter cultures is the bread industry. While brewing strains used to produce alcohol are often tuned for producing ethanol, yeast starter cultures used to make bread are designed for producing CO_2 from sugar or starch[61].

2.11 Review of Literatures on Fermented Soursop Juice and Starters

2.11.1 Production of Formulated Juice Beverage from Soursop and Grapefruit

Grape fruit and soursop are two fruit types that contain a number of health-promoting

compounds. Soursop is prized for its juicy, aromatic, sub-acid, and tasty flesh. Conversely, grape fruit is well known for its fruit, which ranges from semi-sweet to somewhat sour and bitter. However, these raw fruits are frequently consumed while still fresh. To boost the added value of these, the study was conducted to create a unique concentrated fruit juice beverage formulation utilizing grapefruit peel and soursop juice. The following ratios were allowed for its preservation based on our findings: 0.3 percent carrageenan, 30 percent soursop juice to water (70 percent), 15 percent grapefruit peel supplementation, and a final beverage concentration of 65 ^{o}Bx[62].

2.11.2 *Soursop (Annona Muricata)* Properties and Perspectives for Integral Valorization

As an extremely perishable fruit, soursop (*Annona muricata L.*) is frequently rejected by the market because of surface damage or a quick aging process that results in inadequate senescence for further processing. An alternate fermentative starting culture was used in a prior study to optimize the alcoholic fermentation of soursop in order to generate soursop wine[63]. In order to produce soursop vinegar through acetous fermentation and its physicochemical, toxicity, and organoleptic properties, this study was conducted. In comparison to those of other vinegars, soursop vinegar demonstrated significantly ($p \leq 0.05$) higher levels of acetic acid (3.5 ± 0.3 percent), total phenolic content (220 ± 20 ppm gallic acid equivalence), and FRAP (ferric reducing antioxidant power) activity (222 ± 2 µM ascorbic acid equivalence), with lower levels of pH, sugar, ethanol, and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity while retaining ascorbic acid. The median lethality concentration (LC50) for fish embryos, as determined by a toxicity assay using the fish embryo toxicity test (FET), was 11.8 mg/mL, which was deemed non-toxic[63]. However, toxicity with human liver tissue (HepG2) using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay indicated that the

cells were more likely to survive when the soursop vinegar concentration was less than 6.25 percent.

Sensory evaluation was carried out using quantitative descriptive analysis and the soursop \vinegar was less sour and sweeter than commercial vinegar. This study presents an alternate approach of decreasing loss of soursop fruit by the conversion of soursop into vinegar utilizing a mix of alternative alcoholic and acetous fermentations[63].

2.11.3 Lactic Acid Fermentation Beverage from Soursop (*Annona muricata L.*) by *Lactobacillus plantarum*

A common fruit in South East Asia, North Africa, and the Pacific is soursop (*Annona muricata L.*). Because of the fruit's bioactive value and fragrant flavor, making a beverage out of soursop is a possible path, particularly in the form of probiotic fermented fruit juice, which has drawn a lot of interest from customers who are health-conscious. Lactobacillus plantarum is regarded as one of the probiotic strains with several qualities that make it ideal for fermenting fruit juice products[64]. In order to create fermented soursop juice with probiotic qualities and excellent sensory quality while preserving the nutritional and bioactive ingredients, this work sought to improve the fermentation parameters of soursop juice using *L. plantarum*. Several factors were investigated for this purpose, such as the concentration of soursop juice (SJ) in sugar syrup (SS) at 1:1, 1:1.5, and 1:2; the total soluble solids content (TSS) at 14, 16, and 18 ^{o}Bx; the pH index at 4.5, 5.0, 5.5, and 6.0; the fermentation temperature at 20, 30, and 40°C; and the duration of 48, 56, and 64 hours. The ideal conditions were determined to be 10^7 CFU/mL of *L. plantarum* LB-1, a 1:2 SJ:SS ratio, a TSS of 16°Bx, a pH of 5.0, and a 48-hour fermentation period at 30°C. The end result was 0.54 ± 0.02 g/100 mL of total titratable acidity (TTA), 2.13 ± 0.04 g/100 mL of reducing sugar, 14.97 ± 0.47 ^{o}Bx of TSS, 3.95 ± 0.13 pH, 30.54 ± 0.89 mg/100 mL of vitamin C, and 172.62 ± 8.16 mg GAE/100 mL of total phenolic content.

Since the yeast count was below the allowable limit for beverages and coliform and mold were not found, the finished product satisfied the microbiological safety criteria. The amount of lactic bacteria was 6.35×10^7 CFU/Ml [64]. Based on the hedonic rating test findings, which showed an overall acceptance of 7.69 ± 0.56/9.00, the sensory quality of the final fermented juice was assessed as moderate[64].

2.11.4 Low-alcoholic Fermented Beverage from Soursop (*Annona muricata* L.) by *Saccharomyces cerevisiae* and *Saccharomyces bayanus*

Due to its considerable commercial potential, soursop (*Annona muricata L.*), a tropical fruit, has been processed into a variety of goods. Using yeast, this study attempts to create a low-alcohol fermented beverage from soursop. To determine whether strain of yeast was better suited for fermentation, two strains—*Saccharomyces cerevisiae* RV002 and *Saccharomyces bayanus* FD-3—were investigated. After that, more research was done to improve the fermentation parameters[65]. This included using the One Factor at a Time (OFAT) approach to look at the ratios of soursop juice (SJ) to sugar syrup (SS) (0.5:1, 1:1, and 1.5:1) and total soluble solids (15, 17, and 19 °Brix). The following settings produced the best fermentation conditions: After 48 hours of fermentation at 15°C with an SJ:SS ratio of 1:1 and 10^6 CFU/ml of *S. bayanus* FD-3 introduced, the total soluble solids reached 17°Brix and the pH was 4.5. The end product's v/v alcohol concentration was 2.05 percent, its pH was 13.2, its total titratable acidity was 0.4 percent, its vitamin C content was 16.98 mg/100 ml, its total phenolic content was 142.66 mg GAE/100 ml, and its overall acceptability on a 9-point hedonic scale was 7.82±0.33[65].

2.11.5 Research on the Co-cultured Fermentation of Soursop (*Annona muricata* L.) Fruit Juice by *Lactobacillus plantarum* and *Saccharomyces bayanus* Strain

An excellent tropical plant with nutritional and biological potential is soursop (*Annona muricata L.*), a member of the *Annonaceae* family[66]. Because of its many benefits, fermented fruit products are presently a trendy trend in consumption. Multi-strain

fermentation, also known as symbiosis, is being employed more and more in the food production industry these days, particularly in the fruit juice industry, in addition to the widely used fermentation techniques of alcoholic, acetic, and lactic fermentation. In order to change the fermented conditions for this study, a 48-hour lactic fermentation combined with the addition of 10^7 CFU/mL *L. plantarum* bacterial strain at 30°C and a 24-hour combined alcoholic and lactic fermentation with the addition of 10^6 CFU/mL *S. bayanus* yeast strain at 15°C was the proper procedure to use. A dilution ratio of 1:2 between soursop juice (SJ) and water (W), a total soluble solids (TSS) of 16°Brix, and a pH of 5.0 were determined to be the ideal conditions for fermenting soursop fruit juice. Total titratable acidity (TTA) of 0.488 ± 0.026 percent, total sugar content of 14.881 ± 0.189 g/100 mL, TSS of 15.37 ± 0.17 oBrix, pH index of 3.82 ± 0.04, ethanol content of 0.42 ± 0.01 percent v/v, vitamin C concentration of 29.26 ± 0.79 mg/100 mL, and total phenolic content of 95.35 ± 0.64 mg GAE/100 mL were all attained by the final product under these appropriate fermentation conditions. The fermented soursop juice satisfied the necessary microbiological quality criteria upon evaluation. The co-cultured fermented soursop juice received a moderate rating for sensory quality (7.41 ± 0.46 on a 9-point hedonic scale)[66].

2.11.6 Wine Production from Soursop Using Genetically-Identified *Saccharomyces cerevisiae* Isolated from Palm Wine

It is imperative that the raw materials used to produce wine in Nigeria be indigenous, particularly the source of juice and yeast starter cultures. Using genetically identified *Saccharomyces cerevisiae* isolated from palm wine, research was conducted on the generation of wine from soursop fruit[67]. Using 1,000 milliliters of soursop juice, wine was made via extracting, fermenting, racking, stabilizing, pasteurizing, aging, and maturing the juice. To increase the juice's sugar content, 100g of granulated sugar was added. 2.0g of sodium metabisulfite was added to the must to protect it from microbial assault. After

mixing 150 milliliters of yeast biomass with 1,000 milliliters of sterile juice, fermentation started[67]. The durations of primary and secondary fermentation were seven and fourteen days, respectively. The resulting wine sample was subjected to organoleptic, microbiological, and physicochemical studies. Before fermentation, the fresh "must" had a pH of 4.2, a specific gravity of 1.069, and a sugar content of 17 ^{o}Brix. The wine sample's specific gravity, percentage of alcohol (v/v), total acidity as acetic acid, pH, and sugar content were determined by physicochemical analysis to be 0.9800, 9.5 percent V/V, 0.8945 percent, 3.6, and 1.8 ^{o}Brix, respectively. The isolates' colony count, as determined by bacteriological examination, was $2.0x10^{-3}$CFU/ml. After a month of storage, the wine sample's organoleptic test revealed that its look was rated higher than its taste and scent, with a widely agreed score of 95[67].

2.11.7 Mycoflora and Production of Wine from Fruits of Soursop (*Annona Muricata* L.).

The mycoflora linked to the various sections of fresh and decaying soursop (*Annona muricata L.*) fruits was examined, as was the possibility of producing wine using both commercial yeast extract and native yeast flora[68]. The pathogenicity test and fungal isolation were performed using Sabouraud dextrose agar. The decaying fruits had more mycoflora than the fresh ones. *Trichoderma viride* was only isolated from fresh fruits, but *Botryodiplodia theobromae* was only isolated from rotting fruits (skin). *Rhizopus stolonifer* had the largest percentage occurrence (36.39 percent) in the rotting fruit, while *Penicillium sp.* was the most prevalent in all fruit parts of the fresh soursop fruit. The majority of the isolated fungus showed that soursop fruits were home to these common airborne fungi and that they had the ability to cause rot in fresh, healthy soursop fruits while they were being stored. After ten days of fermentation, wine was produced from soursop juice. The alcoholic concentration of the wines made with the commercial and native yeasts differed significantly ($P < 0.05$). The wine with the greatest alcoholic concentration was made from pasteurized, ameliorated soursop juice that had been infected with native yeast. The *Annona muricata* is a suitable source for wine production and single-cell protein based on the nutritional composition of soursop juice, its capacity to promote yeast growth, its high alcoholic content, and its palatability. This particular research spontaneous fermentation process was used first before conditional fermentation was conducted[68].

2.11.8 Preparation and Nutritional Evaluation of Soursop Juice as Partial Replacement for Dairy Milk in Yoghurt Production

A large variety of plant-based milks produced from almonds, oats, cashews, macadamia nuts, coconut, and our beloved soy bean are now available in almost all major grocery shops. Compared to dairy milk, these types of milk usually have less calories and no saturated fat. Therefore, the purpose of this research is to examine, using suitable standard

procedures, the manufacture and nutritional assessment of soursop juice milk as a partial substitute for dairy milk in the manufacturing of yoghurt. According to the data, yogurt enhanced with soursop juice had a slightly higher colour score (7.57) than plain cow milk yoghurt, suggesting that it contributed positively to colour acceptance[69]. Yogurt mixed with soursop received aroma and taste scores over 7. It also received an overall acceptability score of 7.00, which suggests that it has a high level of consumer preference and potential commercial appeal. Additional nutritional value was added by adding soursop juice, which resulted in higher dietary fiber and ash contents of 1.98 and 2.00, respectively. When compared to regular cow milk yoghurt, this enrichment could provide more health advantages. By decreasing whey separation and improving texture, the dietary fiber from soursop juice increased the yoghurt's stability. This implies that adding soursop juice makes the final product more stable and appealing. Compared with normal cow milk yogurt, soursop juice improves the yoghurt's stability, nutritional profile, and sensory qualities. These enhancements imply that yoghurt containing soursop juice might provide a competitive advantage in the market, attracting more customers[69].

2.11.9 Production and Properties of Probioticic Soursop Juice Using *Pediococcus pentosaceus* LBF2 as Starter

Making probiotic soursop juice and assessing the juice samples' characteristics after three weeks of storage at 4°C. *Pediococcus pentosaceus* LBF2 was used as the beginning culture in order to ascertain the characteristics of the soursop juice. Location and Study Period: July–December 2016 in the Department of Microbiology, University of Ibadan, Ibadan, Oyo state, Nigeria[70]. *Pediococcus pentosaceus* LBF2 was used as a probiotic starter in the production of soursop juice, and the viability of the starter as well as the physicochemical, nutritional, and organoleptic characteristics of the samples stored at 4°C for three weeks were assessed. The labels for the probiotic starter-containing soursop juice and the non-probiotic starter-containing soursop juice were Psop and Pcont.. The

probiotic strain was viable in the samples during storage (1.57×10^7 CFU/mL – 7.9×10^7CFU/mL) and the highest viability (1.85×107CFU/mL) was recorded at 2 weeks of storage. The lactic acid content of the samples ranged from 1621.44 - 2450.176 mg/l during storage. There was a general reduction in the pH of the samples. The Total Soluble Solid of the Psop and Pcont samples ranged from 15 – 6.0 and 13.5 - 8.0.The samples were acceptable and strongly liked in terms of sensory attributes in the 1st and 2nd week of storage. The Pcont samples were extremely disliked in the 4th week of storage. The crude protein, crude fat, crude fiber of the samples ranged from 0.58b - 0.65a%, 0.04c – 0.07a% and 0.48c-1.91a%. The highest crude protein was recorded at week 2 and 3 of storage. There was a general reduction in the crude fat, crude fiber and carbohydrate content during storage. In conclusion, the samples had a longer shelf-life, contain viable probiotic candidate, strongly acceptable with good nutritional composition which can confer a strong health beneficial effect on the consumer[70].

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Chapter Three

Methodology

3.1 Research Design

This study employs an experimental design to develop a fermented beverage from soursop pulp using Yeast and LAB isolates as Starters in mono- and co-fermentation experiments.

3.2 Sample Collection

The sample name Soursop –*Annona muricata*.

3.2.1 Area of Collection

The samples were purchased from Lagos fruit Market in Isolo Low-Cost Housing Estate and at Oje fruit market in Ibadan.

3.2.2 Sample Preparation and Juice Extraction

The purchased fruit samples were collected into clean bags and transported via the car immediately to the laboratory after collection. The samples were washed in clean water thoroughly to remove attached dirt and sand. The samples were then washed in the mixture of distilled water and white vinegar in the ratio 1:4 respectively. The washed fruit samples were peeled and the seeds were aseptically removed and blended in the laminar air flow after sterilizing the environment with UV light for 30 minutes. A cleanblender (Nakai Blender Mx (701) series) and fruit juicer (Midea –Juicer 6000 series) were used to blend the pulp and extract the juice . The juice was then filtered through a sieve of about 0.1mm pore size, transferred into a sterile Bama bottle of about 500 mL capacity and stored at 4°C in the laboratory table top refrigerator.

3.3 Spontaneous Fermentation of Soursop Juice

For spontaneous fermentation, 20 mL of the extracted juice was measured into each sterile Falcon tube, replicates of tubes were made and placed on a laboratory shaker incubator at ambient temperature for 5 days.

3.4 Proximate Analyses of the Spontaneously Fermented Juice

Aliquot part of the samples that had undergone spontaneous fermentation for 5 days were stored at appropriate temperature for subsequent proximate composition analyses in a commercial laboratory.

3.5 Microbiological Analysis of Fermented Samples

3.5.1 Preparation of Culture Media and Culturing Procedure for Bacteria Isolates (LAB)

De Man, Rogosa,(5.515g) and Sharpe (MRS) medium-formulated specifically for the cultivation of lactic acid bacteria was dissolved in 100mL of distilled waterand 1.5g of agar was measured along with it into a clean 250 mL conical flask. The contents were gently swirled to ensure uniform mixing, homogenised in the laboratory water bath at 80 °C and then sterilized by autoclaving at 121 °C for 15 minutes after properly corking the flask. After sterilization, the medium was allowed to cool to approximately 45–50 °C to prevent thermal damage during inoculation.At this point, 5mL of fluconazole was aseptically added to the medium to inhibit the growth of any contaminating fungi, ensuring selective cultivation of the bacteria isolate. The medium was then gently swirled to ensure even distribution of the antibiotic.It was subsequently poured into sterile Petri dishes and left to solidify undisturbed in the laboratory laminar air flow[1].

3.5.2 Plating out of Spontaneously Fermented Soursop Juice Samples on MRS Agar Plates.

For each replicate samples, enumeration of the colonies of LAB was done on de Man Rogosa Sharpe (MRS) agar plates at 24 hour interval. To achieve this, serial dilution of

each sample was made using microbiological standards. The tubes that were to be used for serial dilution were each filled with 4.5mL of distilled water and sterilized in the laboratory autoclave at 121°C for 15 minutes at 15lbs pressure. From each sample of fermented soursop juice, 0.5 mL were drawn using a sterile syringe for each sample and transferred into the tube containing sterile 4.5 mL distilled water to make up to 5mL content. Toachieve higher dilution serially, 0.5 mL of the aliquot was transferred into another tube of 4.5mL distilled water, this was done until a satisfactory dilution factor was attained to generate distinct colonies on agar plates of each of the medium used. The dilution factors of 10^{-2}, 10^{-3} and 10^{-5} were selected out of the sets of dilution factors generated by serial dilution. From each of the aforementioned sets of dilution factors 5 microliter of aliquot was drawn with sterile micropipette into each plate medium. On each of the agar plates prepared and kept earlier,5 microliter of aliquot of appropriate dilution factor was drawn with sterile micropipette and introduced onto each MRS agar plate using spread plate method. The inoculated plates were incubated under anaerobic conditions at 37°C in the laboratory anaerobic incubator to promote optimal growth and distinct colony development[2].

3.5.3 Plating out of Spontaneous Fermented Soursop Juice Samples on Yeast Extract Glucose Agar Plates

Approximately 200 mL of yeast extractagar was measured out into a clean conical flask. To enhance the fermentable sugar content, 1 gram of glucose was added to the medium. The mixture was thoroughly swirled to dissolve the components, homogenised in the laboratory water bath at 80 °C after which it was sterilized in an autoclave at 121°C for 15 minutes. Once the sterilization process was completed, the medium was allowed to cool to about 45-50°C. At this point, 1000 mg (1g) of amoxycillin was aseptically added to the medium to inhibit the growth of any contaminating bacteria, ensuring selective cultivation of the yeast isolate. The medium was then gently swirled to ensure even

distribution of the antibiotic within it. Subsequently, about 15 mL of the medium was poured into each sterile Petri dish and left undisturbed to solidify under aseptic conditions.

On each of the agar plates,five microliter of aliquot of appropriate dilution factor was drawn with sterile micropipette and introduced using spread plate method. The inoculated plates were incubated under aerobic conditions at 25±°C in the laboratory incubator for 24 -36 hours to promote optimal growth and distinct colony development.

the 5 yeast isolates initially stored on agar slants was aseptically picked using a sterilized inoculating loop and streaked onto the surface of the prepared plates. The inoculated plates were incubated at 25°C in the laboratory incubator for optimal growth and colony development[2].

3.5.4 Enumeration of Distinct Colony Unit on the Inoculated Plates

After 24 -36 hours of incubation, the plates were observed for growth and the distinct colonies on the plates (MRS and YEGA) were counted using grid method. The plates were divided into sections for easier counting. This procedure was repeated for plating samples from other time durations of 48 hours, 72 hours,96 hours and 120 hours[2].

3.6 Subculture of Distinct LAB Colonies

Sterile inoculating loop was used to touch the tip of a distinct colony in order to pick an inoculum from an MRS culture plate after incubation hours and was streaked across the surface of a newagar plate. The streaking was done from the first portion into new area of the plate and it was repeated for 2 to 4 time. This was necessary to make the bacteria spread out more sparsely on the plate.The inoculating loop was flamed before subsequent use and after use. Isolated colonies were seen as the procedure was repeated,up to when a single/pure isolated colony was successfully cultured. The pure colony was aseptically transferred with sterilized inoculating loop onto a MRS slant bottle, incubated appropriately as earlier mentioned and stored at 4°C as stock cultures for future use. This

same procedure was done for the yeast extract glucose cultures and the obtained pure cultures after incubating for plates and slant bottles at 27 ±2°C under aerobic condition andwere also kept as stock cultures[3].

3.7 Microscopic Observation of Yeast Cells

For microscopic observation of yeast cells was done by introducing a drop of 30% (w/v) lactophenol cotton blue was placed on a clean slide. A small amount of yeast culture was picked by using the tip of a sterile inoculating loop to touch the yeast culture on the culture plate and emulsified into the dye, and the smear was covered with a clean cover slip. The slide was examined under a light microscope at ×40 objective to see typical yeast cell morphology[4].

3.8 Presumptive Identification Tests for LAB Isolates

To do a presumptive test to verify the grouping of the LAB isolates,few microbiological and biochemical tests were carried out on each of the isolates as described below:

3.8.1 Gram Staining Techniques

A drop of distilled water was placed on a clean glass slide, a smear of each isolate was picked using a sterile inoculating loop to touch the tip of its colony and mixed with distilled water on the glass slide using the loop to allow the smear to be well dispersed on the slide. The smear was heat-fixed by passing the slide over a spirit lamp with flame twice. The isolate smear on the slide was stained with two drops of 2% crystal violet solution for 60 seconds, followed by gently rinsing with distilled water. Next, two drops of 1% iodine (50:50 v/v) was added for 60 seconds, followed by rinsing with distilled water. 95% ethanol was added to rinse the glass slide for 5 seconds, followed by immediate gentle rinsing with distilled water. Finally, 3.5% safranin solution was added for less than 30 seconds and rinsed off with distilled water. The slide was then air dried,

after which it was examined under the light microscope using magnification lenses x100 (oil immersion) for the LAB isolates[5].

3.8.2 Catalase Test

Using a syringe, 1 mL of 3% Hydrogen peroxide was transferred into a test tube. A small amount of colony picked by using a sterile inoculating loop to touch a tip of a fresh colony of the isolate in the petri plate and transferred into the Hydrogen peroxide solution and the reaction that followed was recorded[6].

3.8.3 Sugar Fermentation Test

This test determines the ability of the isolates to ferment specific sugars and produce acid and/or gas as metabolic byproducts.

Bromocresol purple, a pH indicator, was added to fermentation broth (peptone water) in each of the McCartney bottle each of the various carbohydrates, including lactose, sucrose, and glucose was added to each tube. In order to collect any gas generated during fermentation, clean Durham tubes were placed inside each sterile McCartney bottle after the medium had been dispensed into each of them in an inverted position. The bottles were sterilized by autoclaving at 121°C for 15 minutes. The desired test sugar was added to each of the McCartney bottles and then filtered aseptically under a laminar airflow cabinet using a sterile membrane filter and a vacuum pump, in order to sterilize the solution through this specialized filtration method. A sterile inoculating loop was used to aseptically inoculate each tube with the test isolate by using the loop to touch the tip of the fresh colony of the test isolate culture in a petri dish. For 48 hours, the tubes were incubated at 37°C in an aerobic environment. The tubes were checked for obvious color changes following incubation[7].

3.9 Molecular Identification of the Bacteria

Twenty grams of SDS were carefully measured into a conical flask and dissolved in 80 mL of deionized water with gentle stirring, followed by the addition of more deionized water until a total volume of 100 mL of SDS solution was achieved[8].

3.9.1 Enzymatic Lysis Buffer

Approximately 0.605 g of Tris base was dissolved in approximately 80 mL of distilled water, and the pH was adjusted to 8.0 using HCl. Separately, 0.372 g of EDTA was dissolved in distilled water and adjusted to pH 8.0 with NaOH. Additionally, 0.584 g of NaCl was dissolved in distilled water. These solutions of Tris-HCl, EDTA, and NaCl were then combined. Finally, 100 mg of lysozyme was added to the combined solution and stirred until completely dissolved.

3.9.2 Chloroform Isoamyl Alcohol Solution

About 96 mL of chloroform and 4mL of isoamyl alcohol were measured separately. These liquids were then combined in an amber bottle and thoroughly mixed to ensure homogeneity of the solution.

3.9.3 TE (Tris-EDTA) Buffer

The process involved measuring 10mM Tris-HCl and adjusting its pH to 8.0 with HCl. Following this, 1mM EDTA was measured and its pH adjusted to 8.0 using NaOH. The Tris-HCl and EDTA solutions were then combined in a clean container, after which distilled water was added to achieve the final desired volume.

3.9.4 DNA Extraction Protocol for LAB Isolates

Ten milliliters Nutrient Broth culture of the isolates were prepared and incubated for 24 hours at 37^{o}C. 5 mLs was aseptically pipetted into clean centrifuge tubes and centrifuged at 10,000 rpm for 10 minutes after which the bacteria cells had collected as a pellet and the bottom of the tubes. The supernatant was discarded by decanting carefully after which

a mixture was prepared by adding 2 mL of sterile distilled water to the pellet and dispersing the pellet by pipetting up and down with a sterile pipette. To this was added 400 microliters of 10mg/mL lysozyme, which was also thoroughly mixed by pipetting up and down and then incubated at 37°C for 30 minutes. Next, 800 microliters of 20% SDS solution was added to the culture and incubated at 60°C for another 30 minutes. Solid NaCl was introduced to achieve a concentration of 1 M, specifically 0.187008 grams in a 3.2 mL volume, and mixed thoroughly by pipetting. An equal volume of Chloroform Isoamyl alcohol was added, and the mixture was centrifuged at 15000 rpm for 5 minutes to facilitate protein separation. Following this, absolute ethanol at a concentration of 67% was added. The DNA precipitate was then carefully removed by spooling it around an applicator stick and allowed to dry for approximately a minute to remove excess alcohol. Finally, the DNA was dissolved in 2 µL of TE Buffer[7].

3.10 Deoxyribonucleic Acid (DNA) Extraction for Extraction of Yeast DNA using Commercial Kit

Genomic DNA was extracted from the cultures received using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005) by following this procedure:

A 24-hour-old culture of the bacterial isolates containing approximately 2 x 10^9 cells were harvested in a microcentrifuge(Eppendorf Centrifuge) tube by centrifuging for 10 minutes at 5000 x g (7500 rpm), after which the supernatant was discarded. The bacterial pellet was then resuspended in 180 µL of enzymatic lysis buffer and incubated at 37°C for 30 minutes. Following this, 25 µL of Proteinase K and 200 µL of Buffer AL (without ethanol) were added to mixture and homogenized by vortexing. An additional incubation at 56°C for 30 minutes followed. Subsequently, 200 µL of 96% ethanol was added to the sample and thoroughly mixed on a vortexing machine to ensure homogeneity. The

resulting mixture was pipetted into a spin column placed in a 2 mL collection tube and centrifuged at ≥6000 x g (8000 rpm) for 1 minute. After this the DNA was resuspended in an aliquot solution[9].

3.11 Polymerase Chain Reaction (PCR) for Amplification of 16SrRNA from the Extracted Genomic DNA of Isolates

The primers used for this step were universal primers: For LAB isolates - 27F and 1492R with the following sequences

27F – AGAGTTTGATCCTGGCTCAG (forward primer)

1492R – CTACGGCTACCTTGTTACGA (reverse primer)

For yeast isolates – TUBUF2 as forward primer and TUBUR as reverse primer

The target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) with the primers presented below:

TUBUF2(FORWARD	CGGTAACAACTGGGCCAAGG
TUBUR1(REVERSE)	CGGTAACAACTGGGCCAAGG

Stock solution of both the forward and reverse primers was made by adding 698µL of T.E buffer to the reverse primer and 644.9µL of the same T.E buffer to the forward primer. A 1:10 dilution of the primers was made with nuclease free water to create a working solution. A 1:10 dilution of each extracted deoxyribonucleic acid (DNA) was also carried out by adding 90µL of nuclease free water to 10µL of DNA in a 1.5mL Eppendorf tube. The mixture was pipetted up and down to achieve a uniform dispersal of the DNA.

A 50 µL polymerase chain reaction cocktail was prepared by mixing 25 µL of master mix, 1 µL of forward primer, 1 µL of reverse primer, 1 µL of the template (DNA) and 22 µL of nuclease free water.

Conditions for polymerase chain reaction by thermocycling were set as follows:

I. Initial and actual denaturation reactions: 94°C for 30 seconds.
II. The annealing reaction: 54°C for 60 seconds.
III. The extension reaction: 68°C for 2 minutes.
IV. The final extension reaction: 68°C for 5 minutes.

The denaturation, annealing and extension occurred repeatedly in 30 cycles. The machine stops the reactions automatically and keeps it at 4°C. The product of PCR was submitted to a commercial laboratory for valuation of product size and sequencing in order to determine the identity of the selected LAB by the base sequence of their 16SrRNA gene and the yeasts by the base sequence of the 18SrRNA gene[10].

3.11.1 DNA Agarose Gel Electrophoresis

PCR products (amplicons) were separated by electrophoresis on a 1% agarose with Tris-acetate-EDTA buffer (TAE). Where 15µL of the PCR product was mixed with 3 µL loading dye and ran at a voltage of 70V for 30min on an horizontal gel well electrophoretic setup. After running the electrophoresis, the gel carefully removed from the well, stained with a mixture of 0.0025%crystal violet (w/v) and 0.0005%methyl orange dye (w/v)in sterile distilled water and left for 30 minutes inside the solution.

The PCR products were run on a gel and gel extracted with the Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Catalogue No. D4001). The resulting gels from both

procedures were later viewed in a trans illuminator to confirm the presence and quality of the bacteria DNA[11].

3.12 Molecular Identification of Yeast Strains

The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3- 100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample. DNAStar was used to analyze the. ab1 files generated by the ABI 3500XL Genetic Analyzer and results were obtained by a BLAST search (NCBI)[12].

3.13 Fermentation of Soursop Juice Using Selected Isolates

3.13.1 Pasteurization of Soursop Juice

More samples of soursop fruit were obtained and treated as earlier stated to obtain the fruit juice. The soursop juice samples were transferred intosterile 500 mL capacity bottles and heated to 80°C for 15 minutes for pasteurization process.To increase the sugar content of soursop juice meant for the fermentation process, 2 g each of fructose and glucose was added to 300 mL of the juice[13].

3.14 Inoculum Preparation

3.14.1 Preparation of Broth Culture of Selected Starters

A yeast isolate and a LAB isolate was used for further work (as starter cultures) from the isolates obtained from the spontaneously fermentedsoursop juice after confirming their identities.

These isolates were each earlier inoculated into each of their broth cultures respectively (yeast in yeast extract peptone broth and LAB in MRS broth) and allowed to grow at the appropriate growth condition for each as earlier stated[2].

3.14.2 Determination of Inoculum Size of Starters

To determine the inoculum size to use for fermentation process, serial dilution of each isolate used was done in normal saline in sterile MacCartney bottles. Into each sterile MacCartney bottle, 9mL of normal saline was introduced while 1 mL of the microbial broth was added to it. Ten microliters of dilution factors of $10^{-2,}$ $10^{-4,}$ $10^{-6,}$ $10^{-8,}$ 10^{-10} of each starter used were plated on on the appropriate agar medium used for culturing each of them in this work.

Plate counts were taken to obtain CFU/mL of each dilution point and optical density of each dilution point was also determined in a spectrophotometer and compared with a standard curve which has optical density values for known cell counts. The standard curve was used to estimate the optical density for 10^5 CFU/mL which was used as the inoculum size of each starter. Starters were used singly and in combination for the fermentation processes[14].

3.14.3 Inoculation of the Pasteurised Juice Samples Jars for Fermentation Process

Each fermentation jar had 300 mL of pasteurized soursop juice and each jar was labelled with a number as 1, 2,4, 5 and 6 for distinction. The Jars labelled 1 was uninoculated with starter while the Jars labelled 4 was unpasteurized and uninoculated with starter. Jars Labelled 2 were inoculated with only the yeast starter for the first 48 hours and then the LAB starter was added to each Jar for the last 24 hours. Jars labelled 5 were inoculated with the LAB starter first for 48 hours and then the yeast isolate was added for the last 24 hours. Jars labelled 6 were each inoculated with appropriate inoculum of the LAB isolate

for 72 hours. The jars were each covered with sterile muslin cloth and incubated at 37°C for 72 hours[15].

3.15 Proximate Analyses of Fermented Samples

One common technique for identifying a sample's basic nutritional components is proximate analysis. Proximate analysis was conducted to determine the basic nutritional composition of the fermented samples, including moisture, ash, protein, fat, fibre, and nitrogen-free extract. Proximate analyses of the starter fermented samples were determined using the same method used for analyzing the spontaneously fermented soursop juice as stated below:

3.15.1 Moisture Content of Fermenting Juice

Moisture content was assessed by measuring the loss in weight of a sample when it was dried to a constant weight in an oven. Procedure: Approximately 2 g of sample was weighed into a pre-weighed, oven-dried silica dish. It was dried in an oven at 65°C for 36 hours to constant weight. After cooling in a desiccator, the dish was weighed again until a constant weight was obtained[16].

Calculation:

% Moisture = (Initial weight - Final weight) / Weight of sample × 100

3.15.2 Ash Determination in Fermenting Juice (Total Mineral Content)

This process helps estimate the mineral composition of the Juice.

Procedure:

Two grams of dried, ground sample was placed in a pre-weighed crucible, ashed in a muffle furnace at 500°C for 4 hours until a grey-white residue appeared. The crucible was cooled in a desiccator and weighed[11].

Calculation:

% Ash = (Weight of crucible + ash - Weight of crucible) / Sample weight × 100.

3.15.3 Crude Protein Determination in Fermenting Juice

Crude protein was determined by first analyzing the nitrogen content of the sample, then multiplying the result by a conversion factor of 6.25. This calculation is based on the assumption that proteins typically contain about 16% nitrogen. The analysis followed the standard Kjeldahl method (AOAC), which consists of three (3) key steps: Digestion, distillation and titration[17].

1. Digestion:

Approximately 2 g of the finely ground sample was weighed into a Kjeldahl flask. 25 mL of concentrated sulphuric acid (H_2SO_4) was added, followed by; 0.5 g copper sulphate ($CuSO_4$) (as a catalyst), 5 g sodium sulphate (Na_2SO_4) (to raise the boiling point), and A speck of selenium tablet (optional catalyst). The flask was heated gently in a fume cupboard, initially at low heat to prevent frothing. Then, it was digested vigorously for approximately 45 minutes, or until the solution became clear pale green. After digestion, the flask was cooled completely. Then, 100 mL of distilled water is added carefully. The flask and contents were rinsed 2–3 times with distilled water, and all rinsing were added to the digest

2. Distillation

A Markham distillation apparatus was used. The apparatus was steamed, and 10 mL of the digested sample was added through the funnel. Immediately, 10 mL of sodium hydroxide (NaOH) was introduced to the flask to liberate ammonia (NH_3). The released ammonia was distilled into a receiving flask containing 50 mL of 2% boric acid solution, with a few drops of screened methyl red indicator[11].

3. Titration: The ammonium borate formed during distillation was titrated against 0.1 N hydrochloric acid (HCl). The volume of acid used (titre value) is recorded and used in the following formula to calculate nitrogen content:

%N = 14 x VA x 0.1 x w x100

w= weight of sample

VA = volume of acid used %crude protein = %N x 6.25.

3.15.4 Ether Extract (Fat Content) Determination in Fermenting Juice

Anhydrous diethyl ether (or petroleum ether) 150 mL with a boiling point range of 40–60°C was added to the round-bottom flask. A feed sample weighing between 2–5 g was placed into a thimble, which was then sealed with cotton wool. The thimble was inserted into the extractor. Upon heating the flask, the ether vapor rose to the condenser, where it cooled and returned in liquid form to the thimble, soaking the sample. The ether dissolved fat-soluble substances and carried them back into the flask through the siphon tube. This continuous extraction process was maintained for at least 4 hours. After extraction, the thimble was removed and the remaining solvent was mostly distilled off. The flask contaidning the ether extract was then placed in an oven at 65°C for 4 hours, cooled in a

desiccator, and weighed to determine the amount of ether extract present [8]%Ether extract = wt of flask + extract – tare wt of flask x100wt of sample[18].

3.15.5 Crude Fibre in Fermenting Juice

Crude Fibre represents the indigestible portion of plant material, mainly cellulose and lignin. It is typically determined from the organic residue left after the sequential extraction of fat from a feed sample. The fat-free sample was transferred into a beaker or flask. 200 mL of pre-heated 1.25% sulfuric acid (H_2SO_4) was added to the sample. The mixture was gently boiled for 30 minutes. The volume was maintained by adding hot water as needed. A Büchner funnel fitted with Whatman filter paper was pre-heated with hot water. The acid-digested sample was filtered hot under vacuum suction. The residue was washed thoroughly with boiling water until it became neutral to litmus paper. The neutralized residue was transferred back to the beaker, and 200 mL of pre-heated 1.25% sodium hydroxide (NaOH) was added. This solution was boiled for another 30 minutes, filtered hot, and the residue was washed with boiling water and twice with ethanol to remove the remaining soluble substances. The residue was then dried at 65°C for approximately 24 hours, cooled in a desiccator, and weighed. Finally, the dried residue was transferred to a crucible and incinerated in a muffle furnace at 400–600°C for 4 hours. It was then cooled in a desiccator and reweighed[11].

3.15.6 Determination of pH Content of Fermented Samples

The pH meter was rinsed with distilled water and then calibrated using a standard buffer solution, which I started by using pH 7 down to pH 4. At each time of analysis, 20 mL of the fermenting juice sample to be analyzed was poured into a sterile beaker and then the electrode of the pH meter was inserted into the sample (Juice) for few minutes. The reading on the pH meterfor each sample was recorded before the electrode was removed

from tested sample and rinsed with a distilled water to prevent cross contamination and fake results[19].

3.15.7 Determination of Total Soluble Solid (TSS) Content in Fermenting Juice

Total soluble solids (TSS) BRIX

TSS was determined using portable digital refractometer (ERMA, Japan) with a scale of 0–32 Brix (least count 0.2°Brix) at room temperature (~30 C)[13].

3.15.8 Quantification of Acetic Acid Produced in Fermenting Juice

3.15.8.1 Experimental Procedure

For the process, samples of fermenting juice were used. 150 mL of dry, clean beaker with 100 mL of NaOH.

Twelve milliliters of fermenting juice were pipetted into the Erlenmeyer flask, and three to four drops of phenolphthalein indicator were added. The mixture was then gently stirred until it became colorless. A tiny bit of the NaOH solution was then used to rinse the burette.

The phenophtalein and fermenting juice combination will be positioned under the burette's tip, and NaOH was added to the flask's contents.

A drop of NaOH was then added while the fluid was stirred (a pink colour may be observed where the NaOH solution drops).

The combination will turn colorless once the end point is reached. In this manner, keep adding the sodium hydroxide solution, turning the flask as you go. The pink hue will last longer as the end point draws near before the mixing turns the solution back into a colorless state. When the well-swirled solution in the flask does not clear to colorless and stays a very faint pink, you have achieved the endpoint. The amount of NaOH used was noted after the end point was achieved. Check and note the NaOH volume.

This is the amount of base needed to neutralize the acid quantitatively.

(Hint: Vf - Vi = VT, where Vf is the final

volume, Vi is the initial volume and VT is the titration volume)

A second titration using a brand-new 10.00 mL sample of your diluted vinegar and phenolphthalein and a refilled burette (where Vi is the original volume and VT is the titration volume). Do a third titration if the titration volumes for these two trials do not agree well and are not within 0.02 mL of one another. Next, the average titration volume was determined. The acid concentration will be determined using the average titration volume[14].

3.15.9 Determination of Vitamin B1

Thiamin and Riboflavin. These vitamins are usually extracted from foods using dilute mineral acids and autoclaving at 121°C, followed by enzymatic hydrolysis to release the bound forms of the vitamins. Riboflavin can be measured directly with fluorescence but thiamin requires conversion to thiochrome with alkaline potassium ferricyanide solution. The latter can be performed either manually prior to injection onto the analytical column, or by post-column derivatization. Reverse phase Liquid Chromatography (LC) analysis of thiochrome invariably involves the injection of high salt concentrations on to the analytical column necessitating frequent washing and much reduced column life. This can be overcome to some extent by the use of guard columns but this may be uneconomic. Alternatively, thiochrome can be selectively extracted into an organic solvent, normally isobutanol, allowing fluorometric determination after a normal phase LC separation of thiochrome from any remaining fluorescing interferences. This approach can give increased sensitivity and rapid automated analysis without the need for post-column derivatization.

Thiamin methods based on the thiochrome reaction after acid hydrolysis and treatment with enzyme to release the phosphorylated forms give total thiamin concentrations. The

separation of thiamin and its phosphorylated forms [thiamin monophosphate (TMP), thiamin di- or pyro-phosphate (TPP) and thiamin triphosphate (TTP)] has also been reported[15].

3.15.10 Determination of Vitamin C (Ascorbic Acid)

Preparation of Starch Indicator:

One g of starch was dissolved in 200 mL of boiling water inside a 250 mL conicalflask.

The solution was allowed to cool before use.

i. Preparation of Iodine Solution (0.01 N):

- Five g of potassium iodide (KI) and 0.268 g of potassium iodate (KIO_3) were dissolved in 200 mL of distilled water.
- 30 mL of 3 M sulfuric acid (H_2SO_4) was added to the solution.
- The final volume was made up to 500 mL with distilled water in a 500 mL conical flask.

ii. Standardization of Iodine Solution:

- To determine the exact normality of the iodine solution, it was titrated against 5 mL of a 1% ascorbic acid standard solution.
- Three – four drops of starch indicator were added to the standard solution.
- The endpoint of the titration was identified by the appearance of a persistent blue-black coloration.

iii. Sample Titration:

- 5 mL of soursop juice sample was measured into a clean conical flask.
- A few drops of starch indicator were added.
- The mixture was titrated with the standardized iodine solution.
- The endpoint was observed as a persistent blue-black color, indicating the presence of unreacted iodine[16].

iv. **Calculation of Ascorbic Acid Content:**The concentration of Vitamin C in the juice sample was calculated using the formula:
Ascorbic Acid (mg/mL) = {V_1}/{V_2} X {known concentration}

where V_1 = titre volume of the sample

V_2 = titre volume of the standard.

3.15.11 Determination of Zinc, Magnessium, Calcium in Fermenting Juice

The investigation was conducted using deionized water (18.2 MΩ cm) from a Polwater water purification system (Labopol-Polwater, Poland). Every chemical was at least analytical quality. All working standard solutions were prepared using stock standard solutions of Ca, K, Mg, and Na (1000 mg L−1) that were purchased from Sigma-Aldrich (Germany). An ACS-grade concentrated HNO_3 (65% m/m) solution (Merck, Germany) was used to acidify the FLC (Flowing Liquid Cathode) solutions. The samples were digested using the same concentrated HNO_3 and a 30% H_2O_2 solution (Merck, Germany) prior to being analyzed by ICP OES (Inductively Coupled Plasma Optical Emission Spectrometry).

The fermenting juices were first centrifuged for 10 minutes at 15000 rpm to get rid of any dissolved particles before FLC (Flowing Liquid Cathode-Atmosperic Glow Discharge) APGD OES (Optical Emission Spectroscpy) tests. A twist-cup container was then filled with suitable aliquots of each of the examined juices, and concentrated HNO_3 was added until the concentration reached 0.1 mol L−1. Ultimately, deionized water was added to each sample to achieve the ultimate 1000-fold dilution of the juices (on the weight basis). These manufactured samples were examined using basic standards solutions in relation to external calibration curves.

Additionally, the usual addition procedure for calibration was used to examine all juice samples. All of the previously described sample preparation procedures were repeated in this instance; however, following acidification, each sample was separated into four

sections, and the last three were subjected to the standard addition using a Ca and Mg mixed solution (10 mg L−1). After that, deionized water was added to each sample to achieve the ultimate 1000-fold dilution of the juices (on the weight basis).

As regards ICP OES measurements, the aforesaid juice samples were wet-digested in order to destroy the organic matrix. For this purpose, samples of 5.0 g of each juice and concentrated HNO_3 (5.0 mL) were transferred into a DigiPrep tube and heated for 180 min at 120 °C. After cooling, 5.0 mL of H_2O_2 (30%) was added and the samples were further heated for 60 min. Afterwards, such prepared samples were filled with deionized water to 50.0 g. After that, appropriate amounts of digested solutions were transferred into a twist-cup container and filled with deionized water up to 30.0 g. Two dilutions of each juice sample were prepared, *i.e.*, 12-fold and 750-fold (on the weight basis).

The aforementioned juice samples were wet-digested to eliminate the organic matrix in order to perform ICP OES tests. Samples of 5.0 g of each juice and 5.0 mL of concentrated HNO_3 were put into a DigiPrep tube and heated to 120 °C for 180 minutes in order to accomplish this. Following cooling, 5.0 mL of 30% H_2O_2 was added, and the samples were then heated for an additional 60 minutes. These prepared samples were then filled to a weight of 50.0 g with deionized water. Subsequently, the proper volumes of digested solutions were poured into a twist-cup container, which was then filled to the brim with deionized water (30.0 g). Each juice sample was made in two dilutions: a 12-fold and a 750-fold dilution (on the weight basis).

Each of the aforementioned samples was made in triplicate, and the final results also took into account the required procedural blanks. Ca, K, and Mg values were determined for each sample using basic standards solutions and external calibration curves[17].

3.15.12 Sensory Analysis of Fermenting Juice

This analysis was performed to evaluate the organoleptic properties of the differently treated soursop juice samples. Ten-man panel was constituted to evaluate the properties

of each of the fermented samples. Each member was pre informed and trained on how the sensory evaluation will be correctly done and recorded prior to the real evaluation and they were allowed to first do a pilot session. About 1 mLof each sample was taken to evaluate the properties (taste, aroma, and appearance) and record. After evaluation of each sample, sterile distilled water was taken to rinse my mouths before carrying out the test for another sample until all samples were evaluated[20].

3.15.13 Statistical Analyses

R Programming Language was used for all the statiscal analyses in this work. The version used version 4.5.1[21].

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Chapter Four

Results and Discussion of Findings

4.1 Results

Table 4.1 displays the findings of the bacterial and yeast colony counts that were isolated from the spontaneously fermented soursop juice at 24-hour interval for 120 hours. The spontaneous fermentation of soursop juice is characterized by a successional change in the dominant microbial population, as detailed in Table 4.1. Initially, yeasts drive the process, but are progressively outcompeted by lactic acid bacteria (LAB) as the fermentation progresses.

Fermentation Dynamics

Initial Phase (24–48 hours)

Yeast dominance**:** At 24 hours, the yeast count was significantly higher than the LAB count (24.8×10^6 CFU/mL versus 4.1×10^6 CFU/mL).

Mid-Fermentation (48–72 hours)

LAB ascendancy**:** The LAB count increased, reaching its peak count at 72 hours (57.8×10^6 CFU/mL). This coincided with a sharp drop in the yeast count to 1.6×10^6 CFU/mL

Fermentation (96–120 hours)

The LAB's count dropped significantly to 15.0×10^6 CFU/mL at 96 hours and further to 2.8×10^6 CFU/mL at 120 hours. Yeast's count also decreased, with the count falling to 0.5×10^6 CFU/mL at 96 hours and showing no growth at 120 hours.

Table 4.1: Yeast and LAB Counts from Spontaneously Fermented Soursop Juice at Different Durations

Fermentation Duration	LAB Count x 10^6(CFU/mL)	Yeast Count x 10^6(CFU/mL)
24 hours	4.1	24.8
48 hours	30.8	20.3
72 hours	57.8	1.6
96 hours	15.0	0.5
120 hours	2.8	No growth

Key: LAB = *Lactobacillus*

Source: Laboratory Work, 2025

Results of morphological and biochemical characteristics of the obtained bacterial isolates are displayed in Table 4.2. For example, the isolate (bacteria) tested negative for the catalase test, indicating no burble generation. In the tests for the fermentation of carbohydrates, the bromocresol's purple changed to yellow, and gas production was evident in the inserted Durham tubes.

For Gram's staining of the bacteria, the result indicated that it is Gram positive because its cell retained the crystal violet stain with purple cocci chains under the x100 magnification of the light microscope. For citrate test, the bacterial isolates are observed to be citrate positive due to change in the color of the medium after 24hours. The medium change from a total green color to blue. When the bacterium was stained with Gram's stain, the cell preserved the crystal violet stain with purple cocci chains under the light microscope's x100 magnification, indicating that the bacteria was Gram positive. The morphology of yeasts under the microscope lens X40 magnification showed spherical cells with buds.

Table 4.2: Presumptive Biochemical Characteristics of LAB Isolate

Isolates' Code	Catalase	Acid ad Gas Production from Lactose Fermentation	Gram Staining
SS1MRS24	**-**	+	Gram + cocci
SS1MRS24	**-**	+	Gram + cocci
SS2MRS48	**-**	+	Gram + cocci
SS1MRS48	**-**	+	Gram + cocci

Key: LAB = *Lactobacillus*

Source: Laboratory Work, 2025

Tables 4.3 and 4.4 show the molecular identities of the bacteria isolates and yeast obtained from spontaneously fermented soursop juice. The LAB isolates were all *Leuconostoc mesenteroides*, details of their information from NCBI database is presented on this Table 4.3 while details of the yeasts are on Table 4.3. Among the yeasts, only *Kurtzmaniella quercitrusa*is not implicated with any infection, it was therefore selected as the most suitable candidate for starter among the other yeast isolates obtained.

Table 4.5 is the proximate composition of spontaneous fermented and unfermented soursop juice for paramters such as moisture, carbohydrate, protein, fat, fibre and ash contents. The proximate composition of the fermented gruel changed with fermentation duration, and the changes were significant for some parameters, but not for others. More details on this is available at the discussion section.

The Table 4.6 is the proximate composition of controlled fermented and unfermented soursop juice for paramters such as moisture, carbohydrate, protein, fat, fibre and ash contents.

Table 4.7 shows the vitamin contents of the controlled fermented juice samples. Sample SC1 had the highest vitamin content among all the samples while samples SC1 and SC5 had the lowest vitamin contents.

Calcium content of SC2 was highest among the control (Starter) fermented juices while the Magnesium and Zinc contents were highest in samples SC1, the content of the latter minerals were within acceptable range in sample SC2 (Table 4.8).

Acetic acid was highest in SC2 and lowest in SC1 while the pH is lowest in SC2 and highest in SC1 as presented on Tables 4.9 and 4.10. Total soluble solid content was highest in SCI and lowest in SC6 samples (Table 4.11)

SC2 was most acceptable based on the sensory evaluation results shown on Table 4.12.

Table 4.3: Molecular Identities of LAB Isolates Obtained from Spontaneously Fermented Soursop Juice

Isolate Code	Identities of Organisms	Sequence Length (bp)	% Identity	Accession No of BLAST Hit	Error (E) Value	Highest Query Coverage (%)
SS1MRS24	*Leuconostoc mesenteroides*	1215	96.34	PX106408	0.0	92
SS1MRS24	*Leuconostoc mesenteroides*	1158	81.66	PQ796731.1	0.0	82
SS2MRS24	*Leuconostoc mesenteroides*	1179	93.57	PX106407	0.0	96
SS2MRS24	*Leuconostoc mesenteroides*	489	91.84	MK774576.1	5e-131	65
SS1MRS48	*Leuconostoc mesenteroides*	1009	93.16	MF164053.1	0.0	46
SS1MRS48	*Leuconostoc mesenteroides*	751	89.92	PX106406	0.0	97

Key: LAB = *Lactobacillus*

Source: Laboratory Work, 2025

Table 4.4: Molecular Identities of the Yeast Isolates Obtained from Spontaneously Fermented Soursop Juice

Serial Number	Isolate Code	Organism	Sequence Length (bp)	% Identity	Accession No of BLAST Hit	E – Value	Highest Query Coverage (%)
1.	**D**	*Candida tropicalis*	528	100.00%	LC605187.1	0.0	100.00%
2.	**G1**	*Kurtzmaniella quercitrusa*	602	100.00%	PX106405	0.0	100.00%
3.	**G2**	*Lodderomyces elongisporus*	558	100.00%	JN606251.1	0.0	100.00%

Source: Laboratory Work, 2025

Table 4.5: Proximate Composition of Spontaneous Fermented Soursop Juice

Traits	Sample	Mean± Standard Deviation
Moisture	120hrs	95.48 ± 0.38
Moisture	24hrs	92 ± 0.33
Moisture	48hrs	95.74 ± 0.01
Moisture	72hrs	95.65 ± 0.01
Moisture	96hrs	96.17 ± 0.07
Moisture	Unf	91.14 ± 0.87
Carbohydrate	120hrs	1.74 ± 0.43
Carbohydrate	24hrs	3.115 ± 0.95
Carbohydrate	48hrs	0.375 ± 0.08
Carbohydrate	72hrs	0.98 ± 0.35
Carbohydrate	96hrs	0.545 ± 0.23
Carbohydrate	Unf	1.315 ± 0.08
Protein	120hrs	0.505 ± 0.09
Protein	24hrs	1.445 ± 0.12
Protein	48hrs	1.16 ± 0.028
Protein	72hrs	1.05 ± 0.127
Protein	96hrs	0.92 ± 0.18
Protein	Unf	1.815 ± 0.09
Fat	120hrs	0.155 ± 0.04
Fat	24hrs	0.69 ± 0.21
Fat	48hrs	0.69 ± 0.22
Fat	72hrs	0.615 ± 0.43
Fat	96hrs	0.515 ± 0.21
Fat	Unf	0.385 ± 0.05
Fibre	120hrs	2.055 ± 0.16
Fibre	24hrs	2.67 ± 0.33
Fibre	48hrs	1.965 ± 0.18
Fibre	72hrs	1.655 ± 0.06
Fibre	96hrs	1.79 ± 0.34
Fibre	Unf	5.24 ± 0.60
Ash	120hrs	0.065 ±0.02
Ash	24hrs	0.08 ±0.42
Ash	48hrs	0.07 ± 0.014
Ash	72hrs	0.05 ± 0.028
Ash	96hrs	0.06 ± 0.014
Ash	Unf	0.10± 0.049

Key: Numbers represent mean ± standard deviation of values obtained

Source: Laboratory Work, 2025

Table 4.6: Proximate Composition of Controlled Fermented Soursop Juice

Traits	Sample	Mean±Standard Deviation
Moisture	SC1	72.04 ± .083
Moisture	SC2	73.06 ± 0.08
Moisture	SC5	71.11 ± 0.15
Moisture	SC6	73.50 ± 0.69
Carbohydrate	SC1	15.91 ± 0.00
Carbohydrate	SC2	14.60 ± 0.16
Carbohydrate	SC5	17.71 ± 0.18
Carbohydrate	SC6	15.90 ± 0.64
Protein	SC1	11.67 ± 0.03
Protein	SC2	12.06 ± 0.03
Protein	SC5	10.84 ± 0.03
Protein	SC6	10.30 ± 0.04
Fat	SC1	0.05 ± 0.06
Fat	SC2	0.04 ± 0.04
Fat	SC5	0.01 ± .001
Fat	SC6	0.01 ± 0.00
Fibre	SC1	0.02 ± 0.00
Fibre	SC2	0.02 ± 0.00
Fibre	SC5	0.03 ± 0.00
Fibre	SC6	0.03 ± 0.00
Ash	SC1	0.32 ± 0.00
Ash	SC2	0.22 ± 0.00
Ash	SC5	0.31 ± 0.00
Ash	SC6	0.26 ± 0.003

Key: Numbers represent mean ± standard deviation of values obtained

SC1- Unfermented soursop juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

Table 4.7: Vitamin Content of Controlled Fermented Soursop Juice

Traits	Sample	Mean±Standard Deviation
Vitamin B1	SC1	0.278 ± 0.000
Vitamin B1	SC2	0.379 ± 0.028
Vitamin B1	SC5	0.279 ± 0.001
Vitamin B1	SC6	0.276 ± 0.001
Vitamin C	SC1	1.733 ± 0.153
Vitamin C	SC2	3.433 ± 0.404
Vitamin C	SC5	1.323 ± 0.064
Vitamin C	SC6	2.170 ± 0.106

Key: Numbers represent the mean ± standard deviation of values obtained

SC1- Unfermented soursop juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

Table 4.8: Mineral Content of Controlled Fermented Soursop Juice

Traits	Sample	Mean±Standard Deviation
Calcium	SC1	173.17 ± 0.17
Calcium	SC2	221.20 ± 0.40
Calcium	SC5	172.10 ± 0.30
Calcium	SC6	218.46 ± 0.35
Magnessium	SC1	382.23 ± 0.30
Magnessium	SC2	333.33 ± 0.70
Magnessium	SC5	375.24 ± 0.70
Magnessium	SC6	333.56 ± 0.81
Zinc	SC1	0.66 ± 0.00
Zinc	SC2	0.58 ± 0.00
Zinc	SC5	0.65 ± 0.00
Zinc	SC6	0.58 ± 0.00

Key: Numbers represent the mean ± standard deviation of values obtained

SC1- Unfermented soursop juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

Table 4.9: Acetic Acid (mg/mL) Content of Controlled Fermented Soursop Juice

Sample	Mean±Standard Deviation
SC1	0.90 ± 0.07
SC2	2.68 ± 0.11
SC5	1.45 ± 0.00
SC6	1.23± 0.04

Key: Numbers represent the mean ± standard deviation of values obtained

SC1- Unfermented soursop juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

Table 4.10: pH of Starter Fermented Soursop Juice

Sample	Mean±Standard Deviation
SC1	4.84 ± 0.01
SC2	4.17 ± 0.01
SC5	4.42 ± 0.01
SC6	4.54 ± 0.01

Key: Numbers represent the mean ± standard deviation of values obtained

SC1- Unfermented soursop juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

Table 4.11: Total Soluble Solid Content of Controlled Fermented Soursop Juice

Sample	Mean
SC1	13.50
SC2	5.50
SC5	10.00
SC6	5.00

Key: Numbers represent the mean ± standard deviation of values obtained

SC1- Unfermented soursop juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

Table 4.12: Sensory Evaluation Scale of Starter Fermented Soursop Juice

Parameters	Sample	Mean
Appearance	SC2	3.60
Appearance	SC5	3.10
Appearance	SC6	2.40
Aroma	SC2	3.40
Aroma	SC5	3.30
Aroma	SC6	3.60
Taste	SC2	4.00
Taste	SC5	2.90
Taste	SC6	2.10

Key: Numbers represent the mean ± standard deviation of values obtained

SC1- Unfermented soursop juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

4.2 Discussion of Findings

The yeast and LAB counts from spontaneously fermented soursop juice at different durations are shown in Table 4.1. For the yeast count at 24 hours, it was higher (24.8 x 10^6 cfu/mL) when compared with the LAB count (4.1 x 10^6 cfu/mL). The yeast count decreased with time until there was no growth at the 120-hour duration. For the LAB count peaked at 72 hours with 57.8 x 10^6 cfu/mL and subsequently reduced from then to 2.8 x 10^6 cfu/mL at 120 hours' duration. The result in Table 4.3a confirmed the identities of the isolates, which were listed as *Leuconostoc mesenteroides*. The samples with higher percentage identities were submitted to GenBank, and the sequences were listed in the appendix, along with the accession numbers obtained. The biochemical characteristics of LAB, as shown in Table 4.2, are consistent with the molecular result. The 4.3b revealed the molecular identities of organisms isolated from the fermented soursop juice. Further work was conducted using only *Kurtzmaniella quercitrusa,* as others are found to be human pathogens. As discussed earlier, the sequence for the *K. quercetina* listed on the appendix and the accession number received from GenBank was PX106405.

The statistical analyses of the proximate analyses of the spontaneously fermented revealed that the parameters such as the ash, carbohydrate, fibre, and protein contents were significantly reduced when compared with the unfermented soursop juice, while the moisture had a significant increase in carbohydrate content in the spontaneously fermented soursop juice when compared with the unfermented soursop juice. This notable difference was seen mainly after the spontaneous fermentation for a 48-hour period when compared with unfermented soursop juice. This revealed that the metabolic activity of the microorganisms increased in the breakdown of the fruit sugar to produce organic acid.

The result proximate analysis of the fruit juice moisture content (82.27 ± 0.510), carbohydrate content (17.33 ± 0.940), protein content (5.08 ± 0.250), crude fat content(1.50 ± 0.001), Ash content(2.44 ± 0.001), crude fibre content(3.99 ± 0.001).The result from my research was in agreement with other authors' work on fermented soursop juice[1, 2].

Moreover, for statistical analyses of the controlled fermentation of soursop juice, the fermentation in this case was just for a 72-hour duration. The result followed a similar pattern to that of spontaneous fermentation. SC2 (co-cultured *Kurtzmaniella quertricusa* and *Leuconostoc mesenteroides*) had significantly reduced ash, and the carbohydrate content of the fermented soursop juice was reduced for SC2, while the fibre, moisture, and protein contents were raised slightly. SC5 (co-cultured *Leuconostoc mesenteroides* and *Kurtzmaniella quertricusa)* had the ash, moisture, and protein content lowered when compared to SC1, which is the unfermented soursop juice, and SC6 (Leuconostoc *mesenteroides*) had the protein, carbohydrate, and ash content decreased and the moisture and fibre increased slightly[1,2,3].

For vitamin B and vitamin C, the co-culture of yeast and LAB was statistically increased (0.379 ± 0.028 mg/100 mL, 3.43 ± 0.43 mg/100 mL) as shown in Table 4.6 when compared with the unfermented soursop juice SC1 (0.278 ± 0 mg/100 mL, 1.73 ± 0.15 mg/100 mL). Also, from the statistical analyses for vitamin B and vitamin C, SC2 had significant differences from SC1, SC5 and SC6[1].The result of the author is not inline with my result for SC2 increased but the authors' result for vitamin C reduced.

The vitamin B content was statistically increased too (0.279±0.0005 mg/100 mL) but not significantly different from SC1, as the value of $p > 0.05$. C6 was significantly different from SC1, as $p<0.05$ when compared with the result for vitamin C for unfermented soursop juice. Also, SC6 vitamin C content was statistically increased too (2.17 ± 0.11

mg/100 mL).The mineral content for the combined culture of yeast and LAB (SC2), as shown in Table 4.7, the calcium, was significantly increased (221.19 ± 0.40 mg/100 mL) in SC2, with a value of $p<0.05$. The calcium content of SC6 (218.45 ± 0.35 mg/100 mL) was significantly increased too when compared to the unfermented soursop juice (173.16 ± 0.17 mg/100 mL) and the value of $p<0.05$. The unfermented juice SC1 values for magnesium and zinc were higher, and they were significantly different when compared with SC2, SC5 and SC6, with a value of $p<0.05$.

The total soluble solid for SC1 was 13.5° Brix, SC2 was 5.5° Brix, SC5 was 10.0° Brix, and SC6 was 5.0° Brix, as shown in Table 4.10. These values listed were reduced during the fermentation of the soursop juice[1,2,3].The acetic acid content was significantly increased across the samples (SC2, SC5 and SC6) when compared with the unfermented soursop juice, as shown in Table 4.8. SC2-(2.675 ± 0.07 mg/100 mL), SC5-(1.45 ± 0 mg/100 mL), SC6 (1.225 ± 0.04 mg/100 mL) and SC1-(0.9 ± 0.05 mg/100 mL).

The pH content was reduced across the samples (SC1 – 4.84 ± 0.005, SC2 – 4.17 ± 0.0057, SC5 – 4.42 ± 0.005, SC6 – 4.54 ± 0.05) when compared with the unfermented soursop juice, as shown in Table 4.9[1,3].The sensory evaluation of the samples of fermented soursop juice, as shown in Table 4.11, found that the aroma of the samples was not significantly affected, as the $p > 0.05$ value across the samples, and the appearance and taste were significantly increased, as the $p < 0.05$ and the p-value < 0.05 for the appearance and taste of the controlled fermented juice, according to Kruskal-Wallis analyses. The pairwise comparison for the post hoc analyses, the value of $p<0.05$ for SC2 and SC6, has a higher mean score for appearance, so SC2 from the result was better. Also for taste, SC2 was better, also having a higher mean score. The hedonic scale appearance

score for SC2 was the highest and was 3.6; the taste score for SC2 was 4, and the aroma score for SC6 was 3.6 and the highest[4,5,6].

Endnotes

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6. T.H., Nguyen, N.C., Nguyen, T.T., Nguyen & V.H., Nguyen. *Research on the cocultured fermentation of Soursop (Annona Muricata L.) fruit juice by Lactobacillus plantarum and Saccharomyces bayanus strain*, **Journal of Food and Nutrition Research**, 12(10), 2024, 399–408, Available Online: https://doi.org/10.12691/jfnr-12-10-1.

Chapter Five

Conclusion

5.1 Summary of Findings

Lactic Acid Bacteria and Yeasts were isolated from spontaneously fermented soursop juice up till the 72nd hour fermentation duration. The obtained LAB were identified to species level as *Leuconostoc messenteroides* while the yeast isolates were *Candida tropicalis,*. *Kurtzmaniella quercitrusa* and *Lodderomyces elongisporus*. *Candida tropicalis* and *Lodderomyces elongisporus* were not used for further work because they are pathogens according to literature.

The LAB isolate used singly andin coculture with one of the yeast isolates were able to ferment the juice to generate a beverage that scored relative well with regard to its nutritional attributes and sensory acceptability.

5.2 Conclusion

In this study, a fermented beverage with relatively acceptable ethanol and lactic acid content, high nutritional attributes and relatively high acceptability was produced using LAB and yeast isolates in combination. With further researches, a functional food may be generated from this initial study.

5.3 Recommendations

1. The stability of the finished beverage's shelf life under various storage circumstances should be investigated in further detail.

2. Production techniques could be scaled up and studies on economy of production of this beverage using the starter could be carried out to enhancethe commercial viability of the project.

3. Recombinant DNA technology can be used to optimize the efficiency of the starter especially with profitability and economy of cost on mind.

4. Other bioactive substances like some volatile nutrient components, antioxidants and other phytochemicals may be evaluated in the beverage generated.

5. Market insights can expand consumer acceptance testing conducted across broader populations.

5.4 Contribution to Knowledge

1. By identifying and isolating bacteria from naturally fermenting soursop juice, the effort adds to the little-known indigenous microbiota in tropical fruit fermentations. Additionally, it describes functioning starters and offers fresh information about their potential for successful soursop fermentation.The data of the isolates from the project has been submiited into GeneBank and the ascension number for the isolates are as follows:

   2_*Leuconostoc mesenteroides*: PX106406

   4_*Leuconostoc mesenteroides*: PX106407

   8_*Leuconostoc mesenteroides*: PX106408

   G1_*Kurtzmaniella quercitrusa*: PX106405

2. This study standardizes traditional fermentation of soursop juice using specific starters, providing a controlled, standardized protocol for replication and scaling. It establishes a direct link between starter cultures and product quality, revealing why certain fermentations may yield superior results.
3. The acceptabilitly of the sensory evaluation data of this project, fermented soursop juice proves that it has the potential of becoming the healthy beverage option in

the market if further work is done on pilot scale to determine the profitability of commercialising it and other informations that needs to be confirmed.

5.5 Suggested Areas of Future Research

1. Studies on enhancing the fermentation process through the investigation of starter culture combinations, modification of fermentation parameters such as temperature, pH, and duration, and the incorporation of nutrient fortification to promote starter growth and generate advantageous compounds, while also examining the synergistic interactions of chosen starter cultures.
2. The research will encompass in-vitro and in-vivo investigations to substantiate the health advantages of fermented soursop juice, focussing on its probiotic effects, antioxidant characteristics, potential anti-inflammatory or antibacteria activity, and bioactive chemical analysis employing sophisticated methodologies.
3. Before fermented soursop juice can be commercialized, the ideal packaging and storage conditions must be determined. The research will investigate methods to maintain quality and microbial viability. New product formulations, such probiotic yoghurts and low-alcoholic drinks, can be made with soursop juice. It is possible to transform by-products like pulp or seeds into fiber or other beneficial materials.

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Appendices

Appendix 1: Nucleotide sequence of Obtained Isolates

>2_Leuconostoc mesenteroides PX106406

AACGcACAGCGAAAGGTGCTTGCACCTTTCAAGTGAGTGGCGaACGGGTGAGT
AACaCGTGGaCAaCCtGCcTCgAGGCTGGGGATAACATTTGGAAACAGAT
GCTaATACCGAATAAAACTTAGTGTCGCATGACACAAAGTTAAAAGGC
GCTTCGGCGTCACCTAGAGATGGATCCGCGGTGCATTAGTTAGTTGGT
GGGGTAAAGGCCTACCAAGACAATGATGCATAGCCGAGTTGAGAGAC
TGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGG
CTGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAAC
GCCGCGTGTGTGATGAAGGCTTTCGGGTCGTAAAGCACTGTTGTATGG
GAAGAACAGCTAGAATAGGAAATGATTTTAGTTTGACGGTACCATACC
AGAAAGGGACGGCTAAATACGTGCCAGCAGCCGCGGTAATACGTATG
TCCCGAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGACGGT
TTATTAAGTCTGATGTGAAAGCCCGGAGCTCAACTCCGGAATGGCATT
GGAAACTGGTTAACTTGAGTGCAGTAGAGGTAAGTGGAACTCCATGTG
TAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGC
GGCTTACTGGACTGCAACTGACGTTGAGGCTCGAAAGTGTGGGTAGCA
AACAGGATTAGATACCCTGGTAgTCCACACCGTAaACGATGAACACTA
GGTGTTAGGAGGTTTCCGCCTCTTAGTGCCGAAGCTAACGCATTAAGT

GTTCCGCCTGGGGAGTACGACCGCAGGTTGAAACTCAaAGGAtTTGACG
GGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCg
aAAGAACCTTACCAGGTCTTGACATCCTTTGAAGCTTTTAGAGATAGAA
GTGTTCTCTTCCGGAGaCAAAGTGACAGGTGTGCATGGTCGTCGTCAaC
TCaTGATCcgGAaAtTGTTGGATTAAGTCCCGCAaCGAGCGCaACcCTTAT
TGTTAGTTtTCAGCATttCAcATGCGAACTCTTAGCGAaAACTGCTGAgGA
aCTCGAGATGTgGACGACGTcCGaATCCATCctTCACCTATAtGTACTTTG
GGcCTcACMACATcGTGgCcAtTACACAT

>4_Leuconostoc mesenteroides PX106407

AGTCGAACGCACaGCGAAAGGTGCTTGCACCtTTCAAGTGAGTGGCGaACgGGT
GaGTAACaCGTGGaCAaCCtGCcTCgAGGCTGGGGATAACATTTGGAAaCa
GATGCTgATACCGAATAAAACTTAGTGTCGCATGACACAAAGTTAAAA
GGCGCTTCGGCGTCACCTAGAGATGGATCCGCGGTGCATTAGTTAGTT
GGTGGGGTAAAGGCCTACCAAGACAATGATGCATAGCCGAGTTGAGA
GACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCtACGGG
AGGCTGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCTGATGGAGC
AACGCCgCGTGTGTGATGAAGGCTTTCGGGTCGTAAAGCACTGTTGTAT
GGGAAgAACAgCTacAATAGGAAATGATTTTAGTTTGACGGTACCaTACC
aGAAAGGGACGGCTAAATACGTGCCAGCAGCCgCGGTAATACGTATGT
CCCGAGCGTTATCCGGATTTATTGGGCgtAaAGCGAGCGCAGACgGTTTA
TTAAGTCTGATGTGAAAGCCCGGAGCTCAACTCCGGAATGGCATTGGA
AACTGGTTAACTTGAGTGCAGtAGAGGtAAGTGGAACTCCATGTGTAGC
GGTGGAATGCGTAGATATATGGAAGAACACCAgTGGCGAAGGCGGCTT
ACTGGACTGCAACTGACGTTGAGGCTCGAAAGTGtGGGTAGCAAACAG
GATTAGATACCCTGGtagTCCACACCGTAAACGATGAACACTaGGTGTTA
GGAGGTTTCCGCCTCTtacTGCCGAAGCTAACGCATTAAGTGTTCCGCCT
GGGGAGTACGACCGCAAGGTTGAAACtCAaAGGAATTGACGGGGACCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCACGCGAAGAACCT
TACCAGTCTTGACATCCTTTGAGCTTTTAGAGATAGAgTgTTCTCTTCGG
AGACAAAGTGACAGTGGTGCATGTCGTCGTCAGCTCGTGcCGTgAaATG
TTGGGTAAGTCCCGCAACGAGCGCACCCTAtTGTTAGTTGCCcGCATCcG
AcGGCACTCTACCGaAACTGCCCGGACACGAAGAGgTGGAACGACcTCG
ATCATCccGCTCCTTtTtGACTCcGGCCTcTA

>8_Leuconostoc mesenteroides PX106408

CCATGCAAGTCGAACGCACAGTTTAAGGtGCTTGCACCCATAAGTGAGTGGCG
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TTTGGAAACaGATGCTAATACCGAATAAAACTTAGTGTCGCATGACAC
AAAGTTAAAAGGCGCTTCgGCGTCaCCTAcAGATGGATCCGCGGCGCAT
TAgtTAGcTGGcGGGGtAAaGGcCTACCAAGACAATGATGCATAGCCGAgT
TGAGAgACTGATCGGCCACATTGGGACTGAgACACGGtCCAAACTCCTA
CGGGAGGCTGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCTGATG
GAcCAACCCCGCgTGTGTGATGAAcGCTTTCGGGTCgTAAAGCACTGTT
GTATGGGAAcAACAtCTAGAATAGGAAATGATTTTATTTTGACGGcACC
ATACCAgAAAGGGACGGCTAAATACgTGCCACCAgCCGcgGtAATACgTA
TGTCCCGAcCGtTATCCGGATTTATTGGGCGCAAAGCgAGCGCATACGG
ACCATTAAGTCTGATGAGAAAGtCCGCACCTCAACTCCCGAATGGCCttG
GAAACTGGTTAACTTGtGTGTtTCCAAGTAAgTGGAACTCCATGTGTACC
GGTGGAATGCATACAaATATGGAaGAACACCAGTGGCGAAGGCGGCTT
ACTGGACTGCAACTGACCTTGAtGCTCGAAAAGAGGGTAGCA

>G1_Kurtzmaniella quercitrusa PX106405

TTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTTTGCCAGCGCTT
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CTTTGGTCTGGCGCAAGTTGGGCCAAAGGTTTATTAAACTTCAATTTTATATT
GAACTGTTATTTTAACTAAAGTCAATTTGTTGATTAAATTCAAAAATCTTCAA
AACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAT
TGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCATTTCT
CTCTCAAATCTTCGGATTTGGTTTTGAGTGATACTCTTAGTCGAACTAGGCGTT
TGCTTGAAAAGTATTGGCAAGAGTGGTACTTTAGGTGCTAAACTGTTTCAATG
TATTAGGTTTATCCAACTCGTTGAATCTGGTTAGTTACTTTAGGGTGCTTAGGC
TCGGCCTTACAACAACAAACAAAGTTTGACCTCAAATCAGGTAGGATTACCC
GCTGAACTTAAGCATATC

Laboratory Work,2025

Appendix 2: ANOVA For Unfermented Soursop and Spontaneously Fermented Soursop Juice at Different Fermentation Durations

Parameter	Effects	Df	Sum Sq	Mean Sq	F value	P value
Ash	Sample	5	0.00366667	0.00073333	0.74576271	0.61750147
Ash	Residuals	6	0.0059	0.00098333		
Carbohydrate	Sample	5	10.0079	2.00158	9.26550168	0.0086467
Carbohydrate	Residuals	6	1.29615	0.216025		
Moisture	Sample	5	48.0738667	9.61477333	55.7270479	6.0018E-05
Moisture	Residuals	6	1.0352	0.17253333		
Fat	Sample	5	0.43496667	0.08699333	1.59134146	0.29243174
Fat	Residuals	6	0.328	0.05466667		
Fibrep;	Sample	5	18.430875	3.686175	34.3432453	0.00024302
Fibre	Residuals	6	0.644	0.10733333		
Protein	Sample	5	2.01654167	0.40330833	29.456482	0.0003764
Protein	Residuals	6	0.08215	0.01369167		

Laboratory Work,2025

Appendix 3: ANOVA For Controlled Fermented Soursop Juice

Parameter	Effects	Df	Sum Sq	Mean Sq	F value	P value
Ash	Sample	3	0.012317	0.0041056 7	469.21904 8	1.504E-05
Ash	Residuals	4	3.5E-05	8.75E-06		
Carbohydrate	Sample	3	9.8173375	3.2724458 3	27.430277 6	0.0039661 5
Carbohydrate	Residuals	4	0.477202	0.1193005		
Fat	Sample	3	0.002282	0.0007606 7	0.6080469	0.6441414 2
Fat	Residuals	4	0.005004	0.001251		
Fibre	Sample	3	0.0002313 8	7.7125E-05	22.851851 9	0.0055929 2
Fibre	Residuals	4	0.0000135	3.375E-06		
Moisture	Sample	3	6.8702173 8	2.2900724 6	17.787229 6	0.0089027 5
Moisture	Residuals	4	0.5149925	0.1287481 2		
Protein	Sample	3	3.79231	1.2641033 3	1130.6827 7	2.6002E-06
Protein	Residuals	4	0.004472	0.001118		

Laboratory Work, 2025

Appendix 4: ANOVA For Mineral Content of the Controlled Fermented Soursop Juice

Parameter	Effects	Df	Sum Sq	Mean Sq	F value	P value
Calcium	Sample	3	6697.89672	2232.63224	21971.9514	5.3367E-16
Calcium	Residuals	8	0.81290267	0.10161283		
Magnessium	Sample	3	6227.37653	2075.79218	4833.20666	2.275E-13
Magnessium	Residuals	8	3.435884	0.4294855		
Zinc	Sample	3	0.01870292	0.00623431	3562.46032	7.701E-13
Zinc	Residuals	8	0.000014	0.00000175		

Laboratory Work, 2025

Appendix 5: ANOVA For the Acetic Acid Content of the Controlled Fermented Soursop Juice

Effects	Df	Sum Sq	Mean Sq	F value	P value
Sample	3	3.60625	1.20208333	274.761905	4.3658E-05
Residuals	4	0.0175	0.004375		

Laboratory Work, 2025

Appendix 6: ANOVA For pH Content of the Controlled Fermented Soursop Juice

Effects	Df	Sum Sq	Mean Sq	F value	P value
Samples	3	0.70795833	0.23598611	7079.58333	4.9457E-14
Residuals	8	0.00026667	3.3333E-05		

Laboratory Work, 2025

Appendix 7: Post Hoc For Carbohydrate Content of the Spontaneous Fermented Soursop Juice at Different Durations

Effects	Df	Sum Sq	Mean Sq	F value	P value
Carbohydrate	120hrs - 24hrs	-1.375	0.0253	-2.51228759	-0.23771241
	120hrs - 48hrs	1.365	0.0331	0.15548384	2.57451616
	120hrs - 72hrs	0.76	0.1651	-0.41871164	1.93871164
	120hrs - 96hrs	1.195	0.0506	-0.00423146	2.39423146
	120hrs - Unf	0.425	0.3958	-0.71228759	1.56228759
	24hrs - 48hrs	2.74	0.0016	1.52593885	3.95406115
	24hrs - 72hrs	2.135	0.0049	0.93576854	3.33423146
	24hrs - 96hrs	2.57	0.0021	1.36048384	3.77951616
	24hrs - Unf	1.8	0.0097	0.62128836	2.97871164
	48hrs - 72hrs	-0.605	0.2547	-1.78371164	0.57371164
	48hrs - 96hrs	-0.17	0.7271	-1.30728759	0.96728759
	48hrs - Unf	-0.94	0.1027	-2.13923146	0.25923146
	72hrs - 96hrs	0.435	0.3854	-0.70228759	1.57228759
	72hrs - Unf	-0.335	0.4982	-	0.80228759

	9/6hrs - Unf	-0.77	0.1605	1.47228759 - 1.94871164	0.40871164

Key:Unf- Unfermented Soursop Juice

Laboratory Work,2025

Appendix 8: Post Hoc For Moisture Content of the Spontaneous Fermented Soursop Juice at Different Durations

Traits	Sample	Difference	Pvalue	LCL	UCL
Moisture	120hrs - 24hrs	3.48	0.0002	2.46362307	4.49637693
	120hrs - 48hrs	-0.26	0.5663	- 1.31339699	0.79339699
	120hrs - 72hrs	-0.17	0.6965	- 1.18637693	0.84637693
	120hrs - 96hrs	-0.69	0.1643	- 1.76173525	0.38173525
	120hrs - Unf	4.34	0.0001	3.28660301	5.39339699
	24hrs - 48hrs	-3.74	0.0001	- 4.81173525	- 2.66826475
	24hrs - 72hrs	-3.65	0.0001	- 4.70339699	- 2.59660301
	24hrs - 96hrs	-4.17	0.0001	- 5.25092653	- 3.08907347
	24hrs - Unf	0.86	0.0838	- 0.15637693	1.87637693
	48hrs - 72hrs	0.09	0.8356	- 0.92637693	1.10637693
	48hrs - 96hrs	-0.43	0.3405	- 1.44637693	0.58637693
	48hrs - Unf	4.6	0	3.51907347	5.68092653
	72hrs - 96hrs	-0.52	0.2713	- 1.57339699	0.53339699
	72hrs - Unf	4.51	0.0001	3.43826475	5.58173525

96hrs - Unf	5.03	0	3.94501168	6.11498832

Key:Unf- Unfermented Soursop Juice

Laboratory Work,2025

Appendix 9: Post Hoc For Fibre Content of the Spontaneous Fermented Soursop Juice at Different Durations

Traits	Sample	Difference	Pvalue	LCL	UCL
Fibre	120hrs - 24hrs	-0.615	0.1096	-1.41665148	0.18665148
	120hrs - 48hrs	0.09	0.7928	-0.71165148	0.89165148
	120hrs - 72hrs	0.4	0.2869	-0.44531449	1.24531449
	120hrs - 96hrs	0.265	0.4631	-0.56585048	1.09585048
	120hrs - Unf	-3.185	0.0001	-4.01585048	-2.35414952
	24hrs - 48hrs	0.705	0.083	-0.12585048	1.53585048
	24hrs - 72hrs	1.015	0.0272	0.16243603	1.86756397
	24hrs - 96hrs	0.88	0.0437	0.03468551	1.72531449
	24hrs - Unf	-2.57	0.0002	-3.37165148	-1.76834852
	48hrs - 72hrs	0.31	0.3948	-0.52085048	1.14085048
	48hrs - 96hrs	0.175	0.6124	-0.62665148	0.97665148
	48hrs - Unf	-3.275	0.0001	-4.12031449	-2.42968551
	72hrs -	-0.135	0.6946	-	0.66665148

96hrs			0.93665148	
72hrs - Unf	-3.585	0.0001	-4.44076765	-2.72923235
96hrs - Unf	-3.45	0.0001	-4.30256397	-2.59743603

Key:Unf- Unfermented Soursop Juice

Laboratory Work,2025

Appendix 10: ANOVA For Vitamin Contents of the Controlled Fermented Soursop Juice

Parameter	Effects	Df	Sum Sq	Mean Sq	F value	P value
VIT.B1	Sample	3	0.02296092	0.00765364	38.752602	4.1179E-05
VIT.B1	Residuals	8	0.00158	0.0001975		
VIT.C	Sample	3	7.5103	2.50343333	49.5729373	1.6365E-05
VIT.C	Residuals	8	0.404	0.0505		

Key: Vit.B1- Vitamin B1

Vit. C – Vitamin C

Laboratory Work,2025

Appendix 11: Post Hoc For Protein Content of the Spontaneous Fermented Soursop Juice at Different Durations

Traits	Sample	Difference	Pvalue	LCL	UCL
Protein Content	120hrs - 24hrs	-0.94	0.0003	-1.24450037	-0.63549963
	120hrs - 48hrs	-0.655	0.0019	-0.95691116	-0.35308884
	120hrs - 72hrs	-0.545	0.0042	-0.84174521	-0.24825479
	120hrs - 96hrs	-0.415	0.0121	-0.70131655	-0.12868345
	120hrs - Unf	-1.31	0	-1.61564459	-1.00435541
	24hrs - 48hrs	0.285	0.0508	-0.00131655	0.57131655
	24hrs - 72hrs	0.395	0.0174	0.09825479	0.69174521
	24hrs - 96hrs	0.525	0.0055	0.22308884	0.82691116
	24hrs - Unf	-0.37	0.0195	-0.65631655	-0.08368345
	48hrs - 72hrs	0.11	0.3835	-0.17631655	0.39631655
	48hrs - 96hrs	0.24	0.095	-0.05674521	0.53674521
	48hrs - Unf	-0.655	0.0017	-0.95174521	-0.35825479
	72hrs - 96hrs	0.13	0.3091	-0.15631655	0.41631655
	72hrs - Unf	-0.765	0.0008	-1.06691116	-0.46308884
	96hrs - Unf	-0.895	0.0004	-	-

					1.19950037	0.59049963

Key:Unf- Unfermented Soursop Juice

Laboratory Work,2025

Appendix 12: Post Hoc For Ash Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L.mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Ash Content	Sample		Difference	Pvalue	LCL	UCL
Ash	SC1	- SC2	0.0985	0	0.09006418	0.10693582
Ash	SC1	- SC5	0.01	0.0278	0.00178713	0.01821287
Ash	SC1	- SC6	0.0555	0.0001	0.04710717	0.06389283
Ash	SC2	- SC5	-0.0885	0	-0.09689283	-0.08010717
Ash	SC2	- SC6	-0.043	0.0001	-0.05121287	-0.03478713
Ash	SC5	- SC6	0.0455	0.0001	0.03728713	0.05371287

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Laboratory Work,2025

Appendix 13: Post Hoc For Carbonhydrate Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L.mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Carbonhydrate Content	Sample		Difference	Pvalue	LCL	UCL
Carbohydrate	SC1 SC2	-	1.309	0.0208	0.32900078	2.28899922
Carbohydrate	SC1 SC5	-	-1.804	0.0064	-2.76298565	-0.84501435
Carbohydrate	SC1 SC6	-	0.008	0.9826	-0.95098565	0.96698565
Carbohydrate	SC2 SC5	-	-3.113	0.001	-4.09801853	-2.12798147
Carbohydrate	SC2 SC6	-	-1.301	0.0197	-2.25998565	-0.34201435
Carbohydrate	SC5 SC6	-	1.812	0.0069	0.83200078	2.79199922

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 14: Post Hoc For Fibre Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L. mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Fibre Content	Sample		Difference	Pvalue	LCL	UCL
Fibre	SC1	-	-0.003	0.1778	-0.00810068	0.00210068
	SC2					
Fibre	SC1	-	-0.01	0.0061	-0.01521245	-0.00478755
	SC5					
Fibre	SC1	-	-0.0135	0.0021	-0.01873914	-0.00826086
	SC6					
Fibre	SC2	-	-0.007	0.0189	-0.01210068	-0.00189932
	SC5					
Fibre	SC2	-	-0.0105	0.0051	-0.01571245	-0.00528755
	SC6					
Fibre	SC5	-	-0.0035	0.1295	-0.00860068	0.00160068
	SC6					

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 15: Post Hoc For Moisture Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L.mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Moisture Content	Sample		Difference	Pvalue	LCL	UCL
Moisture	SC1 SC2	-	-1.0205	0.0467	- 2.01673416	- 0.02426584
Moisture	SC1 SC5	-	0.935	0.0597	- 0.06123416	1.93123416
Moisture	SC1 SC6	-	-1.453	0.0168	- 2.47106394	- 0.43493606
Moisture	SC2 SC5	-	1.9555	0.006	0.93743606	2.97356394
Moisture	SC2 SC6	-	-0.4325	0.2945	- 1.42873416	0.56373416
Moisture	SC5 SC6	-	-2.388	0.003	- 3.41127821	- 1.36472179

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 16: Post Hoc For Protein Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L.mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Protein Content	Sample		Difference	Pvalue	LCL	UCL
Protein	SC1 SC2	-	-0.394	0.0003	-0.48683503	-0.30116497
Protein	SC1 SC5	-	0.832	0	0.73916497	0.92483503
Protein	SC1 SC6	-	1.364	0	1.26913074	1.45886926
Protein	SC2 SC5	-	1.226	0	1.13113074	1.32086926
Protein	SC2 SC6	-	1.758	0	1.66264485	1.85335515
Protein	SC5 SC6	-	0.532	0.0001	0.43916497	0.62483503

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 17: Post Hoc For Vitamin Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L. mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Traits	Sample		Difference	Pvalue	LCL	UCL
Vitamin B1	SC1 SC2	-	- 0.10066667	0	-0.128241	- 0.07309233
Vitamin B1	SC1 SC5	-	- 0.00066667	0.9551	- 0.02712716	0.02579383
Vitamin B1	SC1 SC6	-	0.00166667	0.8881	- 0.02479383	0.02812716
Vitamin B1	SC2 SC5	-	0.1	0	0.0735395	0.1264605
Vitamin B1	SC2 SC6	-	0.10233333	0	0.07413642	0.13053025
Vitamin B1	SC5 SC6	-	0.00233333	0.8501	-0.025241	0.02990767
Vitamin C	SC1 SC2	-	-1.7	0	- 2.14092753	- 1.25907247
Vitamin C	SC1 SC5	-	0.41	0.0559	- 0.01311668	0.83311668
Vitamin C	SC1 SC6	-	- 0.43666667	0.0446	- 0.85978334	- 0.01354999
Vitamin C	SC2 SC5	-	2.11	0	1.65911716	2.56088284
Vitamin C	SC2 SC6	-	1.26333333	0.0001	0.84021666	1.68645001
Vitamin C	SC5 SC6	-	- 0.84666667	0.0022	-1.2875942	- 0.40573913

key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 18: Post Hoc For Mineral Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L. mesenteroides* (PX106406) and *Kurtzmaniella quertricusa* (PX106406)

Traits	Sample		Difference	Pvalue	LCL	UCL
Calcium	SC1	-	-	0	-	-47.397212
	SC2		48.0226667		48.6481213	
Calcium	SC1	-	1.101	0.0029	0.50081003	1.70118997
	SC5					
Calcium	SC1	-	-	0	-	-
	SC6		45.2856667		45.8858566	44.6854767
Calcium	SC2	-	49.1236667	0	48.4840905	49.7632429
	SC5					
Calcium	SC2	-	2.737	0	2.13681003	3.33718997
	SC6					
Calcium	SC5	-	-	0	-	-45.761212
	SC6		46.3866667		47.0121213	
Magnessium	SC1	-	48.9013333	0	47.5864347	50.2162319
	SC2					
Magnessium	SC1	-	6.98566667	0	5.75174183	8.21959151
	SC5					
Magnessium	SC1	-	48.6683333	0	47.3824671	49.9541995
	SC6					
Magnessium	SC2	-	-	0	-	-
	SC5		41.9156667		43.2015329	40.6298005
Magnessium	SC2	-	-0.233	0.6748	-	1.00092484
	SC6				1.46692484	
Magnessium	SC5	-	41.6826667	0	40.4487418	42.9165915
	SC6					
Zinc	SC1	-	0.08466667	0	0.08201245	0.08732089
	SC2					
Zinc	SC1	-	0.012	0	0.00950923	0.01449077
	SC5					
Zinc	SC1	-	0.08433333	0	0.08173772	0.08692895
	SC6					
Zinc	SC2	-	-	0	-	-
	SC5		0.07266667		0.07526228	0.07007105
Zinc	SC2	-	-	0.7655	-0.0028241	0.00215744
	SC6		0.00033333			
Zinc	SC5	-	0.07233333	0	0.06984256	0.0748241
	SC6					

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

Appendix 19: Post Hoc For Acetic Acid Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L. mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Sample		Difference	Pvalue	LCL	UCL
SC1	-	-1.775	0	-	-
SC2				1.96363067	1.58636933
SC1	-	-0.55	0.0013	-	-
SC5				0.73766947	0.36233053
SC1	-	-0.325	0.008	-	-
SC6				0.50864538	0.14135462
SC2	-	1.225	0.0001	1.04135462	1.40864538
SC5					
SC2	-	1.45	0	1.26233053	1.63766947
SC6					
SC5	-	0.225	0.0272	0.04135462	0.40864538
SC6					

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 20: Post Hoc For pH Content of the Controlled Fermented Soursop Juice with Mono and Coculture of *L. mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Effect		Difference	Pvalue	LCL	UCL
SC1 SC2	-	0.67666667	0	0.6650827	0.68825064
SC1 SC5	-	0.42	0	0.4086718	0.4313282
SC1 SC6	-	0.30666667	0	0.29579606	0.31753727
SC2 SC5	-	- 0.25666667	0	- 0.26753727	- 0.24579606
SC2 SC6	-	-0.37	0	-0.3813282	-0.3586718
SC5 SC6	-	- 0.11333333	0	- 0.12420394	- 0.10246273

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 21: Summary of Sensory Evaluation Result For Controlled Fermented Soursop Juice with Mono and Coculture of *L. mesenteroides* (PX106406) **and *Kurtzmaniella quertricusa*** (PX106406)

Parameters	Sample	Mean	Min	Max
Appearance	SC2	3.6	3	4
Appearance	SC5	3.1	3	4
Appearance	SC6	2.4	2	3
Aroma	SC2	3.4	2	5
Aroma	SC5	3.3	3	4
Aroma	SC6	3.6	3	4
Taste	SC2	4	2	5
Taste	SC5	2.9	2	4
Taste	SC6	2.1	2	3

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 22: Kruskal-Wallis Result For Sensory Evaluation of the Controlled Fermented Soursop Juice with Mono and Coculture of *L. mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Parameters	**N**	**statistic**	**df**	**P**
Appearance	30	16.1876254	2	0.000305
Aroma	30	1.07617188	2	0.584
Taste	30	17.7673333	2	0.000139

Source: Laboratory Work,2025

Appendix 23: Post Hoc for the Sensory Evaluation of the Controlled Fermented Soursop Juice with Mono and Coculture of *L. mesenteroides* (PX106406**) and *Kurtzmaniella quertricusa* (**PX106406**)**

Parameters	group1	group2	statistic	P value
Appearance	SC2	SC5	-1.70578398	0.08804831
Appearance	SC2	SC6	-4.00859236	0.00018325
Appearance	SC5	SC6	-2.30280838	0.04257925
Aroma	SC2	SC5	-0.41221581	1
Aroma	SC2	SC6	0.61832372	1
Aroma	SC5	SC6	1.03053953	0.90827042
Taste	SC2	SC5	-2.04707108	0.06039548
Taste	SC2	SC6	-4.2145581	7.508E-05
Taste	SC5	SC6	-2.16748702	0.06039548

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 24: Mean, Standard Deviation and Standard Error of Proximate Analysis of Controlled Fermented Soursop Juice

Traits	Sample	Mean	std	Se
Ash	SC1	0.3175	0.00353553	0.00209165
Ash	SC2	0.219	0.00141421	0.00209165
Ash	SC5	0.3075	0.00353553	0.00209165
Ash	SC6	0.262	0.00282843	0.00209165
Carbohydrate	SC1	15.907	0	0.24423401
Carbohydrate	SC2	14.598	0.15839192	0.24423401
Carbohydrate	SC5	17.711	0.17536248	0.24423401
Carbohydrate	SC6	15.899	0.64912403	0.24423401
Fat	SC1	0.05	0.05656854	0.02501
Fat	SC2	0.04	0.04242641	0.02501
Fat	SC5	0.013	0.00141421	0.02501
Fat	SC6	0.011	0.00141421	0.02501
Fibre	SC1	0.0155	0.00070711	0.00129904
Fibre	SC2	0.0185	0.00212132	0.00129904
Fibre	SC5	0.0255	0.00070711	0.00129904
Fibre	SC6	0.029	0.00282843	0.00129904
Moisture	SC1	72.042	0.0834386	0.25372044
Moisture	SC2	73.0625	0.08131728	0.25372044
Moisture	SC5	71.107	0.145664	0.25372044
Moisture	SC6	73.495	0.69296465	0.25372044
Protein	SC1	11.668	0.0311127	0.02364318
Protein	SC2	12.062	0.0311127	0.02364318
Protein	SC5	10.836	0.0311127	0.02364318
Protein	SC6	10.304	0.03959798	0.02364318

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 25: Mean, Standard Deviation and Standard Error of Vitamin Content of Controlled Fermented Soursop Juice

Traits	Sample	Mean	std	se
Vitamin B1	SC1	0.278	0	0.00811377
Vitamin B1	SC2	0.37866667	0.02809508	0.00811377
Vitamin B1	SC5	0.27866667	0.00057735	0.00811377
Vitamin B1	SC6	0.27633333	0.00057735	0.00811377
Vitamin C	SC1	1.73333333	0.15275252	0.12974334
Vitamin C	SC2	3.43333333	0.40414519	0.12974334
Vitamin C	SC5	1.32333333	0.06429101	0.12974334
Vitamin C	SC6	2.17	0.10583005	0.12974334

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 26: Mean, Standard Deviation and Standard Error of Mineral Content of Controlled Fermented Soursop Juice

Traits	Sample	Mean	std	Se
Calcium	SC1	173.169667	0.17435978	0.18404061
Calcium	SC2	221.192333	0.40299049	0.18404061
Calcium	SC5	172.068667	0.30250014	0.18404061
Calcium	SC6	218.455333	0.34948867	0.18404061
Magnessium	SC1	382.229667	0.30150014	0.37836733
Magnessium	SC2	333.328333	0.69850006	0.37836733
Magnessium	SC5	375.244	0.699	0.37836733
Magnessium	SC6	333.561333	0.80655833	0.37836733
Zinc	SC1	0.66066667	0.00152753	0.00076376
Zinc	SC2	0.576	0.001	0.00076376
Zinc	SC5	0.64866667	0.00152753	0.00076376
Zinc	SC6	0.57633333	0.0011547	0.00076376

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 27: Mean, Standard Deviation and Standard Error of Acetic Acid of Controlled Fermented Soursop Juice

Sample	Mean	std	se
SC1	0.9	0.07071068	0.04677072
SC2	2.675	0.10606602	0.04677072
SC5	1.45	0	0.04677072
SC6	1.225	0.03535534	0.04677072

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work,2025

Appendix 28: Mean, Standard Deviation and Standard Error of pH Content of Controlled Fermented Soursop Juice

Sample	Mean	std	se
SC1	4.84333333	0.0057735	0.00333333
SC2	4.16666667	0.0057735	0.00333333
SC5	4.42333333	0.0057735	0.00333333
SC6	4.53666667	0.0057735	0.00333333

Key: SC1- Unfermented Soursop Juice

SC2- Coculture *Kurtzmaniella quercitrusa* 48 hours with *Leuconostoc mesenteroides* for 24 hours.

SC5- Coculture *Leuconostoc mesenteroides* for 48 hours and with *Kurtzmaniella quercitrusa* for 24 hours.

SC6- Coculture *Leuconostoc mesenteroides* for 24 hours only.

Source: Laboratory Work, 2025

Appenndix 29: Questionnaire on Fermented Soursop Juice

This study is for research purposes only. Therefore, it will be held confidentially. You are served with different samples of fermented soursop to evaluate for attributes such as aroma (pleasant or unpleasant, taste (sourness), appearance (bright and dull cream) and overall acceptability.

Assess the attributes of the samples with respect to your preference for fermented beverages.

Your utmost sincerity in filling out the questionnaire will be highly appreciated. Thank you.

S/N	Sample Names	Panelist ID	Taste Score	Aroma Score	Appearance Score	Overall Acceptability Score
	SC2	I				4
		II				4
		III				4
		IV				4
		V				5
		VI				3
		VII				3
		VIII				4
		IX				4
		X				4
	SC5	I				2
		II				3
		III				3
		IV				2
		V				2
		VI				3
		VII				3
		VIII				3
		IX				3
		X				4
	SC6	I				3
		II				2
		III				2
		IV				2
		V				2
		VI				2
		VII				3
		VIII				3
		IX				2
		X				2

Identification code:

Date:

This is how they made their table on the questionnaire:

Attributes	Sample Code	Score	Overall Comment
Taste			
Aroma			
Appearance			
Overall Score			

Please submit after scoring. Thanks for your cooperation.

Table ---: Overall Acceptability of the Fermented Soursop samples

Author's Field Work, 2025

Biodata

A. Personal Data

1.	Full Names:	**Olatunji AYODELE**
2.	Email	ayo19602003@yahoo.com
3.	Phone no:	07065173248
4.	Address:	2, Roland Omoregbe Street Lagos State
5.	Date and Place of Birth:	8th, Jan.1981.
6.	Nationality:	Nigerian
7.	Name and Address of Next of Kin:	Temitope Olabimpe. No2, Bolaji Street, Adejumo Ibadan.

B. Educational Background with Dates

1. Educational Institutions Attended with Dates and Qualifications

- Lead City University Ibadan, Oyo State 2023
- Olabisi Onabanjo University Ago Iwoye. 2008
- Angus Memorial High School, Yaba Lagos 2002
- Igbobi Primary School, Yaba Lagos. 1992

2. Academic Qualifications Obtained (with dates):

- M.Sc Food Microbiology in view

D. Award and Fellowship (if any): Nil

E. Member of Academic Professional Bodies: Nil

F. Publication (s): Isolation and Antimicrobial Effect of Four Medicinal Plants on Urine Pathogens (Undergraduate Dissertation)

H. References

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Signature **Date**

University Compliance Certificate

This is to certify that the Thesis was written by Olatunji Abiodun AYODELE, with the matriculation number: **LCU/PG/003857** in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Nigeria is in full compliance with the approval format and style.

…… ……..……

Signature **Date**