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PRODUCTION OF WINE FROM BANANA AND SWEET ORANGE

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CERTIFICATION

This is certify that this project is the original carried out and reported by **ND/23/SLT/PT/0144** to the Department Of Science Laboratory Technology, Institute Of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been approved in partial fulfillment of the requirement of the requirements of the award of national diploma (ND) in science laboratory technology (Environmental Biology)

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ABSTRACT

This study focuses on the production of wine from banana (Musa sapientum) and sweet orange (Citrus sinensis) using Saccharomyces cerevisiae as the fermenting agent. The objective was to develop a novel fruit wine by leveraging the high sugar content and nutritional profile of these tropical fruits to create a marketable, value-added product that reduces post-harvest losses. Juice was extracted from ripe bananas and sweet oranges, mixed with distilled water (1:1 w/v), and supplemented with sucrose to enhance fermentable sugars. The must was sterilized using sodium metabisulphite and inoculated with Saccharomyces cerevisiae for primary (aerobic) and secondary (anaerobic) fermentation over 10 and 14 days, respectively. Bentonite was added for clarification, followed by racking to remove sediments. Physicochemical properties, including pH, titratable acidity, total reducing sugar, and alcohol content, were analyzed. The banana wine exhibited a pH of approximately 3.6–3.8, titratable acidity of 2.6–2.9 g/100ml, and reducing sugar of 0.4–0.7 g/100ml, while the orange wine showed a pH of around 4.0, titratable acidity of 2.11–2.13 g/100ml, and reducing sugar of 0.6–0.8 g/100ml. The alcohol content ranged from 8–14% v/v. Sensory evaluation by a panel of testers confirmed the wines' acceptability in terms of flavor, clarity, and overall quality. This research demonstrates the potential of banana and sweet orange as viable substrates for wine production, offering economic and environmental benefits by utilizing surplus fruits.

CHAPTER ONE

INTRODUCTION

Wine production has a rich history spanning millennia, with grapes traditionally serving as the primary source of fermentation. However, the exploration of alternative fruits for winemaking has sparked innovation in recent years. Among these alternatives, bananas and sweet oranges have emerged as intriguing candidates due to their abundant availability and unique flavor profiles.

Banana and sweet orange wine production represents a departure from the conventional grape based approach, offering a distinctive sensory experience for enthusiasts and connoisseurs alike. This introduction aims to provide an overview of the processes involved in crafting wine from these unconventional fruits, drawing upon both traditional winemaking principles and innovative techniques.

In recent years, a growing body of literature has explored the feasibility and potential of banana and sweet orange wines. Studies such as those by Smith et al. (2018) and Garcia-Benitez et al. (2020) have investigated various aspects of fruit selection, fermentation methods, and sensory attributes, shedding light on the intricate dynamics of non-grape winemaking. Furthermore, consumer trends and preferences have evolved, with an increasing demand for novel and exotic beverages. Banana and sweet orange wines, with their vibrant flavors and aromatic profiles, have captured the attention of both casual drinkers and discerning wine enthusiasts, presenting an exciting avenue for exploration within the beverage industry.

Bananas belong to the family Musaceae and genus Musa. Musa spp. already provided man with food, tools and shelter prior to recorded history. Bananas are major crops of West and East Africa and are grown in some 120 countries throughout the developing world. World banana production, according to available statistics, was 80.6 million tons per annum in the early 1990s (Food and Agriculture Organization, 1994), with Africa producing about 30 million tons per annum (Food and Agriculture Organization, 1996). According to the latest FAO statistical records as reported by the International Institute of Tropical Agriculture (IITA, 2003), more than 58 million tons of bananas and 30 million tons of plantains were produced worldwide in 2000.

India is the largest banana producer with an output of 16 million tons per annum and Uganda ranks second to it producing 12 million tons per annum (Sunday Monitor, 2007). South Africa produces

300 000 tons of bananas per annum (De Beer, 2004).

Banana is the fourth most important crop after rice, wheat and maize and international trade in bananas is valued at around US$5 billion per annum (Sunday Monitor, 2007). Traditional banana juice extraction and its subsequent fermentation to produce beer (tonto) is an important social and economic activity among many tribes in East Africa (Stover and Simmonds, 1987; Davies, 1993). Likimani (1991) reported that tonto is a popular traditional beverage in Burundi, Uganda and Rwanda. Banana juice and beer may however contain suspended solids which are not desired by some consumers. Therefore, efforts are made to reduce suspended solids by applying different processing technologies such as the addition of enzymes to pulps. Although bananas have been traditionally dietary staples in many countries in Tropical Africa, they have until recently been relatively neglected by most policy makers and research institutions partly due to high post-harvest losses coupled with difficulty in marketing and processing of these highly perishable commodities (Olorunda, 2000). Generally, the most recent estimates of losses of cooking bananas and plantain differ in different countries and were 0-10% for Kampala in Uganda (Digger, 1994) and as high as 35% for the Ivory Coast (N'Guessan, 1991).

Biotechnology and other related technologies such as genetic engineering have played a significant role in food processing technologies. Enzymes play a pivotal role in the winemaking process. In addition to enzymes that occur in pre-and post fermentation operations, there are many more different enzymes driving the fermentation kinetics that convert grape juice to wine. Commercial enzyme preparations are widely used as supplements since the endogenous enzymes from yeasts and other micro organisms present in must and wine are often neither efficient nor sufficient under winemaking conditions to efficiently catalyse the various biotransformation reactions (for a detailed review on enzymes in winemaking, see Van Rensburg and Pretorius, 2000). Pectolytic enzyme preparations have been used with great success for many years in the fields of food technology (Ough and Berg, 1974). In wine and fruit juices, these enzyme preparations are mainly used to yield more juice and increase the press capacity (Wörner and et al., 1998). The use of pectolytic enzyme preparations has been reported to affect sensory quality, since these preparations often also contain other enzyme activities (for example,

cinnamylesterase, glucosidase, oxidase) that can have a negative effect on wine (Lao et al., 1997). The best wines are produced when the desired enzymatic activities are optimally reinforced and the negative effects restricted to a minimal level (Van Rensburg and Pretorius, 2000).

Banana (Musa Sapientum) is a fruit common in the tropics and is non-seasonal. It is readily available in Nigeria. Due to its high sugar content, it is suitable for the production of wine (Robinson, 2006). Depending upon cultivar and ripeness, the flesh can vary in taste from starchy to sweet and texture from firm to mushy. Both skin and inner part can be eaten raw or cooked. Bananas' flavor is due, amongst other chemicals, to isoamyl acetate which is one of the main constituents of banana oil. Wine is an alcoholic beverage typically made from fermented fruit juice. Any fruit with a good proportion of sugar may be used for wine production and the resulting wines are normally named after the fruit hence banana, apple, orange, pineapple, strawberries and coconut may be used to produce wine. The type of fruit wine to be produced dictates the fruit and strain of yeast to be involved (Alexander and Charpenter, 2004). Wine production has not been a major market in Nigeria although institutions such as NIFOR (Nigerian Institute for oil palm research) have been involved in production of bottled palm wine using chemical preservatives. Bioaccumulation of chemical preservatives poses potential dangers due to either toxicity or pro-toxicity (Idise and Izuagbe, 1988; Svans, 2008). It is thus pertinent to search for means of producing wines devoid of chemical additives. Banana possesses desirable qualities – high fiber-content which helps restore normal bowl action, stimulates the production of hemoglobin in the blood, contains potassium and has a low salt content which helps to lower blood pressure as well as control stroke and when consumed along with other fruits and vegetables, banana was observed to be associated with reduced risk of colorectal cancer (Deneo Pellegrini et al., 1996) and in women, breast cancer (Zhang, 2009) and renal cell carcinoma (Rashidkhani et al., 2005).

An orange specifically, the sweet orange is the citrus (*Citrus sinensis*) and its fruit. It is the most commonly grown tree fruit in the world. The orange is a hybrid of ancient cultivated origin, possibly between pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*). It is an evergreen flowering tree generally growing to 9–10 m in height (although very old specimens have reached 15 m). The leaves are arranged alternately, are ovate in shape with crenulate margins and are 4–10 cm long. The orange fruit is a hesperidium, a type of berry (Ramachandra and Arun, 2005).

Orange trees are widely cultivated in tropical and subtropical climates for the sweet fruit, which is peeled or cut (to avoid the bitter rind) and eaten whole, or processed to extract orange juice, and also for the fragrant peel. In 2008, 68.5 million tons of oranges were grown worldwide, primarily in Brazil and the US states California and Florida (Idise, 2012). The fruit of *Citrus sinensis* is called sweet orange to distinguish it from *Citrus aurantium*, the bitter orange. The name is thought to derive ultimately from the Sanskrit for the orange tree, with its final form developing after passing through numerous intermediate languages. In a number of languages, it is known as a "Chinese apple", e.g., Dutch sinaasappel ("China's apple") or appelsien, or northern German Apfelsine. In English, however, "Chinese apple" generally refers to the pomegranate (Idise, 2012)

Plate 1: Orange blossoms and oranges on tree

Among the greatest and genius invention of man is the incessant production of vivacious and edible alcoholic beverages from fermentation of natural fruit juice such as grape in wine - making. Wine has been part of human culture for over 6,000 years, serving dietary and socio religious functions. Its production takes place on every continent and has been enjoyed by many people from peasants to kings and its chemical composition is profoundly influenced by

enological techniques **(**Nikhil *et al.,* 2009).It is produced by fermentation of juice of ripe grapes using a microscopic single-celled organism called yeasts (*Saccharomyces cerevisae),* digest sugars found in fruit juice, producing alcohol and carbon dioxide gas in the process. Other naturally occurring microorganisms may grow in the must or juice, affecting the flavors and aromas of the finished wine. For example, lactic acid bacteria use the acids in wine as a source of energy, reducing the wine's acidity. These bacteria also produce other aromas and are responsible for the buttery smells that can be found in wine. Sometimes the wine maker restricts the growth of lactic acid bacteria, especially if the wine is already low in acidity or if the buttery character would clash with other aromas of the wine. *Acetobacter,* another type of bacteria, can spoil the wine by converting ethanol to acetic acid to make vinegar. Wine naturally contains about 79 to 89 percent water, 9 to 21 percent alcohol, less than 1 percent fruit acids, and hundreds of aroma and flavor components in very small amounts. Wines are categorized using a number of different methods. Sometimes they are grouped into different categories by grape variety, region of origin, by color, by the name of the wine maker or viticulturist, or by production technique (Bisson *et al.,* 2009).There are two types of wines namely Natural wines with 9-14% alcohol and Fortified (Dessert and appetizer) wines with 15 to 21% alcohol (Nduka, 2007). The nutritional role of wine is important since its average contribution to total energy intake is estimated to be 10 to20% in adult males (Macrae *et al.*, 1993). Although grapes are the most common fruit used to make wine for the past few decades. In lieu of this several studies have investigated the suitability of other fruits as substrates for the purpose of wine production (Okunowo *et al.*, 2005). Moreover, the non-availability of grapes, which is usually the fruit of choice for wine production in the tropics has necessitated the search for alternative fruit source in tropical countries; Nigeria (Alobo and Offonry, 2009). Wine is also made from the fermented juice of pears, apples, berries, blackcurrants and even flowers such as dandelions are sometimes used. However, are usually referred to as fruits or country wine (Bisson *et al.*, 2009). Any fruit with good proportion of sugar may be used in producing wine and the resultant wine is normally named after the fruit. The type of wine to be produced dictates the fruit and strain of yeast to be involved (Amerine and Kunkee, 2005). In contrast to most foods and beverages that spoil quickly or that can spread diseases, wine does not spoil if stored properly. This implies that the production of wine from fruits is a form of extending the shelf life of the fruit hence, preservation.

Wine composition and quality are functions of many different intrinsic and extrinsic variables, many of which are microbiologically mediated. A large diversity of microbes are inherent to winemaking including various yeasts, bacteria and fungi. Prominent in this process are *Saccharomyces* species (predominantly *Saccharomyces cerevisiae*), which dominate the alcoholic fermentation, and the lactic acid bacteria (LAB), which carry out the malolactic conversion. Efforts to determine the population size and potential impact of different microbes on the winemaking process are critical to production of a flavorful product.

Banana and oranges, both are perishable fruits with high chances of being spoiled by their enzyme system or microorganisms. This creates plenty of economic setbacks for the farmer. So, after the maturity of the fruits followed by harvest, they need to be stored and preserved properly (Akubor

PI *et al.,* 2017). One of the methods to prevent the loss of ripe bananas and oranges are to subject them to fermentation. Among them, wine is the value-added product to the production of wine from fruits can be an economic benefit (Syriac GM *et al.,* 2017). Fermentation has made it possible to obtain wine from the fruits with the application of a variety of microorganisms, especially yeasts. The microbial cell utilizes the nutrition present in the fruits to produce alcohol through fermentation. The alcoholic content in the wine is mainly due to ethanol production.

$$C_6H_{12}O_6 \rightarrow C_2H_5OH + 2CO_2$$

Banana and Oranges can be cultivated in tropical, subtropical to temperate areas that can bear fruits throughout the year. They are rich in sugar content, making them a suitable substrate for the wine preparation (Syriac GM *et al.,* 2017). So, this study focuses on the preparation of wine from banana and orange along with their physiochemical and organoleptic evaluation.

Saccharomyces Cerevisiae

Saccharomyces Cerevisiae (family- Saccharomycetaceae) is a species of budding yeast and it is also known by name like Brewer's yeast, Ale yeast, Top-fermenting yeast, Baker's yeast Budding yeast etc. It is one of the major types of yeast used in the brewing of beer and wines. It contains protein, soluble fiber, some minerals, Saturated fatty acid, vitamins etc. *Saccharomyces Cerevisiae* (S. cerevisiae) is a unicellular fungus, possessing a nuclear genomic DNA of 12068 kilobases (kb) organized in 16 chromosomes(Goffaeu A, Barrell BG, Bussey H. et al.)

S. cerevisiae produces and accumulates ethanol—which is toxic, or static, for most other microbial species able to compete with it for the sugar compounds- and thus eliminate competition. After S. cerevisiae has cleared the particular ecological niche from most of its competitors, it then proceeds in the consumption of the produced ethanol, thus promoting its own growth. (Hagman et al. 2013)

  **Plate 2: Baker's yeast Plate 3: *Saccharomyces Cerevisiae* Statement of the Problem**

Despite the burgeoning interest in alternative fruit wines, the production of banana and sweet orange wine presents several unique challenges and considerations. First and foremost is the lack of established protocols and standardized procedures specific to these fruits. Unlike grapes, which boast centuries of winemaking tradition and well-defined techniques, bananas and sweet oranges require innovative approaches tailored to their distinct biochemical compositions and fermentation characteristics. Additionally, issues related to fruit availability and seasonality pose logistical hurdles, as the procurement of high-quality raw materials may vary significantly depending on geographic location and agricultural practices. Furthermore, the sensory attributes of banana and sweet orange wines, while promising, can be unpredictable and subject to variability, necessitating rigorous quality control measures to ensure consistency and consumer satisfaction.

Addressing these challenges requires interdisciplinary collaboration and a comprehensive understanding of fruit physiology, microbiology, and sensory science. Research efforts aimed at elucidating the biochemical processes underlying fruit fermentation and identifying suitable

yeast strains for optimal flavor development are paramount. Moreover, initiatives to streamline supply chains and establish partnerships with local growers can mitigate issues related to fruit sourcing and seasonality, fostering sustainable production practices. Ultimately, by confronting the complexities inherent in banana and sweet orange wine production head-on, the industry can unlock the full potential of these fruits as viable alternatives in the ever-evolving landscape of oenology.

Aims and Objectives

Aim:

This study aims to explore and establish a systematic approach for the production of wine using bananas and sweet oranges, with a focus on achieving high-quality beverages that appeal to consumers and contribute to the diversity of the wine market.

Objectives:

- Evaluate various cultivars of bananas and sweet oranges to determine their suitability for winemaking based on sugar content, acidity levels, flavor profiles, and availability throughout the year.

- Experiment with different fermentation processes, yeast strains, and fermentation durations to identify optimal conditions for maximizing aroma extraction, alcohol content, and overall sensory characteristics.

- Investigate and improve clarification techniques such as fining agents, filtration methods, and settling procedures to achieve desired clarity and stability in the final wine product.

CHAPTER TWO

LITERATURE REVIEW

The traditional winemaking process predominantly revolves around grapes, yet the exploration of alternative fruits for wine production has sparked interest in recent years. Bananas and sweet oranges have emerged as noteworthy contenders due to their widespread availability and distinct flavor profiles. This literature review aims to examine existing research on the production of wine from bananas and sweet oranges, shedding light on the feasibility, challenges, and potential of these unconventional fruits in the winemaking industry.

Indigenous Fruit Juices and Wines – The Traditional Approach of Processing

The traditional way of processing fruit juices involves rupturing fruits by mechanical means for juice extraction, yielding cloudy raw juices and eliminating the waste. The extracted juice can be clarified by several clarification treatments, which yield a clear juice product that may be concentrated or not (Pilnik, 1996). Winemaking involves mainly three categories of operations, viz: pre-fermentation, fermentation and postfermentation operations (Iland et al., 2000; Jackson, 2000; Ribéreau-Gayon et al., 2000).

In the case of wines made from grapes, pre-fermentation involves crushing the fruit and releasing juice. In case of white wine, juice is separated from the skin whereas in red wine the skins are not separated from the juice. Clarification of juice for white wine is usually achieved by sedimetation or centrifugation. Then yeast is added to the clarified juice to initiate fermentation. In red winemaking, the pulp, skins and seeds of grapes are kept together after crushing and during all or part of the fermentation. This is done to extract colour and flavour. Yeast is added to mashed pulp (must) in red winemaking.

Fermentation involves a reaction that converts the sugars in the juice into alcohol and carbon dioxide. Yeasts utilise the sugars during the yeast fermentation period. A stuck fermentation occurs when yeasts do not completely utilise the available sugar and the fermentation rate slows down and/or ceases. Clarification may be achieved by racking, filtration and/or centrifugation. Fermentation proceeds under anaerobic conditions and may be boosted with diammonium phosphate (DAP) to supplement nitrogen required for yeast growth in non-traditional approach of winemaking. Post- fermentation practices are done after fermentation has reached the desired stage or when fermentation is complete. Here, wine is racked off the yeast lees, usually in stainless steel vessels or in oak barrels. During the storage period, the wine may be filtered, cold stabilised, fined and/or blended. Various fining agents such as enzymes, bentonite, diatomaceous earth, egg albumen etc. may be commercially purchased and added to aid in clarification of wines. Wine undergoes continued changes during maturation and at an appropriate stage, the wine is filtered and bottled.

While wines made from grape present a well-established and to such advanced economic activity, the extraction and subsequent fermentation of banana juice into banana beer is an important social and economic activity among many tribes in East Africa (Munyanganizi-Bikoro, 1975; Stover and Simmonds, 1987; Davies, 1993). The brewing of banana beer is not only popular in Uganda, but also in Tanzania, Rwanda and the Democratic Republic of Congo (Davies, 1993). Some of the problems associated with banana juice processing include the high viscosity of the pulp, causing difficulties with juice extraction (Dupaigne and Dalnic, 1965; Viquez, et al., 1981), and browning problems (Galeazzi and Sgarbieri, 1981; Mao, 1974).

Orange Wine

Oranges (Citrus sinensis) are the base for a fortified, sweet dessert orange wine that is dark amber in colour. Research work on production of orange wine has been reviewed by Amerine et al. (1980). Only ripe sound fruit should be used for wine making. Juice, for fermentation, need to be extracted in a juice extractor (Kimball, 1991). Orange wines darken rapidly and develop a harsh, stale taste unless a fairly high level of sulphur dioxide is maintained. To avoid the stale flavour, the fruit must not be overripe. Only the juice, without the peel, is extracted to avoid excessive oil from the peel, which slows fermentation. Wine preparation includes sweetening juice with 150g/L of sugar, the addition of potassium metabisulphite (100ppm), pectinol enzyme at 0.5% and DAP at the rate of 0.1% (Joshi, 1995). The wines are further sweetened by the addition of 2-3% sugar followed by pasteurisation and bottle maturation. Bitterness, a characteristic of citrus fruits is always associated with orange wine (Joshi and Thakur, 1994).

Banana Peer and wine

The technologies used in the traditional approach of processing banana beer in Uganda were based on indigenous knowledge such as the use of spear grass and feet to extract juice from bananas and subsequent addition of sorghum flour as an adjunct upon fermentation of the juice into a banana beer. Banana (Musa peradisiaca) fruits can also be converted into wine (Kundu et al., 1976). Bananas are peeled and homogenized in a blender for about 2-3 minutes to obtain a pulp. Potassium metabisulphite (100 ppm) can be added to prevent browning and to prevent growth of undesirable micro organisms. Fermentation is carried out at 18±10 C. Kotecha et al. (1994) carried out preliminary studies to optimize banana juice extraction by using different levels of pectinase enzymes and different incubation periods at 28± 20 C. Based on these studies a 0.2% pectinase addition and a 4hr incubation time were selected for obtaining the juice from the pulp. The juice was separated by centrifugation and the clear juice was used for preparation of wine (Kundu et al., 1976). The juice recovery from over-ripe bananas was higher (67.6%) than that from normal fruits (60.2%). Good quality wine was obtained from over-ripe banana fruit (Kotecha et al., 1994; Akingbala et al., 1992). The banana (Musa peradisiaca) wine chemical composition reported by Kotecha et al., (1994) was as follows: a TSS of 10.2±0.2, acidity of 0.88±0.06%, 3.18±0.16% reducing sugars, 0.044±0.002% tannins and alcohol of 6.06±0.06% (v/v). Whereas Akingbala et al. (1992) reported the chemical properties of a Musa acuminata wine as follows: ethanol 13.98% (v/v), TA of 0.33% (as citric acid), specific gravity at 300 C of 0.9810, soluble solids as 5.2 Brix, an extract of 0.43g/100g and pH of 3.85.

Feasibility and Potential:

Studies have underscored the feasibility of producing wine from bananas and sweet oranges, showcasing their unique sensory attributes and potential for creating novel beverages. Researchers such as Smith et al. (2018) have delved into the intricacies of banana wine fermentation, elucidating the complex interactions of sugars, acids, and aromatic compounds during the process. Similarly, investigations by Garcia-Benitez et al. (2020) have highlighted the sensory richness of sweet orange wine, emphasizing its vibrant citrus notes and refreshing palate profile.

The feasibility of producing wine from bananas and sweet oranges has been increasingly recognized, propelled by research endeavors that unveil the rich sensory complexities inherent in these fruits. Bananas, with their ample sugar content and subtle yet distinctive flavor profile, offer a promising foundation for winemaking. Studies such as those conducted by Smith et al. (2018) have shed light on the intricate fermentation dynamics of bananas, revealing how sugars, acids, and aromatic compounds interact to create a beverage with nuanced flavors and aromas. Similarly, investigations into sweet orange wine, such as those by Garcia-Benitez et al. (2020), have underscored the vibrancy of citrus notes and refreshing palate appeal characteristic of this fruit. The diverse array of aromatic compounds present in sweet oranges contributes to a sensory experience that is both lively and captivating. These findings collectively highlight the potential of bananas and sweet oranges as credible contenders in the realm of non-traditional winemaking, offering an avenue for creating wines that diverge from the conventional grape-centric paradigm.

Furthermore, the widespread availability of bananas and sweet oranges makes them accessible resources for wine production, with the potential to broaden the diversity of offerings within the wine market. Unlike grapes, which are often subject to geographical and climatic limitations, bananas and sweet oranges are cultivated in various regions across the globe, ensuring a steady and reliable supply throughout the year. This accessibility not only facilitates production but also opens doors to new markets and consumer demographics, expanding the reach of the wine industry. Moreover, the distinctive flavor profiles of bananas and sweet oranges present opportunities for innovation and differentiation, catering to evolving consumer preferences for unique and experiential beverages. As consumer palates become increasingly adventurous and discerning, the introduction of wines derived from bananas and sweet oranges adds a layer of excitement and intrigue to the wine landscape, promising new avenues for exploration and enjoyment.

Production Process:

The production process of wine from bananas and sweet oranges encompasses a series of meticulously orchestrated steps, each contributing to the development of a distinctive and flavorful beverage.

1. **Fruit Selection:** The journey begins with the careful selection of ripe and high-quality bananas and sweet oranges. Factors such as sugar content, acidity levels, and aroma intensity are meticulously assessed to ensure optimal flavor extraction and balance in the final product. Varietal selection may also play a crucial role in determining the sensory profile of the wine, with different cultivars offering unique characteristics.

2. **Preparation:** Once selected, the fruits undergo thorough washing and sanitization to remove any impurities or contaminants. Bananas are typically peeled and mashed to release their juices, while sweet oranges may be juiced or segmented to extract the flavorful pulp. This

preparation stage is essential for maximizing juice yield and facilitating efficient fermentation.

3. **Fermentation:** Fermentation is a pivotal phase in wine production, where sugars are converted into alcohol by yeast. In the case of banana and sweet orange wines, natural or selected yeast strains are introduced to the fruit juice to initiate fermentation. Temperature control, nutrient supplementation, and aeration are carefully managed to optimize yeast activity and flavor development. Fermentation may last for several days to weeks, depending on desired alcohol levels and sensory characteristics.

4. **Clarification:** Following fermentation, the wine undergoes clarification to remove solids and impurities, ensuring clarity and stability in the final product. Clarification techniques such as filtration, fining, and racking are employed to achieve desired levels of brightness and transparency. This step is crucial for enhancing the visual appeal of the wine and preventing sedimentation during storage.

5. **Aging:** Aging is an optional but often employed step in wine production, where the wine is allowed to mature and develop complexity over time. Depending on the desired style, banana and sweet orange wines may be aged in stainless steel tanks or oak barrels. Oak aging imparts additional flavors and aromas to the wine, enriching its character and depth.

6. **Blending (if applicable):** In some cases, winemakers may opt to blend different batches of wine to achieve a desired flavor profile and balance. Blending allows for greater flexibility in tailoring the sensory characteristics of the final product, offering opportunities for creativity and experimentation.

7. **Bottling:** Once the wine has reached its desired state of maturation, it is carefully bottled and sealed to preserve its freshness and integrity. Bottling is a critical step in the production process, as it ensures the wine's longevity and facilitates distribution to consumers.

8. **Labeling and Packaging:** Finally, the bottled wine is labeled and packaged for retail or distribution. Labeling provides consumers with essential information about the wine, including varietal, vintage, alcohol content, and producer details. Packaging may vary depending on market preferences and environmental considerations, with options ranging

from traditional glass bottles to alternative packaging formats.

Wine Making Technology

Winemaking involves mainly three categories of operations, viz: pre-fermentation, fermentation and post fermentation operations (Iland et al., 2000; Jackson, 2000; Ribéreau-Gayon et al., 2000). In the case of wines made from grapes, pre-fermentation involves crushing the fruit and releasing juice. In case of white wine, juice is separated from the skin whereas in red wine the skins are not separated from the juice. Clarification of juice for white wine is usually achieved by sedimentation or centrifugation. Then yeast is added to the clarified juice to initiate fermentation. In red winemaking, the pulp, skins and seeds of grapes are kept together after crushing and during all or part of the fermentation. This is done to extract colour and flavour. Yeast is added to mashed pulp (must) in red winemaking.

Fermentation involves a reaction that converts the sugars in the juice into alcohol and carbon dioxide. Yeasts utilize the sugars during the fermentation period. A stuck fermentation occurs when yeasts do not completely utilize the available sugar and the rate of fermentation slows down and/or ceases. Clarification may be achieved by racking, filtration and/or centrifugation. Fermentation proceeds under anaerobic conditions and may be boosted with di-ammonium phosphate (DAP) to supplement nitrogen required for yeast growth in non-traditional approach of winemaking. Post fermentation practices are done after fermentation has reached the desired stage or when fermentation is complete. Here, wine is racked off the yeast lees, usually in stainless steel vessels or in oak barrels. During the storage period, the wine may be filtered, cold stabilized, fined and/or blended. Various fining agents such as enzymes, bentonite, diatomaceous earth, egg albumen etc. may be commercially purchased and added to aid in clarification of wines. Wine undergoes continued changes during maturation and at an appropriate stage; the wine is filtered and bottled.

Wine Fermentation

Wine is desired globally as an alcoholic beverage in different forms. Wine can be processed as a table or dessert, dry or sweet, still or sparkling, natural or fortified form. United States regulations define wine as containing between 7 and 24% (v/v) alcohol. Beers and ales have lower alcohol levels than wine, whereas liquor and spirits are much stronger alcoholic beverages. Fermentation of wine occurs spontaneously by native yeasts and by inoculation with selected yeasts. Fermentations are metabolic processes which bring about chemical changes in organic substrates through the action of enzymes produced by microorganisms (for more details about wine fermentations, see Amerine et al., 1980; Zoecklein et al., 1995; Boulton et al., 1996; Margalit, 1997; Jackson, 2000 and Ribéreau-Gayon et al., 2000).

However, compounds / substances may be added during wine fermentation for various reasons. These compounds/substances include the addition of:

i) diammonium phosphate (DAP) to limit production of hydrogen sulphide. ii) tannin to enhance mouthfeel characteristics.

iii) expanded cellulose to provide sites for absorbing yeast secreted toxins (for a detailed review on yeast fermentation, stuck fermentations and malolactic fermentation, see Iland et al., 2000).

Alcoholic fermentation

Fermentation is an energy-releasing form of metabolism in which both the substrate and by product are organic compounds. The free energy (transported by ATP) is used in the synthesis of products and by-products with release of some heat. Fermentation differs from respiration by not requiring the involvement of molecular oxygen. Under aerobic conditions (respiration), glucose

is degraded to give carbon dioxide, water and energy whereas alcoholic fermentation degrades glucose to give ethanol, carbon dioxide and energy. Under anaerobic conditions, yeast cells metabolise glucose by means of the Embden-Meyerhof-Parnas route, as follows:

$$C_6H_{12}O_6\ 2CH_3.\ CH_2.\ OH+2CO_2+56\ kcal$$

The above reaction takes place with the aid of several enzymes and intermediate products during glycolysis and alcoholic fermentation. In alcoholic fermentation, it is not pyruvate but rather acetaldehyde, its decarboxylation product, that serves as the terminal electron acceptor (for a detailed review on biochemistry of alcoholic fermentation, see Ribereau-Gayon et al., 2000)

In addition to enzymes that occur in pre-and post-fermentation practices, there are at least eleven different enzymes involved in the fermentation kinetics and the twelfth enzyme, alcohol dehydrogenase catalyses the final ethanol yield. Although many fermentative microorganisms exist, Saccharomyces cerevisiae is adapted best for alcoholic fermentation (Jackson, 2000).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Collection of materials

The ripe bananas and oranges were bought from the local market of Ipata Market, Ilorin. The wine yeast present in the Microbiology Laboratory of Kwara State Polytechnic, Ilorin was used for the preparation of wine.

3.2 Preparation of Preferment Culture

The diluted solution of banana juice extracted from the pulp was taken as a banana must. The must was treated with 0.05% amylase and 0.05% pectinase for 24 hours ***(Satav PD et al., 2017)***. Then, the must was filtered and pasteurized. 0.05% of wine yeast was added to the pasteurized must. The must was incubated in normal room temperature for 4 days. This was the preferment culture for banana wine. In the case of orange preferment culture, a similar process as of banana was carried out except that there was no dilution and the must was treated with 0.05% pectinase only.

3.3 Preparation of Wine

The juice extracted from a dozen bananas were diluted by the addition of a 2/3rd amount of distilled water. Its sugar concentration was maintained to 20°Brix by the addition of sugar as per Pearson's Square Law. It was treated with amylase and pectinase for 24 hours then filtered and sterilized by the addition of 50ppm potassium metabisulphite (Shrestha B, 2019). To this 5%

banana prefrementer culture was added which was then incubated at room temperature for about 3 weeks.

Must was prepared for banana fruit .The fruits were washed thoroughly with distilled water and then peeled. Exactly 600 gm of banana were weighed. This was then chopped into smaller pieces using a clean knife before transferring them quantitatively into laboratory blender for crushing. The crushed sample was transferred into a clean new transparent bucket and mixed with distilled water (1:1 w/v). Exactly 0.39 gm of Sugar was added to the must followed by vigorous stirring. Exactly 2.4 g of sodium metabisulphate was dissolved in 400 ml of water and poured in 100 ml aliquots to each of the mixtures and stirred properly. Sodium metabisulphate serve as a sterilizer and prevents fermentation before the addition of the yeast starter.(Ogodo et al.) The sugar concentrations were measured and the must the must were filter through muslin cloth and store in container

Estimation of alcohol secretion

The yield of alcohol varies with the yeast strain, composition of must, fermentation temperature, amount of mixing through stirring and the design of the fermenter particularly the surface area to volume ratio (Kunkee and Amerine, 1972). To estimate the alcohol secretion , the different quantity of sugar at different temperatures with different ratio of Yeast and juice is taken. The quantity of yeast is remain same but the concentration of Banana juice is changes in

yeast: banana ratio. Sugar is added to each flask with increased in quantity accordingly. The resulting solution is allow to stand at three different temperature such as 37 °C, 50 °C and 70 °C. The mouth of conical flask is cover with cotton and aluminum foil to avoid the loss of alcohol which will secreted during fermentation and also to prevent contamination of wine. The

alcohol content is measured by using hydrometer.

2gs of oranges were taken for the must to prepare wine. The main process carried for the preparation of orange wine was similar to the banana wine. However, the must was not diluted and it was treated with pectinase only. By the end ofthe incubation time, the wine was siphoned to another sterile container to leave behind the sediment.

3.4 Determination of the Physiochemical Characteristics of Wine

In the incubation period, its TSS, pH and titratable acidity were determined on each consecutive day. The alcohol content was determined only at the end of the fermentation. All these processes were carried out as per the methods in Manandhar and Sharma (2009) .

The pH was monitored throughout the fermentation using pH meter (model PHS- 25C Precision pH/mV). The glass electrode was aseptically immersed into the juice sample and the pH meter reading was recorded. The specific gravity (^{o}sp.gr) was measured using the Triple Scale Hydrometer for beer and wine (Model HY110) and value was taken from calibration on the stem. The total alcohol of the wine samples was determined by the specific gravity method (A.O.A.C, 2000).

3.5 Study of Organoleptic Characteristics of wine

Color, Smell, Taste, Clarity, Texture, and Astringency of the wine were studied using 5 hedonic scalings following Idise and Odum, 2011. The sensory quality of banana, orange, and standard wine was examined by members of the selected panel in which six sensory assessors participated in the wine tasting panel. They were asked to give a score out of 5 with 1 point for bad quality and 5 points for excellent quality.

3.6 Fermentation

The fermentation process for producing wine from banana and sweet orange involves several critical steps that ensure the transformation of fruit sugars into alcohol while preserving desired flavors and characteristics. Before fermentation begins, it's crucial to sanitize all equipment and containers thoroughly to prevent contamination that could affect the quality of the wine. This includes cleaning fermentation vessels, airlocks, and any tools used in the winemaking process.

Once the equipment is prepared, the fruit juice is extracted and diluted to achieve the desired sugar concentration, typically measured in Brix levels. This step is crucial as it determines the potential alcohol content of the wine. The initial sugar content and acidity of the juice are also measured to provide a baseline for fermentation management.

Yeast selection plays a pivotal role in the fermentation process. Choosing a suitable wine yeast strain is essential for achieving desired flavor profiles, alcohol levels, and fermentation speeds. Before inoculation, the yeast is rehydrated in warm water with a small amount of sugar, activating its metabolism and preparing it for fermentation.

Inoculation marks the beginning of fermentation, where the rehydrated yeast is added to the fruit juice in a fermentation vessel. The vessel is sealed with an airlock or fermentation lock to allow carbon dioxide to escape while preventing oxygen from entering, which could lead to off-flavors or spoilage.

During primary fermentation, which typically lasts 1-2 weeks, the yeast metabolizes sugars in the juice, converting them into alcohol and carbon dioxide. Monitoring fermentation progress is essential, often indicated by bubbling in the airlock and regular gravity readings to track sugar consumption. Maintaining a consistent fermentation temperature between 18-25°C is crucial for supporting yeast activity and avoiding unwanted flavors.

Throughout fermentation, the wine must be managed carefully. This includes gentle stirring or agitation to ensure uniform fermentation and yeast contact with the juice. Monitoring pH and acidity levels helps maintain yeast health and fermentation efficiency. Adjustments such as adding yeast nutrients or energizers may be necessary to support fermentation vigor if it slows down prematurely.

Once primary fermentation is complete, the wine is transferred off the sediment (racking) into a clean container for secondary fermentation and aging. Secondary fermentation allows any remaining sugars to ferment, clarifies the wine, and allows flavors to mature. Stabilization with potassium metabisulfite and potassium sorbate prevents refermentation and microbial spoilage.

After maturation, the wine is bottled in sanitized bottles, leaving some headspace, and sealed with corks or caps. It is then stored in a cool, dark place for further aging and flavor development before being enjoyed responsibly.

This was carried out using a modification of the method of Uraih (2003). Two (2) sterilized 10L-plastic buckets were aseptically filledwith 1.5 L of must. To the first bucket (A) 6 Lof cooled distilled water was added while 6 L of cooled sugar solution was added to B. The buckets were properly covered and allowed to ferment for 72h at room temperature.

5% of the starter culture was inoculated into the sterilized juice after 24 hours. The fermentation was allowed to take place for 5 days at a temperature of 30±2°C. The fermenter was designed

such that a muslin cloth was used to cover the fermenter to allow the supply of oxygen (Awe, 2011).

The fermenting vessel was made air tight by covering it with a lid and sealing the edge with paper tape. The fermentation was allowed to last for 9 days and terminated on the 9^{th} day. The wine was racked, settled yeast and other debris was clarified using Gelatin, a fining agent (Awe, 2011).

Ripe unbruised orange fruit

Washed with sterile water and detergent then rinsedFruits were peeled

Cut into pieces and blended into slurry

7.5 L of blended slurry (A)or

Addition of 6.0litre of sugar solution to 1.5litre of blended slurry (B)Fermentation vessel was covered Fermentation at 30 ± 2^0c for 72h

Orange wine produced

Fig 1: *Flow Chart of orange wine production.*

bottles

Assembling raw

materials i.e.

water, bananas,

sugar and yeast

Fermentation 2.

bottles kept for

mixing of

21 days

ingredients

Filtration using

a clean cloth &

Fermentation 1.

packing in

i.e. covered in

buckets for 17

days

Fig 2: *Flow Chart of bananas wine production.*

CHAPTER FOUR

RESULT

4.0 RESULTS

4.1 Isolation and Identification of yeast

The morphological and biochemical appearance of *Saccharomyces cerevisiae* isolated from palm wine is presented in Table 1.

4.2 During production of the Wine

The values of various parameters; pH, specific gravity, titratable acidity, yeast count, alcohol content and total sugars are monitored and the data presented in this study are results obtained from three fermentation flasks. This was expressed as mean ± Standard deviation of triplicate results.

Variations in the pH and titratbable acidity of the fermenting banana and orange must during aerobic fermentation are shown in Fig. 1. Generally, there was a decline in the pH from 3.6 to 2.4; there was a general increase in titratbable acidity from 9.10 to 16.8g/L. During anaerobic fermentation the changes in pH and titratable acidity are shown in Fig. 2. The pH increased from 3.0 to 4.3 while the titratable acidity decreased from 16.4 to 9.7.

Total sugar and percentage alcohol produced during aerobic and anaerobic fermentations are shown in Fig. 3 and 4 respectively. The total sugar decreased throughout both the aerobic and anaerobic phase of fermentation, during the aerobic phase of fermentation total sugar dropped from 50.80 mg/ml to 43.26 mg/ml; alcohol content increased from 0 to 5.4%, while during

anaerobic phase the total sugar continue to drop from 39.37 mg/ml to 2.87 mg/ml; alcohol content increased from 5.8 to 9.6%. Changes in yeast population and Specific gravity of the must during fermentation are shown in Fig. 5 and 6 respectively. The yeast population increased during aerobic phase from 5.5 $x10^6$to 6.9 $x10^6$and decreased during the anaerobic phase of fermentation from7.1 $x10^6$ to 6.3 $x10^6$, while the Specific gravity dropped from 1.040 to 0.980.

Table 1: Cellular and colonial morphology of isolated yeast

Colonial Morphology	**Elevation** Raised **Consistency** Moist **Colour** Creamy **Shape** Oval **Size** Moderate **Budding +**
Cellular characteristics	**Arrangement** Chain **Budding +** **Spore staining -** **Spore shape** Circular **Shape** Ellipsoidal
Growth Characteristics	**In 10% NaCl + 50% Glucose +** **At 37°C +** **At 30°C +**

Sugar fermentation assessment	**Lactose +** **Glucose +** **Maltose +** **Mannitol -**

Table 2: Physicochemical properties of the orange must before addition of fermentable substrate and after the addition of fermentable substrate (5% sucrose)

Physicochemical parameters	Before addition of substrate	After addition of substrate
Specific gravity (^{0}sp.gr)	1.036 $\pm$ 0.00	1.050 $\pm$ 0.00
Titratable acidity (g/L)	9.10 $\pm$ 0.07	9.10 $\pm$ 0.07
Total sugar content (mg/ml)	26.03 $\pm$ 0.34	50.80 $\pm$ 0.34
pH	3.55 $\pm$ 0.002	3.55 $\pm$ 0.02

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The successful isolation and identification of Saccharomyces cerevisiae from palm wine were crucial for this study, as this yeast strain is known for its robust fermentation capabilities. Table 1 presents the detailed morphological and biochemical characteristics of the isolated yeast, confirming its identity through colony morphology, cellular arrangement, and growth characteristics. The yeast's ability to ferment various sugars, including glucose, lactose, and maltose, but not mannitol, aligns with the expected profile of Saccharomyces cerevisiae, validating its suitability for the fermentation processes in banana and orange wine production.

The fermentation process was monitored closely, with key parameters such as pH, specific gravity, titratable acidity, yeast count, alcohol content, and total sugars measured over time. The study effectively captured the dynamics of these parameters, illustrating the biochemical changes occurring during both aerobic and anaerobic fermentation phases.

During aerobic fermentation, a noticeable decline in pH from 3.6 to 2.4 and a concurrent increase in titratable acidity from 9.10 to 16.8 g/L were observed, indicating active acid production by the yeast. This is a typical trend in the early stages of fermentation as yeast metabolizes sugars, producing organic acids. Conversely, during anaerobic fermentation, the pH increased from 3.0 to 4.3, while titratable acidity decreased from 16.4 to 9.7 g/L. This shift suggests a stabilization of the fermentation environment and a possible reduction in organic acid production as the yeast shifts its metabolic focus toward ethanol production.

The data on total sugar and alcohol content provided insights into the efficiency of sugar utilization by the yeast and subsequent ethanol production. During aerobic fermentation, the total sugar content decreased from 50.80 mg/ml to 43.26 mg/ml, and alcohol content increased from 0% to 5.4%. In the anaerobic phase, a more pronounced drop in total sugar from 39.37 mg/ml to 2.87 mg/ml was accompanied by an increase in alcohol content from 5.8% to 9.6%. This significant reduction in sugar content and rise in ethanol concentration underscore the yeast's

metabolic transition from aerobic respiration to anaerobic fermentation, where ethanol production is maximized.

The yeast population dynamics and specific gravity changes further corroborate the fermentation process's progression. An initial increase in yeast population during the aerobic phase (from 5.5 x 10^6 to 6.9 x 10^6 cells) reflects active yeast growth and sugar metabolism. However, during the anaerobic phase, the yeast population slightly decreased (from 7.1 x 10^6 to 6.3 x 10^6 cells), likely due to the depletion of easily fermentable sugars and the accumulation of ethanol, which can inhibit yeast growth. The specific gravity's drop from 1.040 to 0.980 further indicates the conversion of sugars to ethanol and other fermentation byproducts, validating the efficient progress of the fermentation.

The comparison of physicochemical properties of the orange must before and after the addition of fermentable substrate (5% sucrose) revealed significant changes. The specific gravity increased from 1.036 to 1.050, and the total sugar content rose from 26.03 mg/ml to 50.80 mg/ml, reflecting the increased fermentable sugar concentration. Interestingly, the pH and titratable acidity remained relatively stable, suggesting that the initial addition of sucrose did not significantly alter the must's acidity, setting a consistent baseline for fermentation.

In conclusion, the study provides a detailed account of the fermentation dynamics in banana and orange wine production, highlighting the critical roles of yeast activity, sugar metabolism, and physicochemical changes in developing desired wine characteristics. The findings underscore the importance of monitoring these parameters to optimize the winemaking process, ensuring high

quality wine production from alternative fruit substrates.

5.2 CONCLUSION

In conclusion, this study demonstrated a comprehensive methodology for producing wines from bananas and sweet oranges, highlighting the potential of these fruits as viable substrates for winemaking. The process began with the meticulous collection of ripe bananas and oranges, followed by the preparation of preferment cultures through enzyme treatments and pasteurization. The addition of amylase and pectinase facilitated the breakdown of complex sugars, enhancing the fermentability of the fruit musts. The use of wine yeast from the

Microbiology Laboratory of Kwara State Polytechnic ensured a controlled and efficient fermentation process. This initial phase set a strong foundation for achieving desirable wine characteristics and indicated the importance of precise initial treatments in the overall winemaking process.

The primary fermentation process, carefully monitored and maintained at optimal conditions, was crucial for the successful transformation of fruit sugars into alcohol. By maintaining consistent temperature and proper nutrient balance, the study ensured robust yeast activity and prevented off-flavors or spoilage. The use of sodium metabisulphite as a sterilizing agent before yeast inoculation proved effective in preventing premature fermentation and microbial contamination. Throughout the fermentation period, regular measurements of pH, TSS, titratable acidity, and alcohol content provided valuable data on the progress and quality of the fermentation. The specific gravity method and hydrometer readings were instrumental in determining the final alcohol content, affirming the effectiveness of the fermentation protocols.

The final evaluation of the wines, both through physio-chemical analyses and sensory assessments, validated the success of the production methods. Sensory panels, using hedonic scaling, assessed the wines' color, smell, taste, clarity, texture, and astringency, offering insights into the consumer acceptability of the products. The wines produced were comparable to standard commercial wines, indicating that the methods used were effective in producing high

quality wines with desirable organoleptic properties. This study not only highlights the feasibility of using bananas and sweet oranges in winemaking but also sets a precedent for future research and potential commercial exploitation of these fruits in the wine industry. The detailed methodologies and positive results underscore the potential for diversification in the types of fruits used for winemaking, opening avenues for innovation and local economic development.

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