

Review

# Microbial and Chemical Diversity of Traditional Non-Cereal Based Alcoholic Beverages of Sub-Saharan Africa

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Received: 18 December 2017; Accepted: 17 March 2018; Published: 1 May 2018



**Abstract:** Fermentation remains an important food preparation technique of health, cultural and economic importance throughout the world. In Sub-Saharan Africa, traditional alcoholic fermentation of cereal and non-cereal based substrates into alcoholic beverages is deeply rooted in the society. Although a multitude of traditional alcoholic beverages from cereal substrates are well researched and documented, their non-cereal based counterparts, mostly produced from indigenous, inexpensive substrates, remain less well studied. In addition, reports of health problems associated with non-cereal based alcoholic beverages produced from spontaneous fermentation are a major cause of concern. This review aims to highlight the microbiological and chemical profiles of these non-cereal based alcoholic beverages with a focus on the Sub-Saharan region. Here, we underscore the importance of the microbial repertoire and the substrates thereof in attaining aromatic complexity and a characteristic taste in these beverages. These aspects are an important starting point towards the potential commercialization of these complex aromatic non-cereal based traditional beverages.

**Keywords:** non-cereal; fruit; beverage; fermentation; microbes; metabolites

## 1. Introduction

Fermentation is the oldest and most popular technology used by humankind, dating back to the Neolithic period. Its exploitation was mostly used in dairy products, baking, wine making, and brewing. With the advent of new technologies, modern exploitation of fermentation now extends to the production of renewable fuels and biopharmaceuticals. From the traditional viewpoint, food fermentation is vital, as it improves the nutritional composition of foods, their sensory properties, and their acceptability, as well as it prolonging the shelf life of food [1]. In addition, fermentation reduces toxic or anti-nutritional food components such as phytic acids, polyphenolic compounds, and tannins [2] in food and beverages from plant material. To date, different parts of the world boast of a multitude of different alcoholic beverages. Due to cultural differences, these beverages are either cereal or non-cereal based. For example, in the Sub-Saharan region, non-cereal based alcoholic beverages are produced from inexpensive substrates such as wild as well as cultivated fruits, tree saps, roots, and tubers whereas cereal-based alcoholic beverages, are made from small grains; maize, sorghum, and millet [3,4].

Several cereal-based alcoholic beverages in the Sub-Saharan region have been well documented [5–8]. The fermentative microbes and chemical profiles of the resulting alcoholic beverages are well known. These include; *chikokivana*, a spirit produced from a mixture of maize meal, and millet malt popular in Zimbabwe [9], *togwa* of Tanzania, produced from sorghum, maize, and millet [10,11], *burukutu*, and *pito*—both popular indigenous alcoholic beverages of Nigeria produced from sorghum

(*Sorghum vulgare* and *Sorghum bicolor*) [6] as well as *borde* of Ethiopia, produced from maize, barley or wheat and their malts [12]. One of these cereal-based alcoholic beverages—*chibuku*—another sorghum-based beer, has made it to the commercial markets in Botswana, Zimbabwe and South Africa [5]. A consortium of yeasts and bacteria is known to carry out fermentation and contribute to unique sensorial properties of these beverages. Fermentation in the production of *burukutu* is carried by *Saccharomyces cerevisiae*, *Saccharomyces chavelieri*, *Leuconostoc mesenteroides*, *Candida* spp. and *Acetobacter* spp. [13] whilst traditional opaque beers (such as *doro* and *chikokivana*) of Zimbabwe are fermented by *Saccharomyces cerevisiae*, *Issatchenkia occidentalis*, *Kluyveromyces marxianus*, *Candida glabrata*, *Sporobolomyces holsaticus* and *Rhodotorula* spp. [14].

Currently in the Sub-Saharan Africa region, there is less documentation of microorganisms involved in fermentation of non-cereal-based alcoholic beverages as compared to cereal-based alcoholic beverages. The deficiencies in research of the rich biodiversity of microorganisms and sometimes unique substrates that are present in sub-Saharan Africa pose a challenge for commercialization and even improvement of the standard of product produced at household level. There is an increasing trajectory of research output in different countries on their non-cereal-based alcoholic beverages. The research includes the microbial and chemical diversity of their traditional beverages which are produced from different substrates. Examples of the traditional alcoholic beverages include *cachaça* (produced from fresh sugar cane juice), *taruba* (produced from cassava) and *caxiri* (produced from cassava and sweet potatoes) of Brazil [15–19], *kefir* (produced from goat, cow and sheep milk) of Russia [20,21], *koumiss* (produced from milk) of Russia and Mongolia [20,22,23], *pulque* (produced from *Agave* plants) of Mexico [15–17], *toddy* (produced from palm tree sap) of India [24], and *airag* (produced from mare or camel milk) of Mongolia [25] to list a few.

Non-cereal-based alcoholic beverages can pose health problems like food poisoning and food intoxication [26]. Although similar issues have also been observed with regards to cereal-based alcoholic beverages, there have been more media reports of direct deaths from non-standard fermentation of non-cereal-based alcoholic beverages. Knowing the fermentative microorganisms and resultant chemical profiles of these non-cereal-based alcoholic beverages could benefit the development of starter cultures for both household and commercial purposes, thus improving the final product quality and safety as well as poisoning these beverages well for potential commercialization. This review aims to highlight the microbiological and chemical profiles of non-cereal-based alcoholic beverages with a focus on the Sub-Saharan region.

## 2. Non-Cereal-Based Alcoholic Beverages

Non-cereal-based beverages are less commonly consumed in comparison to the cereal-based beverages in the Sub-Saharan region. The abundance of a variety of cereals across the continent could explain the discrepancy. These non-cereal based beverages can be either non-alcoholic or alcoholic. Although non-alcoholic beverages are important based on the nutritional benefits that they offer, the sensory properties of alcoholic beverages make them more popular. In sub-Saharan Africa, fermented alcoholic beverages are produced from various inexpensive local raw materials such as palm sap, *marula* fruit and *mogwana*. Figures 1–3 show examples of cheap substrates normally used. Some brews have even been commercialized such as *A. marula* liqueur from the *marula* fruit (Figure 2) [5] and *tej* (Figure 4) [27,28]. *Tej* is a home processed, honey wine of Ethiopia produced from a mixture of honey and sugar as major fermentable substrates. Traditional alcoholic beverages are usually preferred over their western counterparts as they are inexpensive to produce at household levels and affordable for consumers, especially for low-income earners.



**Figure 1.** The *Hyphaene petersiana* (*mokolwane/moxao*) tree and the ripe fruits during the summer season.



**Figure 2.** The *marula* tree (*Sclerocarya birrea* sub-species *caffra*) and the *marula* fruits.



**Figure 3.** *Grewia flava* tree (*mogwana*) and *khadi*.



**Figure 4.** *Tej*—a cloudy unrefined honey wine.

The mass production of non-cereal-based alcoholic beverages of Sub-Saharan Africa relies on uncontrolled spontaneous fermentation and exploits inexpensive substrates that are available in the locality, viz. palm tree fruits (*Arecaceae* family) and exudates, sugarcane (*Saccharum officinarum*), banana pulp (*Musa acuminata*), watermelons (*Citrullus lanatus*), *Hyphaene petersiana* (*mokolwane/moxao*) (Figure 1), *marula* fruit (*Sclerocaryabirrea*) (Figure 2), *Grewia flava* (*mogwana*) (Figure 3), *Grewia occidentalis* (*moretlwa*), *Grewia flavascens* (*mokgomphatha*), *Popowiaobovata*, *Balanites aegyptiaca*, *Berchemia discolor*, *Ziziphus mauritiana* (*masau*), *Kedrostis hirtella* (*mogakangwaga*), *Khadia acutipetala*, cassava (*Manihot esculenta*), and honey [5,9,29,30]. Some of the beverages made from non-cereal substrate include *muchema*, palm wine, *setopoti*, *bojalwa-ja-morula*, *khadi*, *mukumbi*, *urwagwa*, pineapple wine, and *tej*. The characteristics of the substrates are summarized in Table 1. The fermentation of non-cereal-based beverages, although not all fully characterized, is reported to be carried out by yeasts, lactic acid and acetic acid bacteria during a spontaneous fermentation production procedure. Such a technique associated with mixed culture fermentation, as shown in Table 2, may influence the distinctive flavor complexity of these traditional non-cereal-based alcoholic beverages. Table 2 summarizes the microbial diversity of non-cereal-based alcoholic beverages of sub-Saharan Africa region as well as their secondary metabolites.

**Table 1.** Characteristics of substrates commonly used in the production of non-cereal-based alcoholic beverages.

Substrate	Total Sugar Content *	Sugars	pH	Nitrogen Sources	References
Honey	79.5%	Glucose (38.19%), fructose (31.28%), sucrose (1.31%), maltose, isomaltose, trehalose, trisaccharides, raffinose, melezitose, tetrasaccharides and pentasaccharides	3.4–6.1	Proline, glutamic acid, aspartic acid, glutamine, histidine, glycine, arginine, tryptophan, and cysteine	[31–33]
Palm sap ( <i>Palmae</i> family)	10–18%	Sucrose (36%), glucose (33%), fructose, cellobiose, dextran, maltose, xylose, hamnose, arabinose and galacturonic acid	7–7.4	Valine, threonine, lysine and phenylalanine	[34–36]
<i>Masau</i> ( <i>Ziziphus mauritiana</i> )	13.7%	Glucose (6.7%) and fructose (6.8%)	5.6–6.6		[37]
Pineapple ( <i>Ananas comosus</i> )	7.98%	Sucrose, fructose and glucose	3.5		[38,39]

Table 1. Cont.

Substrate	Total Sugar Content *	Sugars	pH	Nitrogen Sources	References
Plantain ( <i>Musa paradisiaca</i> )	20–27%	Glucose, fructose and sucrose	4.26	Arginine, aspartic acid, glutamic acid and methionine	[40,41]
Banana ( <i>Musa acuminata</i> )	14.20–20.18%	Glucose, fructose and sucrose	4.78	Aspartic acid, histidine, leucine and valine	[42,43]
Cassava ( <i>Manihot esculenta</i> )	4.04–18.47%	Sucrose (1.98–15.40%), maltose, fructose, and glucose	6.2–6.9	Valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, threonine, histidine and lysine	[44–48]
Marula juice ( <i>Sclerocarya birrea</i> )	8.2%	Sucrose (5.9%), fructose and glucose	4.10	Methionine, cysteine, leucine, phenylalanine, lysine, and threonine	[49–51]

\* The percentages of the fermentable sugars are per 100 g.

**Table 2.** Microbial diversity of non-cereal-based alcoholic beverages.

Alcoholic Beverage	Raw Materials/Substrate	Sensory Properties	Nature	Fermentative Microbes	Secondary Metabolites	Country	References
Tej	Honey	Sweet	Effervescent, yellow and cloudy	<i>Saccharomyces cerevisiae</i> , <i>Kluyveromyces bulgaricus</i> , <i>Debaromyces phaffi</i> , <i>K. veronae</i> , <i>Zygosaccharomyces rouxii</i> , <i>Hansenula subpelliculosa</i> , <i>S. norbensis</i> , <i>K. vanudenii</i> , <i>Endomycopsis burtonii</i> , <i>Lactobacillus</i> spp., <i>Streptococcus</i> spp., <i>Leuconostoc</i> spp., and <i>Pediococcus</i> spp.	Ethanol, methanol, esters and organic acids	Ethiopia	[11,27,52]
Urwagwa	Banana pulp	Pungent, fruity, herbaceous and astringent/acidic taste	Cloudy	<i>Saccharomyces cerevisiae</i>	Ethanol, 1-propanol, 2-hexanol, acetic acid, 5-hexanoic acid, benzoic acid, propanoic acid, formic acid, ethyl acetate, butanoic acid, ethyl ester, 1-Butanol 3-methyl-ethyl ester and hexanoic acid ethyl ester	Rwanda, Burundi, Uganda, Tanzania and Kenya	[53]
Palm wine	Palm tree sap	Sour, heavy and astringent taste	Whitish, milk-white opalescent liquid	<i>Staphylococcus</i> spp., <i>Micrococcus</i> spp., <i>Serratia</i> spp., <i>Bacillus</i> spp., <i>Streptococcus</i> spp., <i>Saccharomyces cerevisiae</i> , <i>S. pombe</i> , <i>Candida tropicalis</i> , <i>C. krusei</i> , <i>Candida</i> spp., <i>Pichia</i> spp., <i>S. chevalieri</i> , <i>Zymomonas mobilis</i> , <i>Hanseniaspora guilliermondii</i> , <i>H. uvarum</i> , <i>Lactobacillus plantarum</i> , <i>Leuconostoc mesenteroides</i> , <i>L. nagelii</i> , <i>L. sucicola</i> , <i>Acetobacter pasteurianus</i> , <i>A. indonesiensis</i> and <i>Gluconobacter</i> spp.	Lactic acid, citric acid, tartaric acids, ethanol and acetic acid, esters, carbonyls, higher alcohols, phenols, sulphur compounds, terpenes, hydrocarbons, acetals, nitrogen compounds and lactone	Nigeria, Bukina Faso and Cameroon	[34,35,54,55]
Khadi	<i>Grewia flava</i> , <i>Grewia occidentalis</i> , <i>Grewia flavascens</i> , <i>Kedrostis hirtella</i> and <i>Khadia acutipetala</i>	Sweet	Reddish color	No literature on fermentative microbes	Ethanol, 2-methyl-1-propanol, 2/3-methyl-1-butanol, ethyl lactate and ethyl acetate	Botswana	[56]
Mukumbi	Marula fruits	Thick creamy liquor	Yellowish-brown	<i>Saccharomyces cerevisiae</i> , <i>Pichia anomala</i> , <i>P. guilliermondii</i> , <i>Candida tropicalis</i> , and <i>C. intermedia</i>	Ethanol	Namibia, Botswana, Swaziland, Zimbabwe and Zambia	[9,57]

Table 2. Cont.

Alcoholic Beverage	Raw Materials/Substrate	Sensory Properties	Nature	Fermentative Microbes	Secondary Metabolites	Country	References
<i>Muchema</i>	Palm tree sap	Sour	Brownish to colorless	No literature on the microbial diversity	Ethanol	Botswana and Namibia	[57]
<i>Pineapple wine</i>	Pineapples	Fruity, sweet and cream/fatty notes	Golden yellowish	<i>Hanseniaspora guilliermondii</i> , <i>Pichia anomala</i> , <i>Meyerozyma guilliermondii</i> , <i>H. uvarum</i> , <i>Wickerhamomyces anomalus</i> and <i>H. opuntiae</i>	Acetic acid, ethyl acetate, isobutanol, 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl 2-methylpropanoate, 3-methyl-1-butyl acetate, ethyl hexanoate, methyl benzoate, 2-phenylethanol, methyl 2-methylheptanoate, ethyl benzoate, diethyl succinate, ethyl octanoate, ethyl phenylacetate, 2-phenylethyl acetate, ethyl 9-decenoate and ethyl decanoate	Angola	[38,58–60]
<i>Kachasu</i>	Masau fruits ( <i>Ziziphus mauritiana</i> ), <i>Adansonia digitate</i> and <i>Tamarindus indica</i>	Sour, acidic	Clear distillate	<i>Saccharomyces cerevisiae</i> , <i>Issatchenkia orientalis</i> , <i>Pichia fabianii</i> , <i>Aureobasidium pullulans</i> , <i>Lactobacillus agilis</i> , <i>L. minor</i> , <i>L. confusus</i> , <i>L. fructosus</i> , <i>L. bifementans</i> , <i>L. divergens</i> , <i>L. fermentum</i> , <i>L. hilgardii</i> and <i>Streptococcus</i> spp.	Isoamyl alcohol, isobutanol, methanol, acetaldehyde, acetone, ethyl acetate, and furfurals	Zimbabwe	[5,9]

### 3. Commercialization of Non-Cereal-Based Alcoholic Beverages

Non-cereal-based alcoholic beverages have a great potential for commercialization that is comparable to common European and Mediterranean alcoholic beverages. As such, these beverages need to be duly studied and documented, and their production processes standardized and modernized towards optimization. Local producers are the bearers of the indigenous knowledge associated with processing of the non-cereal-based alcoholic beverages. The inclusion of the locals that produce these beverages in the research and commercialization processes will result in benefits such as employment and contribution towards the countries' economic development. One of the challenges that exists with these beverages however, is that they age or mature very rapidly thus losing flavor, microbial stability, as well as their palatability, hence making their commercialization challenging [38].

It is without doubt that nutritional value and safety could be improved by research and commercialization of traditional alcoholic beverages. The natural microflora of the substrates can also serve as a source of novel non-conventional yeast strains with extra supplementary health benefits and aromatic complexity features. Extensive sensory properties of fermentation products from non-conventional yeast strains have been proposed to enhance beer flavor [61].

### 4. Advancements in Fermentation Strategies

Traditional fermentation processes are customarily uncontrolled and depend on the microorganisms from the environment or the normal microflora of the substrate to initiate fermentation. Such fermentation processes often result in variable yield and quality of the beverage [62]. The most common traditional beverages are products of spontaneous or batch accelerated fermentation. Fermentation in the former is mostly due to the presence of resident yeast flora of the fruit surfaces, or from brewing equipment, whereas the latter involves pitching of the mixture to be fermented with a previously fermented brew. The production of traditional non-cereal-based alcoholic beverages usually involves use of traditional fermentation vessel which are typically clay pots (Figure 5) covered with a cloth, producing wines and spirits, if distilled. The cloths create a slightly anaerobic environment but in some instances, the fermentation vessel is completely closed to create stricter anaerobic conditions. Repurposed polyethylene drums are also increasingly used as fermentation vessels in traditional settings.



**Figure 5.** Floor mounted fermentation vessels in Zimbabwe.

Natural or spontaneous fermentation does not consistently produce products with desirable flavors. The natural microbial flora in the raw material (substrate) may not always be the same just as the substrate will vary depending on different environmental conditions. The secondary metabolites formed during oxidation of the fruit acids and alcohols post-fermentation lead to difficulty in production of a product with consistent characteristics [63,64]. This variance in taste and aroma is inherent and akin to differences

of wine bouquets. Other factors that might influence reproducibility include temperature, contamination, type of fermentation vessel, and fermentation time.

A drawback of natural or spontaneous fermentation is that there is a possibility of product failure due to a reduced fermentation progression rate or an uncharacteristic product formation due to growth of undesirable flora and disease-causing microbes [32,33]. When back-slopping is used, the previous fermentis usually dominated by fermentative microorganisms, which solves to some extent the problem of variability of the product [5,9]. This is the most common practice at household level, in the production of many traditional alcoholic beverages. One disadvantage of this technique however, may be that toxic compounds that are normally less toxic in smaller amounts keep concentrating to toxic levels in the subsequent brews.

Batch-to-batch variability of alcoholic fermentation can be minimized by introduction of a pure commercial starter culture. Due to increasing customer preference for a variety of products, a consortium of different microbes can also be used. Common consortia harbor yeasts, acetic acid, and lactic acid bacteria [34]. The diversity in carbon metabolism pathways among the different microbes in the consortia, allows mixed culture fermentation towards the production of different primary and secondary metabolites that influence the overall aromatic complexity of the alcoholic beverage [65–67]. Such mixed culture fermentation has become common in the wine and beer industries in an effort to improve the distinctive flavors of the alcoholic beverages. For example, the use of *S. cerevisiae* and *T. delbrueckii* lead to the production of beverages with elevated levels of higher alcohols, ethyl acetate and isoamyl acetate, and lower levels of ethyl hexanoate and ethyl octanoate [61] thus enhancing the taste and flavor. In the wine industry starter cultures are dominated by *Saccharomyces* species, but the need for new tastes to sort customer demands have presented great potential for non-*Saccharomyces* species to be used as part of the microbial consortia for fermentation [63,68]. To improve product quality and facilitate the production of a consistent product, the commercialization of non-cereal-based alcoholic beverages would therefore require cultures made up of microbial consortia.

## 5. Microbial Consortia for Fermentation

### 5.1. Yeasts

Yeasts are the most predominant fermentative microorganisms responsible for spontaneous fermentation of non-cereal-based alcoholic beverages. This is probably because yeasts are naturally found on the tree barks, leaves, and skins of fruits [69]. Grapes for example, harbor a number of fermenting yeasts on their skins such as *Kloeckera* spp., *Hanseniaspora* spp., *Candida* spp., *Pichia* spp., *Kluyveromyces* spp., *Metschnikowia* spp., and *Cryptococcus* spp. [67,70,71] with *Hanseniaspora uvarum*, *Pichia terricola*, and *Metschnikowia pulcherrima* being the three most representative species isolated to date [69]. The most well-known and best fermenting yeast, *S. cerevisiae*, however, is found in very low frequencies on some fruits such as the grape skins, and in vineyard soils [72]. Documented evidence suggest that in the early stages of fermentation, fruit surfaces are dominated by non-*Saccharomyces* yeasts, bacteria, and filamentous fungi [73,74] but *Saccharomyces* yeasts dominate the microbial population at the end of fermentation in anaerobic conditions. This suggests that *Saccharomyces* yeasts are the most dominant alcoholic fermentation microorganisms [75–77]. Even in the presence of oxygen, *S. cerevisiae*, is the predominant yeast species responsible for the production of ethanol [78,79]. The production of ethanol, toxic to other microbes, has been hypothesized to be a niche defense mechanism for *S. cerevisiae* [42].

Alcoholic fermentation is not exclusive to *S. cerevisiae* [19,26,47]. An important attribute of a brewer's yeast—an ability to ferment simple sugars, even in the absence or presence of excess O<sub>2</sub> [80], is present in non-conventional yeasts. Examples of non-conventional yeasts that have been isolated from alcoholic beverages include *Saccharomyces ludwigii* [81], *Candida tropicalis* [82], *Torulaspora delbrueckii*, *Zygosaccharomyces rouxii* [83], and *Pichia kluyveri* [22].

*Brettanomyces* spp., *Candida* spp., *Cryptococcus* spp., *Debaryomyces* spp., *Dekkera* spp., *Galactomyces* spp., *Geotrichum* spp., *Hansenula* spp., *Hanseniaspora* spp., *Hyphopichia* spp., *Issatchenkia* spp., *Kazachstania* spp., *Kluyveromyces* spp., *Metschnikowia* spp., *Pichia* spp., *Trichosporon* spp., *Rhodotorula* spp., *Rhodospiridium* spp., *Saccharomyces* spp., *Saccharomycopsis* spp., *Schizosaccharomyces* spp., *Saccharomycodes* spp., *Sporobolomyces* spp., *Torulopsis* spp., *Torulasporea* spp., *Zygosaccharomyces* spp., and *Yarrowia* spp. are among several species of non-conventional yeast strains isolated from fermented foods and alcoholic beverages elsewhere in the world [11]. A list of yeasts species responsible for fermentation of alcoholic beverages in the Sub-Saharan region is shown in Table 2.

## 5.2. Bacteria

Bacteria are known to ferment simple sugars but the end product is usually non-alcoholic if allowed to ferment exclusively. Exceptions of alcohol producing bacteria such as *Zymomonas mobilis*, *Clostridium acetobutylicum*, and *Klebsiella pneumonia* exist. *Z. mobilis* is a natural ethanologen which possess desirable characteristics such as high specific productivity, high alcohol tolerance, a broad pH range for production (pH 3.5 to 7.5) and it is generally regarded as safe (GRAS) [84]. The bacterium is mostly utilized for biofuel production [85] although it has also been isolated from some alcoholic beverages such as *pulque* [11,54] and palm wine [86] where it is responsible for the production of ethanol, CO<sub>2</sub>, acetaldehyde and H<sub>2</sub>S [87]. *C. acetobutylicum* ferments sugars into a mixture of organic solvents namely acetone, butanol, and ethanol [88,89] while *K. pneumonia* produces ethanol, lactate, and acetate [90]. However, their applications in alcoholic beverages are not ideal for human consumption because they produce high levels of acetone and butanol which are harmful to humans.

Lactic acid bacteria (LAB) are known for the sour taste and low pH they confer to the product due to lactic acid production. LAB are present in a significant number of fermented alcoholic beverages (Table 2) probably because of their ubiquity in nature. In the brewing of alcoholic beverages, these bacteria bring about multistep transformation of the raw material (carbohydrate substrates), which would be otherwise impossible to accomplish with yeasts alone. For example, when a substrate cannot be utilized by yeasts alone, the LAB can break it down into simple monomers thus allowing better utilization of the substrate. Major genera of the LAB species isolated from various fermented beverages include *Alkalibacterium* spp., *Carnobacterium* spp., *Enterococcus* spp., *Lactobacillus* spp., *Lactococcus* spp., *Leuconostoc* spp., *Oenococcus* spp., *Pediococcus* spp., *Streptococcus* spp., *Tetragenococcus* spp., *Vagococcus* spp., and *Weissella* spp. [91].

Acetic acid bacteria are also found in most traditional alcoholic beverages contributing to the pH and taste of beverages through the production of acetic acid [92]. The combination of lactic acid and acetic acid have been reported to enhance the flavor of alcoholic beverages [9,60,93,94]. Acetic acid is produced from the conversion of ethanol by *Acetobacter* spp. under aerobic conditions making the alcoholic beverage sour due to an increase in titratable acidity as well as a lower pH of the beverage [10,12,62].

## 6. Microbial Identification Methods

The isolation and correct identification of microbial isolates from a complex microbial community associated with the substrate used for fermentation as well the product of fermentation is a very important starting point for commercialization of non-alcoholic beverages. Recovery of microbes from substrates involves classical extensive sampling, incubation in media of choice depending on the purpose of the study, and then subsequent selection of colonies for further characterization. There are a number of reviews giving comprehensive details on how microbes involved in fermentation are isolated [11,95–97]. Herein we outline some of the identification methods available and their advancements.

Classical identification methods (such as culturing, enumeration, isolation, substrate utilization test, substrate assimilation test, staining and biochemical tests, to list a few) entailing the characterization of morphological, biochemical and physiological traits, have been widely accepted and described in the following texts [9,34,98,99]. The classical identification methods have several limitations including incorrect identification, poor resolution and inconsistent reproducibility due to

the dependence on the physiological state of the cells when compared to molecular identification methods. Molecular identification methods can be classified into culture dependent and culture independent techniques.

## 7. Culture Dependent Identification of Microbes

The use of culture media to isolate microbes from substrates used for fermentation as well as microbes found in alcoholic beverages is the most prominent method. The method involves aseptic acquisition of material and growing microbes in culture and choice before identification of the colonies of choice using DNA. The principles that underlie molecular methods have been extensively addressed [99]. In brief, the amplification, restriction analysis as well as sequencing of ribosomal DNA has been used to quantify and characterize the microbial consortia in alcoholic beverages. The field is evolving fast as extensively reviewed elsewhere [100]. Non-cereal-based alcoholic beverages such as *tej*, *burukutu*, *agadagidi* and *mukumbi* have had their microbial characterization performed using culture dependent molecular methods [13,27,101,102].

The culture-dependent methods have added value by giving insight on the sugars the microorganisms can utilize during fermentation. For example, some of the yeasts isolated from *masau* fruits are able to utilize glucose, sucrose, maltose, galactose and raffinose while others were able to assimilate glucose, galactose, cellobiose, lactose, maltose, saccharose, trehalose, melezitose, raffinose, L-lysine, cadaverine and creatine [9]. The assimilation test is essential as it signifies which is the best chemical media to use for the isolated microbe at industrial scale, ensuring maximum utilization of the substrate to produce the product. The pitfall of culture-dependent methods however, it that they underestimate the species richness of non-culturable microorganisms that may play major roles in production of fermented beverages. Examples of culture-dependent identification methods include DNA extraction from cultured microbes and the use of species-specific PCR primers for species level identification [11].

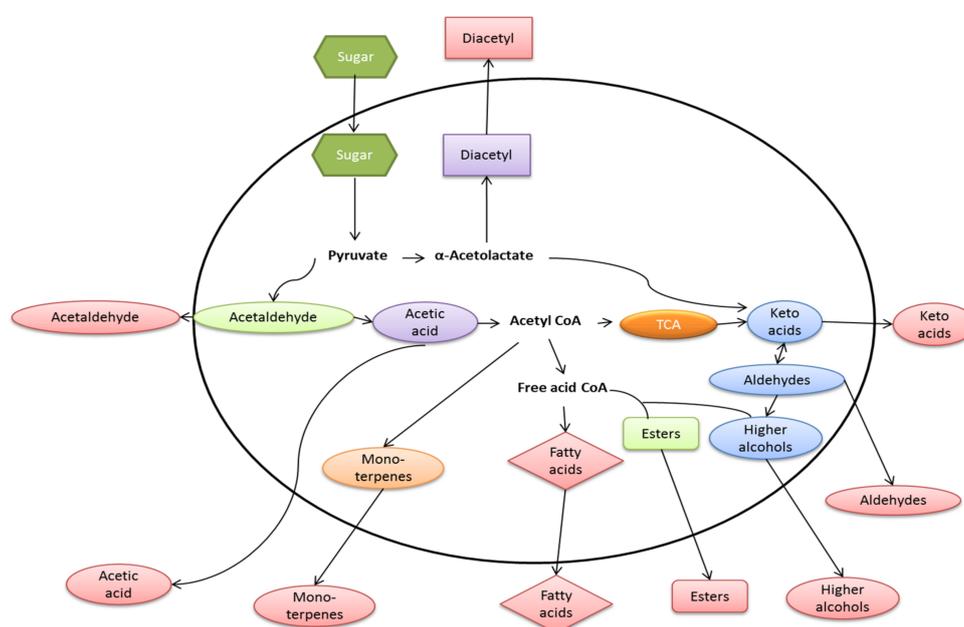
## 8. Culture-Independent Identification of Microbes

While microbial consortia involved in fermentation include bacteria, only 1% of bacteria are cultivable. Profiling of both culturable and non-culturable microbial populations from fermented beverages is therefore attractive. Culture-independent methods entail the use of molecular methods without first culturing microbes on media. Culture-independent methods such as direct DNA extraction from fermented products that represent the ecological niche, have therefore become common [35,85,103,104]. The method allows for detection of more species that would otherwise not be detected by culture-dependent methods. Therefore a less biased picture of species richness is possible [103,105]. For best results, both culture-dependent and culture-independent methods are used simultaneously. For instance in the work performed by Stringini et al. on palm wine, culture-dependent methods detected *S. cerevisiae* (as the predominant species), *S. ludwigii* and *Z. bailii* while a culture-independent method employing denaturing gradient gel electrophoresis (DGGE) analysis further detected *H. uvarum*, *C. parapsilopsis*, *C. fermentati* and *P. fermentans* [81] from the same sample.

## 9. Chemical Products of Fermentation

During alcoholic fermentation, a multitude of aromatic primary and secondary metabolites are produced [105]. Primary metabolites are products of metabolism that are produced during the growth phase of an organism. These perform the physiological functions and support in the overall development of the cell such as hormones, glycerol and enzymes [106–108]. Secondary metabolites, on the other hand, are the end products of primary metabolism that are synthesized after the growth phase has been completed. Such metabolites are vital in the ecological function of the cell. Examples of secondary metabolites include esters, alkanols, organic acids, phenols, aldehydes and ketones (Figure 6). The combination, variety and abundance of aromatic molecules are pertinent to sensory properties of non-cereal-based alcoholic beverages and thus their unique flavor profile.

Different substrates have different types and amounts of fermentable sugars and amino acids. The presence of different microorganisms in different concentrations during fermentation, each with their varying fermentable sugar preference and primary metabolic requirements leads to various species-specific pathways being turned on and resulting in production of different secondary metabolites. This is shown in both Tables 1 and 3. These attributes affect the overall taste and flavor of alcoholic beverages. Differential production of higher alcohols amongst microorganisms contributes to the distinctive beer flavors. Examples of higher alcohols that intensify flavor characteristics of beers include n-propanol, iso-butanol, 2-methylbutanol and 3-methylbutanol [109]. Due to their known antifungal activity, higher alcohols can also increase shelf life of the alcoholic beverage by acting as a preservative. Some of the secondary metabolites such as 2-methyl-1-propanol, 2/3-methyl-1-butanol, ethyl lactate and ethyl acetate, which are typical aromatic volatiles reported in beer, wines and spirits, have been isolated in non-cereal-based alcoholic beverages such as *khadi* [56]. Figure 6 summarizes secondary metabolites produced by yeasts.



**Figure 6.** Basic yeast metabolism. Secondary metabolites are shown in (pink) and metabolism intermediates (lime, orange, purple and blue) are also shown.

Esters are formed through esterification of activated fatty acids and alcohols [109]. The amount of esters produced during fermentation depends on the abundance of the corresponding alcohols and acyl CoAs [67]. Esters can either be found in the starting material albeit at lower concentrations [110] or formed intracellularly by microorganisms. This is the most important group of secondary metabolites essential for aromatic characteristics as they impart a fruity and sweet note to alcoholic beverages during fermentation. Esters are divided into acetate esters (synthesized from a higher alcohol or ethanol with acetic acid) and medium-chain fatty acid ethyl esters (made from a medium-chain fatty acid and an ethanol radical) [111,112].

Some secondary metabolites act as preservatives, some of which include organic acids such as oxalic, citric, tartaric, malic, ascorbic, fumaric, lactic, and acetic acids. Organic acids are responsible for the rapid acidification of alcoholic beverages [113]. They lower pH to below 4.6, which inhibits the growth of unwanted spoilage microorganisms [10].

Not all secondary metabolites contribute positive traits to the alcoholic beverages. For example, acetaldehyde has an unpleasant pungent odor at higher concentrations, imparting a freshly cut green leaves flavor to the beverage [114]. Methanol and its metabolites such as formaldehyde and formic acid,

are produced during microbial fermentation of alcoholic beverages like *kachasu* of Zimbabwe [37] and pose health challenges due to their toxicity effects [115]. The toxicity of *kachasu* due to the presence of isoamyl alcohol, iso-butanol, acetone, methanol, ethylacetate, and furfurals has led to the prohibition of *kachasu* in Zimbabwe since 1971 [5]. Methanol toxicity in distillates has also been reported in *kanyanga* (although a cereal-based distillate) of Burundi [116]. Secondary metabolites of alcoholic fermentation are summarized in Table 3. Their contribution to the flavor of the alcoholic beverage is also briefly noted.

**Table 3.** Contribution of secondary fermentation metabolites to flavor and aroma of alcoholic beverages.

Metabolite Class	Examples of Compounds	Contribution to Flavor and Aroma	References
Organic acids	Succinic acid, acetic acid, lactic acid, citric acid	These compounds contribute to the astringency of fermented beverages. The presence of some acids, notably lactic acid, can indicate undesirable bacterial spoilage.	[72,73,117]
Higher alcohols	Isoamyl alcohol, phenylethanol, isopropanol	These impart desirable flavor and aromas to fermented beverages but only within certain limits	[114]
Carbonyl compounds	Acetaldehyde, aldehydes, ketones	Above its flavor threshold in beverages, this compound can impart a “grassy” or “green apple” (related to acetaldehyde) flavor but this can be removed by secondary yeast fermentation during conditioning.	[72,76]
Vicinal diketones	Diacetyl, pentane-2,3-dione	Diacetyl in most beverages is undesirable, imparting a rancid-butter or “butterscotch” flavor.	[118–120]
Polyols	Glycerol	This compound is produced during normal yeast metabolism or when yeasts are confronted with osmotic stress. Glycerol contributes desirable viscosity to fermented beverages, notably wines, the body of the wine per se.	[21,76,121]
Sulfur compounds	Hydrogen sulphide, dimethyl sulphide, sulphur dioxide, Thiols	These are important beverage flavor and aroma compounds. For example, in beer, dimethyl sulphide (DMS) in low concentration is a desirable attribute of lagers, but higher concentration imparts off-flavors.	[67,122]
Esters	Ethyl acetate	Associated with a fruity aroma and floral flavors and aromas to fermented beverages.	[79,80]

## 10. Influence of Ecology on Fermentation Microbes

The substrates used in fermentation provide ecological niches for different microbial species [70]. An ecological niche is an ecosystem that comprises of microbial species within a particular environment. The precise ecological niche of a microbe is primarily determined by the specific metabolic properties of that organism. The microbes are characterized by different physiological traits, which account for a plethora of effects upon production of the beverages. Microbial consortia found in beverages after fermentation are only those that manage to survive and reproduce in the beverage.

The abundance of the microbes before and after fermentation is generally not proportional. Due to limited literature, the ecological niches are not well understood but microbial diversity on the substrates suggests that the substrates have enough sugars that help the microbes survive and influence fitness for competitive survival of particular species. Additionally, the pH levels of these substrates (Table 1) and the resultant chemical ecology produced by the fermentation process would also have a great influence on the microflora that would dominate fermentation.

Some of the studied ecological niches associated with non-cereal based beverages include the *masau* fruits (*Ziziphus mauritiana*) [9], unfermented palm sap [123], *marula* fruits [124] and honey [125]. The *masau* fruits (*Ziziphus mauritiana*), used to produce *kachasu*, have had their ecology studied using unripe, ripe, dried and fermented fruits. The fruits harbored different microbes which included fermentative and non-fermentative yeasts. *A. pullulans*, *Cryptococcus flavus* and *R. mucilaginosa* were isolated from the unripe fruits, *S. cerevisiae*, *I. orientalis*, *A. pullulans*, *Cryptococcus magnus*, *Z. hellenicus*, *C. parapsilosis*, and *C. pyralidae* were isolated from the ripe fruits, *P. fabianii*, *S. fibuligera*, *A. pullulans* and *P. ciferrii* were isolated from the dried fruits and, *S. cerevisiae*, *I. orientalis*, *P. fabianii*, *S. fibuligera*, and *H. opuntiae* were isolated from the fermented fruits [9]. The above variations show

the different fermentative and non-fermentative strains (some of which included non-conventional yeasts) isolated from different ecological niches.

## 11. Microbial and Chemical Characteristics of Selected Alcoholic Beverages

The chemical complexity of the non-cereal-based alcoholic beverages is due to mixed culture fermentation as illustrated in Table 2. The beverages are produced from varying raw materials which impart unique flavors to the brews. Details of the microbial and chemical characteristics of selected non-cereal based alcoholic beverages are given below.

## 12. Palm Wine

Palm wine is an alcoholic beverage produced from the spontaneously fermented sap of tropical plants of the *Palmae* family [123]. The African oil palm (*Elaeis guineensis*), date palm (*Phoenix dactylifera*), nipa palm (*Nypa fruticans*), kithul palm (*Caryota urens*), and raffia palm (*Raphia hookeri*) are among the examples of palm trees from which the carbohydrate-rich sap can be obtained. Palm wine has an alcohol content ranging from 1.5% to 7.1% (*v/v*) and a pH from 4.0 to 5.5 [113]. Palm wine is known the numerous following names in West Africa; *mu*, *bandji*, *ogogoro*, *nsafufuo*, *nsamba*, *mnazi*, and *yongo*. The beverage is not unique to Africa, as it also popular in India where it is known as *toddy* or *tari* [11]. Palm tree sap is tapped (either through inflorescence tapping or stem tapping) and allowed to undergo spontaneous fermentation [35]. Like any other alcoholic beverages, the conversion of the sugary substrate leads to increased nutritional components. The aromatic complexity of the wine is due to the production of compounds produced such as esters, carbonyls, alcohols, phenols, acids, sulphur compounds, terpenes, hydrocarbons, nitrogen compounds, and lactone [63,79,80,126].

Nwachukwu et al. [54] reportedly isolated *Staphylococcus* spp., *Lactobacillus* spp., *Micrococcus* spp., *Serratia* spp., *Bacillus* spp., *Streptococcus* spp., *Saccharomyces cerevisiae*, and *Candida tropicalis* from Nigerian palm wine. Similar work on the microbial profile of palm wine-isolated *S. cerevisiae*, *S. chevalieri*, *Zymomonas mobilis*, *Hanseniaspora guilliermondii*, *H. uvarum*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *L. nagelii*, *L. succicola*, *Acetobacter pasteurianus*, *A. indonesiensis*, and *Gluconobacter* spp. which were detected using both culture-dependent and culture-independent methods [35]. Some of these isolated microbes have also been detected from the unfermented palm sap, meaning they are responsible for the fermentation of the palm wine [81]. Collectively, these investigations suggest that palm wine has diverse fermenting microbes that cooperatively influence the flavors and aromas of the final product.

### 12.1. Urwagwa

*Urwagwa* is an alcoholic beverage produced from the fermentation of bananas (*Musa acuminata*). The banana beverage is common in Rwanda (*urwagwa*) [42], DR Congo (*kasiksi*), Burundi (*isongo*) [116], Uganda (*tonto*) [127], Tanzania (*mbege*) [128] and Kenya (*urwaga*) [127] where it is known by other local names. There have been reports of *urwagwa* having an alcoholic content ranging from 7% to 18.1% (*v/v*) [34,84]. The process of fermentation [42] involves the extraction of the banana juice from ripe bananas (which contain glucose, sucrose and fructose) by addition of water to the banana juice in the ratio of 3:1. Filtration of the diluted smooth mixture is carried out through grass held in a clay pot or wooden tank. Germinated, dried and ground sorghum is added to the diluted banana juice and the fermentation broth is then covered with banana leaves and split banana stems and incubated for 2 to 4 days. *Saccharomyces cerevisiae* is reported as the major fermenting microorganism [116] as well as lactic acid bacteria, yeast, molds and aerobic mesophilic bacteria, but these have not been identified at species level [42].

Spontaneous fermentation rarely is a result of a single fermenting microorganism. The other sources of fermenting microbes in this beverage are likely the clay pot, the grass sieve, the banana leaves, as well as the non-sterile preparation of the fermenting carbohydrate sap. All these sources of spontaneously fermenting microorganisms are never a pure culture. Therefore, further microbial determination needs

to be done to identify the fermentative microorganisms. The aromatic profile of the beverage has been linked to secondary metabolites such as esters (ethyl isocyanoacetate, ammonium acetate, ethyl ester and ethyl acetate), alcohols (methyl alcohol, ethanol, 1-propanol, propane-1,3-diol and cyclohexanol) and acids (acetic acid, propanoic acid, methyphosphonic acid, benzoic acid and 5-hexanoic acid) [53].

### 12.2. Tej

Tej is a home processed, but also commercially available wine produced from honey. The honey is supplemented with sugar before fermentation. A very high alcoholic content of the product has been reported to be in the range of 8.94% to 13.16% (*v/v*) and pH ranges from 3.56 to 4.45 are common [28]. The honey wine is also produced and consumed in Cameroon where it is known as *kuri* [129]. Tree barks or roots of some plants or herbal ingredients are added to improve the flavor whereas the fermentation pot is seasoned by smoking over smoldering *Rhamnus prinoides* stems and olive wood [17,63]. Fermentation is run for 5 days, in warm weather, or for 15 to 20 days, in colder cases [27].

The mixture is stirred daily and finally filtered through a cloth to remove sediment and *R. prinoides* residues [7]. A yellow, effervescent and cloudy brew is a characteristic of good quality tej (Figure 4). Commercial honey wine (mead) is made using pure cultures of yeasts and they produce aromatic volatile compounds that include alcohols, organic acids, esters, volatile fatty acids, carbonyl compounds, and volatile phenols [111,130]. Aromatic volatile compounds are responsible for the unique flavor of mead as well as its overall sensory characteristics [75,86].

In contrast to the commercial honey wine, *S. cerevisiae*, *Kluyveromyces bulgaricus*, *Debaromyces phaffii*, *K. veronae*, *Zygosaccharomyces rouxii*, *Hansenula subpelliculosa*, *S. norbensis*, *K. vanudenii*, *Endomycopsis burtonii*, *Lactobacillus* spp., *Streptococcus* spp., *Leuconostoc* spp., and *Pediococcus* spp. have been isolated from the homemade beverage using culture-dependent methods [11,17]. Honey naturally contains various osmotolerant yeasts that prefer low pH environments [120]. Some of which include *Saccharomyces* spp. (widely found), *Rhodotorula* spp., *Debaryomyces* spp., *Hansenula* spp., *Lipomyces* spp., *Oosporidium* spp., *Pichia* spp., *Torulopsis* spp., *Trichosporon* spp., *Nematospora* spp., *Schizosaccharomyces* spp., *Schwanniomyces* spp., *Torula* spp., and *Zygosaccharomyces* spp. [125]. These yeasts have been observed to possess the ability to convert glucose and fructose from honey into ethanol and acids. Other species in the genera *Saccharomyces* spp., *Debaryomyces* spp., *Hansenula* spp., *Lipomyces* spp., *Pichia* spp., *Schizosaccharomyces* spp., *Torula* spp., and *Zygosaccharomyces* spp. have been reported to have been isolated from honey wine [120].

### 12.3. Pineapple Wine

Pineapples (*Ananas comosus*), are cultivated in Angola and they have a significant proportion of sugar and acids that make them ideal for wine production [38]. The production of wine involves fermentation of peeled, sliced and pressed pineapple. The juice is allowed to ferment for 3 days yielding a product with a final alcoholic content of 6.0% to 7.0% (*v/v*) [38,60] and a pH of approximately 3.78 [59]. *Hanseniopsis guilliermondii*, *Pichia anomala*, *Meyerozyma guilliermondii*, *H. uvarum*, *Wickerhamomyces anomalus*, and *H. opuntiae* have been reported to be responsible for fermentation [38]. Pineapple wine aroma complexity has been reported to be from compounds such as acetic acid, ethyl acetate, iso-butanol, 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl 2-methylpropanoate, 3-methyl-1-butyl acetate, ethyl hexanoate, methyl benzoate, 2-phenylethanol, methyl 2-methylheptanoate, ethyl benzoate, diethyl succinate, ethyl octanoate, ethyl phenylacetate, 2-phenylethyl acetate, ethyl 9-decenoate, and ethyl decanoate [87,88]. These compounds influence the overall flavor and taste of the wine.

### 12.4. Muchema

Muchema is an alcoholic beverage produced from the spontaneous fermentation of the *Hyphaene petersiana* (*mokolwane/moxao*) tree sap, popular in *Shorobe*, Botswana. Palms grow well in areas with a shallow, slightly saline water table such as salt pans [131]. *Hyphaene petersiana* is therefore a common tree species in such areas of Botswana, Namibia, Zimbabwe, Zambia, Tanzania, Angola, Rwanda, Burundi

and many other Sub-Saharan countries. The sap that is used to make the beverage is harvested and allowed to ferment for 3 to 5 days. The drink is known as *omalunga* in Namibia but its production is forbidden [57]. There is no literature available with regards to the microbial and chemical profile of this beverage, and thus more research needs to be undertaken.

### 12.5. Khadi

*Khadi* is a reddish-brown traditional alcoholic beverage made from the fermentation of *Grewiaflava* (*mogwana*) fruits (Figure 3). The beverage has been reported to contain an alcohol content of between 1.66% and 5.71% (*v/v*), with a pH from 2.87 to 3.16. The beverage has been compared favorably to commercial wine [56]. Brown sugar is added to the fruit juice before fermentation. One would posit that the sugar is required to aid fermentation of the juice or increase alcohol content. However, there is no available literature documenting the sugar content in the *Grewia flava* fruits. The other fruits used in *khadi* fermentation include *Grewia occidentalis* (*moretlwa*), *Grewia flavascens* (*mokgomphatha*), *Kedrostis hirtella* (*mogakangwaga*), and *Khadia acutipetala* [29]. The most well-known technique used in brewing *khadi* is the back-slopping fermentation technique where an already fermented *khadi* beverage is used as a source of starter culture for the brewing. A Global Status Report on Alcohol by the World Health Organization (WHO) (2004) reported that the mixing in of mashed wild pumpkins, wild fruits, wild tuberous roots, can occur in the production of *khadi* [132].

Unorthodox production of this beverage has been known amongst the small scale producers to also include marijuana, car battery acid, tobacco, dagga, during preparation [26]. The reasons why such a harmful additives are used are not documented although some villagers claim that the battery acid increases intoxication [26]. The aromatic complexity of this beverage has been reported and it is said to contain 2-methyl-1-propanol, 2/3-methyl-1-butanol, ethyl lactate, and ethyl acetate [56]. These aromatic volatile compounds have also been reported in commercial beer, wines and spirits as flavor compounds [56]. Although the beverage is seemingly popular amongst *Batswana*, there is no literature available documenting the microbiological characteristics of *khadi*. The characterization of microbiological diversity and consequent chemical profiling of *khadi* will be important to ascertain the resultant variations of the beverage.

### 12.6. Setopoti

*Setopoti* is an alcoholic beverage made from watermelons (*Citrullus lanatus*). The watermelon pulp is collected in a clay pot which is then tightly closed. The preparation is then allowed to spontaneously ferment for 72 h undisturbed. After fermentation, the alcoholic beverage is sieved to remove seeds and solids, and served as a smooth fermented beverage. The alcoholic beverage is commonly consumed in the Central district of Botswana, more specifically in the *Tswapong* region. Watermelons that were not fresh (regarded as waste) were traditionally used to reduce post-harvest losses. Nowadays, fresh watermelons are also being used. The biochemical and microbiological diversity of *setopoti* is yet to be determined as there is no literature available regarding the alcoholic beverage.

### 12.7. Mukumbi

*Mukumbi* is a non-cereal-based alcoholic beverage consumed in many Zimbabwean villages as well as in semi-arid regions like Botswana. *Mukumbi* is traditionally prepared by spontaneously fermenting a mash prepared from ripe fruits of the *marula* plant (*Sclerocarya birrea* sub-species *caffra*) [133]. This alcoholic beverage is also produced and consumed in many SADC countries such as Namibia, Botswana, Swaziland, Zimbabwe and Zambia [9], and known by different names such as *Omagongo* in Namibia [57], *buganu* in Swaziland [134] and *bojalwa-jwa-morula* in Botswana. The *marula* fruits are peeled using a knife and put into a clay pot with the peels and pounded to extract the sugary juices. The juice is then allowed to spontaneously ferment for 3 days. This suggests that the microflora from the fruit surfaces, the clay pots as well as from the hands of the handlers are involved in fermentation.

Once fermentation is complete, the alcoholic beverage is sieved to remove the residual fruit skins. The product is then served with a final alcohol content of about 15% (v/v) [57].

The microorganisms isolated through culture-dependent methods from *mukumbi* include *Saccharomyces cerevisiae*, *Pichia anomala*, *P. guilliermondii*, *Candida tropicalis*, and *C. intermedia* [101]. *Aureobasidium pullulans*, *Geotrichum capitatum*, *Trichosporon brassicae*, *Rhodotorula mucilaginosa*, *Hansenula anomala*, *H. jadinii*, and other *Hansenula* species have been isolated from ripe *marula* fruits [124], and some are involved in the fermentation of the *marula* juice. The *marula* fruit naturally contains sucrose, fructose, and glucose, as such, the yeasts isolated from the *mukumbi* were tested to ascertain their ability to ferment these sugars. The results showed they (*Saccharomyces cerevisiae*, *Pichia anomala*, *P. guilliermondii*, *Candida tropicalis*, and *C. intermedia*) could ferment glucose, galactose, maltose, sucrose, raffinose with an exception of *P. guilliermondii* which could not ferment galactose, raffinose, and maltose and, all the strains could not ferment lactose [101]. The unique sensory properties associated with this beverage are due to mixed culture fermentations, the unique tasting fruit and the resultant secondary metabolites that are yet to be fully elucidated.

### 12.8. Kachasu

*Kachasu* (also known as *tototo*, *lukutu* or *nipa*) is a traditionally fermented, highly intoxicating distilled alcoholic spirit with an alcohol content of 9% to 41% (v/v). *Kachasu* has been reported to be similar to *waragi* of Uganda and *chang'aa* of Kenya [5,127]. It is usually brewed using maize meal but bulrush or finger millet meal, various fruits such as *masau* (*Ziziphus mauritiana*), *Adansoniadigitata*, *Tamarindusindica* [37,133] and banana peels may be used as alternative sources of carbohydrates [5]. The carbohydrate source such as *masau*, is added to warm water in a pot with a hole drilled on the side, which is used later during the distillation of the spirit. The mixture is stirred into a slurry and allowed to simmer for a few minutes before the pot is removed from the fire. Sugar and yeast are added after the slurry has been cooled to ambient temperature. The hole in the pot is sealed with clay and the mixture allowed to ferment for 4 to 7 days at ambient temperature.

The fermented brew is distilled over a small fire and the clear distillate is collected from the end of the pipe into bottles. Toxicity may be attributed to several co-generic alcohols such as isoamyl alcohol, iso-butanol, methanol, and other volatile compounds [5]. The other organic compounds, which have been identified in *kachasu* include acetaldehyde, acetone, ethyl acetate, and furfurals [5]. *Kachasu* production uses *masau* fruits (*Ziziphus mauritiana*) as a substrate which naturally contains microbes such as *Saccharomyces cerevisiae*, *Issatchenkia orientalis*, *Pichia fabianii*, *Aureobasidium pullulans*, *Lactobacillus agilis*, *L. minor*, *L. confusus*, *L. fructosus*, *L. bifementans*, *L. divergens*, *L. fermentum*, *L. hilgardii* and *Streptococcus* spp. [9].

## 13. Plantain Beverages

*Agadagidi* is an alcoholic beverage produced from the fermentation of ripe plantain pulp (*Musa paradisiaca*) consumed in the South-western Nigeria and Cameroon, with a 6.57% (v/v) alcohol content and pH of 3.6 [92,102]. *Agadagidi* production involves packing, peeling, slicing of plantains/bananas in an earthenware pot, and covering with water. The pot is then covered tightly and the product allowed to ferment at room temperature for a period of 1 day to 5 days after which the juice is strained and the *agadagidi* is ready for consumption [102]. Plantain can also be used to produce wines with a pH from 4.1 to 4.4 and spirits with a pH from 2.8 to 3.3 [30]. The plantain wines are distilled and can have a reported final alcohol content of 47.4% (v/v) which is later decreased to 19.8% (v/v) [30]. The fermentation of *agadagidi* is carried out by *S. cerevisiae*, *S. chevalieri*, *Lactococcus lactis*, *Bacillus subtilis*, *Brettanomyces intermedius*, *Kloeckera apiculata*, *Candida tropicalis*, *C. krusei*, *Hansenula anomala*, *Pediococcus cerevisiae*, *L. mesenteroides*, *Staphylococcus epidermidis*, and *Micrococcus leteus* [102,135]. There is no literature on the chemical characterization of these beverages.

## 14. Cassava Brews

Cassava (*Manihotesculenta*) is an important nutritional crop for many people in Africa [45]. Additional to its dietary consumption, cassava can be used as a substrate for fermentation. Cassava is used in the production of traditional spirits with an alcohol content of 7.8% to 26.3% (v/v) and a pH from 3.5 to 4.3 [30]. The production of alcoholic beverages from cassava is common in Africa as well as Brazil [136]. The cassava tuber is cleaned and soaked in water for 7 days. After 7 days, the tubers are peeled and grinded to form a pulp which then has water added to it before being left for 3 days to ferment [137]. Upon completion of fermentation, the product is sieved to remove any solids, and the filtrate is ready for consumption. Traditional production of cassava spirits uses cassava flour, sorghum grains, and baker's yeast. The process follows the steps of sorghum beer production (malting, souring, boiling, mashing, and straining) but using cassava flour and sorghum flour. The fermentation is carried out for 4 days by baker's yeast and the final product is distilled using a drum at the outlet pipe sunk in cold water [137]. The chemical and microbial diversity of traditional African alcoholic cassava brews is yet to be performed and documented.

In the Brazilian beverage *caxiri* (produced from cassava and sweet potatoes), researchers isolated *Saccharomyces cerevisiae*, *Pichia membranifaciens*, *P. guilliermondii*, *Cryptococcus luteolus*, *Sphingomonas* spp., *Pediococcus acidilactici*, *Bacillus pumilus* and *B. subtilis* [138]. In another Brazilian beverage namely, *taruba*, they isolated *L. plantarum*, *L. brevis*, *L. mesenteroides*, *B. subtilis*, *Torulaspora delbrueckii*, *P. exigua*, *Candida rugosa*, *C. tropicalis*, *P. kudriavzevii*, *Wickerhamomyces anomalus*, *C. ethanolica*, *Bacillus amyloliquefaciens*, *B. licheniformis*, *Hanseniaspora uvarum*, and *Chitinophaga terrae* [136].

Cassava in the form of *gari* (a starchy powder produced from cassava) is added to the sorghum malt during the production of *burukutu* as an adjunct [87]. Adjuncts are starch or sugar containing materials added in addition to the carbohydrates in the malt to boost the level of fermentable carbohydrate. *Burukutu* (brown colored suspension with a vinegar-like flavor) is an indigenous alcoholic beverage produced and consumed in the Republic of Benin, Ghana, Northern Guinea and Savannah region of Nigeria [139]. The substrates are fermented by *Saccharomyces cerevisiae*, *S. chevalieri* and *Leuconostoc mesteroides* [6].

## 15. Conclusions

Non-cereal carbohydrate substrates for the production of alcoholic beverages are an attractive commercialization initiative. Although cereal-based beverages are well known in different parts of the African continent, there is an immense diversity of alcoholic beverages made from non-cereal fermentable substrates in the sub-Saharan African region. A limitation is that non-cereal-based alcoholic beverages are less documented, although they exhibit very rich aromatic properties that are attractive for commercialization. Their unique sensory properties could be largely influenced by a rich microbial diversity in the sub-Saharan region which stems from its well-known undisturbed ecosystems. Such an immense microbial biodiversity in the region is applicable for use as fermentation consortia. The lack of robust documentation and minimal exploration remains an untapped potential of the sub-Saharan region.

Diverse microbial species with a preference for different fermentable sugars and ecological niches result in distinct aromatic and flavor profiles. Effort needs to be directed towards ascertaining the interplay of particular combinations of aromatic molecules produced by microbial consortia as it is a major factor for the production of distinctively flavored beverages. Equally significant, is the climatic differences amongst the Sub-Saharan countries which could also be an added advantage as such variances affects wild and cultivated fruit, tree sap, honey, root and tuber sugar compositions and hence leads to differential sensory profiles of beverages. The commercialization of such beverages in the modern Africa could be of much needed economic importance. The increased demand for a variety of beverages and lifestyles clearly calls for the scientific community to work towards meeting consumer demands. The use of mixed consortia which produce metabolites such acetic acid and

lactic acid and other natural food preservatives which prolong the shelf life of fermented traditional alcoholic beverages and reduce the use of chemical food preservatives is an added advantage.

The mining of literature, the harnessing of traditional beverage processing knowledge among diverse communities and conducting research to document the microbial and chemical profiles of non-cereal-based alcoholic traditional beverages of the Sub-Saharan region is important for establishment of knowledge towards commercialization of non-cereal based alcoholic beverages.

**Conflicts of Interest:** The authors declare no conflict of interest.

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