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Determination of Sugars, Amino Acids, pH and Alcohol in Bamboo Beverage from Southern Highlands, Tanzania

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Abstract
The amounts of sugars and amino acids play significant roles in defining the fermentation process and quantifying the alcohol levels in beverages, while pH affects the biological stability, colour, oxidation rate, and protein stability of alcoholic beverages. This study investigated the sugar content, amino acids, alcohol levels, and pH of bamboo beverage from Tanzania's southern highlands. During storage, the sugars significantly decreased ($p < 0.05$), especially when kept at room temperature from 52.96 to 0.00 (source 1), 53.35 to 0.00 (source 2) and 53.57 to 0.00 (source 3) g/L for fructose, from 47.93 to 14.78 (source 1), 47.23 to 14.91 (source 2) and 47.61 to 14.77 (source 3) g/L for glucose, and from 0.40 to 0.00 (source 1), 0.36 to 0.00 (source 2) and 0.37 to 0.00 (source 3) g/L for sucrose after six days of storage. A total of 15 amino acids were determined from the bamboo beverage with tyrosine being the most prevalent (597.68 mg/L for source 1, 599.44 mg/L for source 2 and 597.83 mg/L for source 3), followed by valine (261.13 mg/L for source 1, 261.24 mg/L for source 2 and 262.54 mg/L for source 3), threonine (76.69 mg/L for source 1, 76.91 mg/L for source 2 and 77.13 mg/L for source 3), and serine (66.37 mg/L for source 1, 67.23 mg/L for source 2 and 66.68 mg/L for source 3). After six days of storage at room temperature, there was a significant decrease in pH from 4.04 to 3.63. Alcohol content ranged from 3.11 to 9.05% v/v at the room temperature storage. These results might facilitate the optimal use of bamboo beverages, which have been neglected due to lack of scientific information such as amino acid and sugar levels.

Keywords: Bamboo beverage, *ulanzi*, amino acids, sugars, alcohol content.

Introduction

Alcohol is an organic molecule that can be identified by one or more hydroxyl (OH) groups linked to an alkyl group's carbon atom. Alcohols come in a variety of forms, including propyl alcohol, methyl alcohol, ethyl alcohol, and butyl alcohol. However, for the production of alcoholic beverages, ethyl alcohol (ethanol) is the type used. Some of the other forms of alcohols may cause blindness or death when consumed (Christianah 2018). Since ancient times, alcoholic beverages have been a distinguishing element of many societies (Egea et al. 2016). In the past, humans produced various kinds of traditional, local and indigenous alcoholic beverages worldwide that were manufactured and consumed by local communities (Kubo 2014). Since the second millennium BC, literatures have provided ample evidence on human consumption of fermented foods and beverages, including alcoholic beverages (Kubo 2014). Wine is one of the most popular alcoholic drinks that have been consumed as beverage and health drinks. Along with the Bible, ancient Greek and Latin literatures have all cited this. In wine

fermentation, yeast strains with specific characteristics are needed, for instance, alcohol tolerant yeasts (Maicas 2020). Homemade alcoholic beverages like beer and wine may be the most popular alcoholic drinks consumed in various nations and have substantial economic impacts. Production techniques and consumption of these traditional beverages are also very localized (Fentie et al. 2020).

The entire African continent is dominated by traditional drinks. For instance, the alcoholic beverage burukutu is popular in Nigeria's rural communities and in impoverished urban neighbourhoods because it is less expensive than commercially made beer (WHO 2004). Domestically made beverages from the "informal sector" continue to dominate the market and the local drinking culture in Tanzania. These traditional alcoholic beverages are significant parts of Africans' everyday social, economic, nutritional, and cultural lives. In Tanzania, there are diverse traditionally processed beverages that exist, such as *mbege, denge, gongo (chang'aa), kiambule, kimpumu, komoni,* o*rubisi/amarwa, togwa, waini* and *ulanzi* (bamboo beverage) (Tarimo and Kaale 2022). These varieties of beverages produced are due to plenty of availability of different sources of raw materials including cereal grains, tubers, honey, plant sap and fruits. These beverages although not adequately accounted for, run into millions of litres per annum (WHO 2004). Unfortunately, their production is associated with wide quality variability, unpredictability and lack of routine scientific quality leading to uncertainties in the contents and quality of what is being consumed. These have caused different challenges such as deaths and various diseases due to consumption of local and traditional alcoholic beverages (Nikander et al. 1991). Characteristics of traditional alcoholic beverages are determined by their chemical, biological and physical compositions (Debebe et al. 2016). There is therefore, a need of establishing composition standards for the traditional alcoholic beverages in terms of parameters such as pH, alcohol content, types and compositions of sugars and amino acids, and toxicity. In addition, there is a need to establish quality control mechanisms associated with health risks such as the formation of unwanted fermentation by-products like methanol, butanol, acetaldehyde, and furfural.

Bamboos are fast-growing species in the grass family (*Poaceae*) and mainly grow in temperate and tropical regions of the world (Wang et al. 2017). Bamboos have more than 1500 uses and constitute one of the most economically important plants in the world (Nongdam and Tikendra 2014). Furthermore, bamboos play and have always played significant roles in the socioeconomic activities of rural and ethnic people. Among the widely used bamboo products are paper and handicrafts, homes, furniture, water pipes, storage containers, snacks, beverages (such as bamboo juice), and other essential household items (Nongdam and Tikendra 2014). Bamboo alcoholic beverage, a local beer (*ulanzi*), is one of the natural beverages consumed in the southern highlands part of Tanzania. The major raw material for this beverage is bamboo juice which is being harvested from bamboo shoots.

In Tanzania's southern highlands, the bamboo beverage has significant economic impacts. According to Haule (2015), the average annual household income in Songea in 2007 was TZS 599,794 and the average annual income from the bamboo beverage business, for households involved in the business of selling bamboo beverages, was TZS 418,823. This implies that approximately 69.7% of the household's annual revenue comes from the bamboo beverage (ulanzi) business. Despite being an important traditional product that greatly boosts Tanzania's economy, particularly in the southern highlands where it thrives, bamboo has experienced huge postharvest losses as a result of lack of important scientific information on its value chain. Lack of good handling practices, quality of yeast which is friendly in the fermentation process, untreated water, uncontrolled fermentation, lack of specifications as well as the absence of defined routine, makes the quality of this traditional local beer highly variable and

unpredictable both qualitatively and quantitatively. As a result, the traditional beer quickly degrades, giving it a limited shelf life and increased concentrations of toxic constituents. Information on bamboo beverage production, processing and preservation as well as its physical and chemical characteristics in the country is largely missing. Yet, such food characterisation knowledge can shed light on the significance of bamboo juices and the bamboo wine business, which is more of a way of living than an economic activity. There is a need of promoting this crop by implementing a methodological (holistic) approach throughout its value chain, i.e., from production point, characterization, processing, preservation, packaging, transportation, storage and marketing.

Few studies have been done to establish various contents in bamboo beverages such as alcohol content, acid content, and microbial contamination (Bangu et al. 2006). Moreover, the chemical composition of the beer, particularly the type of sugars, and amino acids of the bamboo juice have yet to be established.

Bamboo juice is a key element in the production of ulanzi. Therefore, understanding the amounts of sugars and amino acids in bamboo juice is crucial because it can help the processors of ulanzi beverages to produce a better quality beverage. The sugar content has a crucial role in determining the fermentation process and, subsequently, in quantifying the alcohol levels of the beverages after alcoholic fermentation (Berthels et al. 2004, Guillaume et al. 2007, Berthels et al. 2008). Fructose, glucose, maltose, sucrose, and maltotriose are the main fermentable sugars (Debebe et al. 2018). These fermentable sugars are transformed into alcohol and carbon dioxide by the yeasts during fermentation. The amino acids in the must, on the other hand, are quite significant since they will determine the flavour of the beverage. The must is a growth medium that includes fermentable sugars (fructose, sucrose, maltose and maltotriose), nitrogenous materials (amino acids, peptides, and proteins) (Ferreira and Guido 2018), nutritional components (vitamins, minerals, and polyphenols), and organoleptic properties (flavour and precursors) (Mas et al. 2014). Changes in the concentrations of amino acids in must will influence the nitrogen metabolism because the yeast amino acid is principally derived from the must amino acid (Ferreira and Guido 2018).

To the best of the researcher's knowledge, there are no data on sugars and amino acids in the regional beer, *ulanzi*, made in Tanzania's southern highlands. The indigenous people have named the bamboo beverage *ulanzi* (bamboo wine). Yet, there is no reliable data for the beverage to support its classification as a wine. Wine is typically a fermented product made primarily of grapefruit juice, plant sap, honey, or other fruit juices with an alcohol content that ranges from 9 to 13% by volume (Ozturk and Anli 2014, Bozoglu et al. 2015). Factors affecting alcoholic fermentation including pH, alcoholic content, amount of sugars and amino acids need to be established. Therefore, the purpose of this study was to determine the alcohol content, amino acid composition, pH, and sugar content of bamboo juice and *ulanzi* local beer.

Materials and Methods

Study location and production of bamboo beverage

This research was carried out in Tanzania's Southern Highlands in the Mlowa Ward, Makambako Council, Njombe District, in Njombe Region (Figure 1). The four main regions in Tanzania's southern highlands where bamboo shoots are grown for the production of bamboo beverages are Njombe, Iringa, Mbeya and Songwe. The bamboo shoot needs three years from planting to beverage production. These resemble trees that, once planted, remain in place forever. Bamboo shoots growing close to a homestead only naturally produce bamboo beverage from December to May, however those grown in river valleys do so all year round because of the easy access to water.

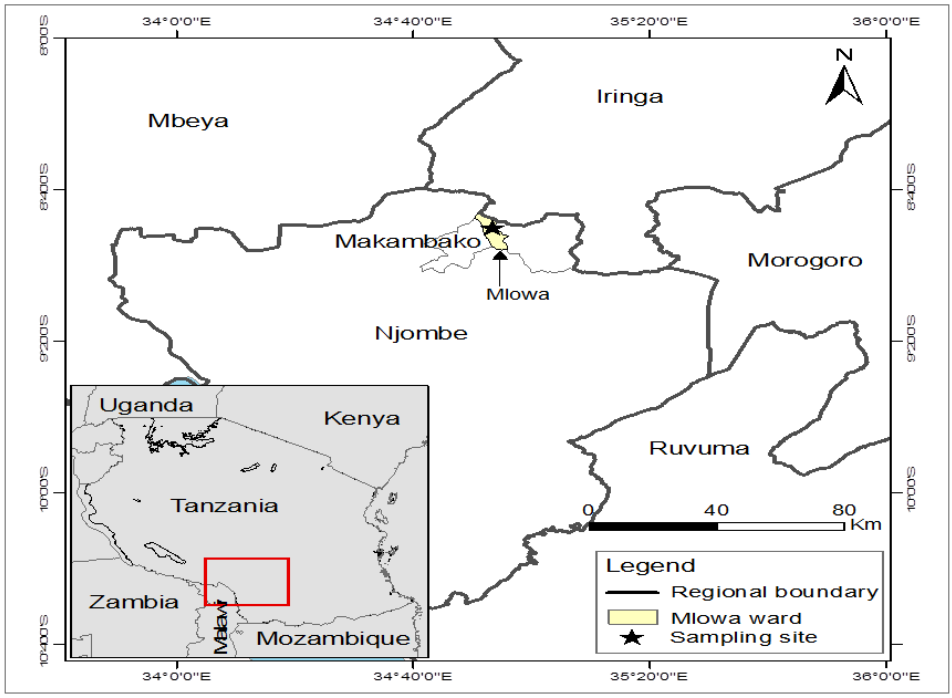

Figure 1: Map of Tanzania showing the location of sampling site in Njombe Region (source: National Bureau of Statistics–Tanzania).

In producing the bamboo beverage (juice), the tips of the young shoots are cut off and the stem is bruised every morning and evening. Before cutting the shoot (old shoot) and allowing new shoots to sprout, the bamboo beverage can be collected from the same shoot for a month. The new shoots will take five weeks to mature and begin producing bamboo beverage. The exudates from each cut and bruised shoot are then collected in a container called *mbeta* (Figure 2). Each shoot has a daily production capacity of 1.5 litres. After bamboo beverage is collected, the only step needed is filtering the beverage to remove any foreign particles such remnants, insects, bees, etc. The beverage is then collected in a bucket and allowed to ferment naturally to become *ulanzi*, a highly cherished alcoholic drink among locals in the vicinity and some townships.

Sample collection

Bamboo sap was harvested on 10th August 2021 from Mlowa ward, Makambako Council, Njombe District in Njombe Region, Southern Highlands of Tanzania. About 21 litres of fresh bamboo beverage were collected from three different farmers (sources).

Bamboo shoot | Bamboo juice tapping process using *mbeta* | *Ulanzi*

Figure 2: Harvesting and fermentation of bamboo beverage to obtain local beer (*ulanzi*).

Samples were packed in previously sterilised amber coloured airtight glass bottles, labelled, and put in cool boxes surrounded with iced and transported from Njombe to Dar es Salaam Region using an air-conditioned vehicle for experimental analyses. Nine litres (9 L) (3 L from each source) of the bamboo beverage were delivered to the Tanzania Medicine and Medical Devices Authority (TMDA) quality control laboratory in Dar es Salaam, Tanzania for analysing sugar content, 9 L were delivered to the Department of Food Science and Technology laboratory, University of Dar es Salaam for analysing pH and amino acids and 3 L was delivered at the Tanzania Breweries Limited (TBL) laboratory, for analysing alcoholic content. Samples were stored at room temperature of about 28 °C and chilled temperature of 4 °C throughout the experimental analyses.

Sample analyses

Chemicals and reagents

Sugar standards: Glucose, fructose and sucrose. **Amino acid standards:** L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, L-glutamine, L-glycine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine; Phenyl iso-thiocyanate (PITC); Trimethylamine (TEA); Methanol HPLC and reagent grade; distilled water; acetonitrile HPLC grade, and acetate buffer pH 6.2. All reagents and chemicals were obtained from Sigma Aldrich, Spruce Street, Saint Louis, MO 63103, USA.

Sugar composition analysis

The method for analysing bamboo beverage sugar composition was adapted from Tihomirova et al. (2016) with slight modifications. Samples were left to attain room temperature before filtration (Chromafil Etra disposable syringe filter 0.45 µm) into an HPLC amber glass vial ready for injection into HPLC. The analysis was performed using HPLC (Nixera2 LC-30AD), equipped with an auto-sampler (SIL-30AC) set at 25 µL injection volume, pump (LC-30AD) set at 1.0 mL/min, Degaser (DGU-20A3R), column oven (CTO-201AC) set at 30 °C and refractive index detector (RID-20A) all from SHIMADZU-Tokyo, Japan. Separation of the sugars was achieved in an amino column (NH_2) Agilent Zorbax Hilic plus column (4.6 x 100 mm, 5 µm). The mobile phase was 75:25 acetonitrile: Ultra-pure water that runs isocratic mode with a run time of 22 min. Glucose ((D) BDH AnalaR, England) Fructose (D-CarloEbra Reagents, SA) and sucrose (D (+) Fulka Biochemica 99–100%, Germany) standard were prepared to get a mixed standard, each with 60, 80, 2 g/L, respectively. This solution was used to prepare 10, 20, 40, 60 and 80 g/L for fructose, 7.5, 15, 30, 45 and 60 g/L for glucose and 0.25, 0.5, 1.0, 1.5 and 2 g/L for sucrose.

Quality control for sugar analysis

Quality control in sugar analysis, fructose, glucose and sucrose standards (Sigma Aldrich, Spruce Street, Saint Louis, MO 63103, USA) were used to prepare a mixed stock solution of 100, 80 and 60 g/L, respectively. The linearity was evaluated by running 10, 20, 40, 60, 80 and 100 g/L for fructose; 8, 16, 32, 48, 64 and 80 g/L for glucose; and 6, 12, 24, 36, 48 and 60 g/L for sucrose using the prepared stock solution. Blank sample (distilled water) was also analyzed using similar steps as in the analysis of samples. Recoveries were studied after mixing bamboo beverage with 200, 500 and 800 µL (duplicate) using the prepared stock solution. Recoveries were calculated as:

$$Recovery\ (\%) = \frac{[Spiked\ sample] - [Unspiked\ sample]}{Expected\ concentration} * 100\%$$

Amino acids composition analysis

The method for analysing bamboo beverage free amino acids was adapted from Klikarova et al. (2021) with slight modifications. Five millilitres (5 mL) of fresh bamboo juice was mixed with 5 mL of extracting solvent (75% methanol in distilled water) and shaken for 2 min. The mixture was transferred to a centrifuge tube and stored at 4 °C for 60 minutes, then centrifuged at 4,000 rpm for 60 min using a centrifuge (Multifuge I.S.R. Heraeus, Kandro laboratory products, Germany). The supernatant was filtered using 0.22 µm Whatman filter paper. To enhance detection with UV-detector, pre-column derivatization was done using a derivatizing agent which comprises methanol, phenyl iso-thiocyanate (PITC), triethylamine (TEA) and deionized water in the ratio of 7:1:1:1 v/v and stored at -18 °C when not in use. The mobile phase "**A**" was an aqueous buffer prepared by adding 0.5 mL/L TEA to 0.14 M sodium acetate and pH adjusted to 6.2 with glacial acetic acid. The mobile phase "**B**" was prepared with acetonitrile and distilled water with a ratio of 60:40 acetonitrile/water. Before analysis, 800 µL of the derivatizing solution was added to 200 µL of amino acid standard/bamboo juice extract in the vial tube of 2 mL. Vortex mixing for 3 seconds was done to enhance mixing and derivatization.

Amino acid contents in standards and bamboo juice were quantified using an Agilent Technologies HPLC, GERMANY (Series 1260, infinity II) with an isocratic pump (G7111A Series DEAEX01679), UV-detector (G7114A Series DEACX10455) and auto-sampler (G7129A Series DEAEQ21126), a chromatographic Poroshell 120 Eclipse EC-C18 column (4.6 × 100 mm, 4 µm of particle size) used to separate. The analytical conditions were ACN/ H_2O (60:40 v/v) and acetate buffer pH 6.2 mobile phases, 25 µL injection volume, 1.0 mL/min flow rate for 15 min, at 254 nm and 30 °C.

Quality controls for amino acids

Amino acid standard (Sigma Aldrich, Spruce Street, Saint Louis, MO 63103, USA) with eighteen amino acids each with a concentration of 0.5 µmol/mL except L-cystine at 0.25 µmol/mL in 0.2 N sodium citrate was used. The linearity of the test method was evaluated by running the standard using four calibration points, i.e., 0.0375, 0.05, 0.075 and 0.1 µmol/mL for all amino acids except L-cystine. The L-cystine calibration levels were 0.01875, 0.025, 0.00375 and 0.05 µmol/mL. Blank sample (derivatizing agent which comprised methanol, phenyl iso-thiocyanate (PITC), triethylamine (TEA) and deionized water in the ratio of 7:1:1:1 v/v) was also analyzed using similar steps as in the analysis of samples. The recoveries were evaluated by spiking 50 and 100 µL of 0.5 µmol/ml for all amino acid (except L-cystine) into the bamboo beverage sample to obtain concentrations of 0.025 and 0.05 µmol/mL, respectively. For L-cystine of concentration of 0.25 µmol/mL, the spiking volumes 50 and 100 µL yielded concentrations of 0.0125 and 0.025 µmol/mL, respectively. Recoveries were calculated using the same formula as for sugar analysis.

Determination of the alcohol content

Fifty (50) mL of the sample were measured using a round-bottomed flask followed by 100 mL ultra-pure water and then fitted into a condenser with cold water (cooled by the Eyela cooling system Kikakikai Co Ltd, China). This mixture was boiled (Electro thermal, UK) and the distillate was collected in a 50 mL volumetric flask. The refractive index of the distillate was measured by a refractometer (Bellingham+, Stanley Ltd RFM 340). The refractive index and temperature readings from each sample were converted to % of the alcohol content using reference tables (AOAC 2005).

pH measurement

The pH of the sample was measured by a pH meter (Mettle Toledo S213, pH and conductivity meter, Switzerland) at 20 °C.

Results and Discussion

Quality control for sugar analysis

Fructose showed the highest correlation coefficient compared to glucose and sucrose. However, the correlation coefficients were >0.97 indicating strong positive relationships between peak areas and concentrations of the respective sugars. Recoveries for sugar analysis ranged from 97.94 to 105.82%, whereby sucrose showed the highest recovery followed by fructose and then glucose. Weiß and Alt (2017) observed similar results of recoveries in sugar analysis using similar concentrations. Sugar analysis parameters were within the recommended range for the method level of 1 g/kg as specified by FDA Foods Program Regulatory Science Steering Committee (2019).

Sugar content in the bamboo juice and *ulanzi*

Figure 3 shows sugars (glucose, fructose and sucrose) of the bamboo juice. The sugar composition is very crucial in wine quality since it determines the alcohol content of the wines (Jordão et al. 2015). Glucose and fructose are the main sugars in the bamboo beverage (Figure 3). In this study, the amount of fructose, glucose and sucrose analysed on day 1 after harvesting ranged 52.96–53.57 (sources 1), 47.23–47.93 (source 2) and 0.36–0.40 g/L (source 3), respectively and the total sugar ranged from 100.89 to 101.55 g/L which is below the range of ripe grape juice that varies from 150 to 250 g/L (Ozturk and Anli 2014). The sugars significantly decreased ($p < 0.05$) from day 1 to day 6 of storage, particularly when stored at room temperature, from 52.96 to 0.00 (source 1), 53.35 to 0.00 (source 2) and 53.57 to 0.00 (source 3) g/L for fructose, from 47.93 to 14.78 (source 1), 47.23 to 14.91 (source 2) and 47.61 to 14.77 (source 3) g/L for glucose and from 0.40 to 0.00 (source 1), 0.36 to 0.00 (source 2) and 0.37 to 0.00 (source 3) g/L for sucrose (Tables 1, 2 and 3).

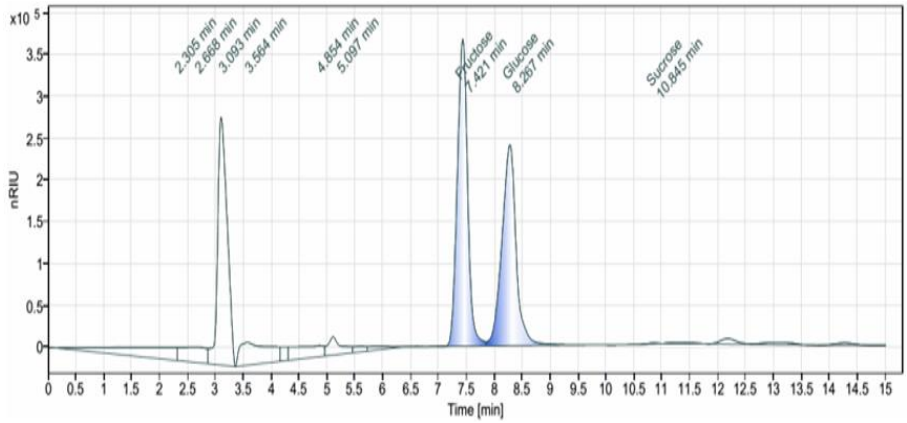

Figure 3: High-performance liquid chromatography bamboo beverage peaks for fructose, glucose and sucrose on day one (1) stored at room temperature (source 3).

Likewise, in chilling storage, there was a significant change ($p < 0.05$) as sugars were decreasing from 52.96 to 49.76 (source 1), 53.35 to 49.66 (source 2) and 53.57 to 49.56 (source 3) g/L for fructose, from 47.55 to 39.41 (source 1), 47.82 to 39.17 (source 2) and 47.40 to 39.98 (source 3) g/L for glucose (Tables 1, 2 and 3).

There was no significant difference ($p > 0.05$) in sucrose levels, which ranged from 0.36 to 0.38 (source 1), 0.38 to 0.38 (source 2) and 0.37 to 0.38 (source 3) g/L (Table 3). There were significant differences ($p < 0.05$) in sugars between the room and chilled storage temperatures. The temperature has a significant bearing on glucose and fructose consumption (Tronchoni et al. 2009). Additionally, both monosaccharides are co-fermented by yeasts throughout the wine fermentation process to produce a variety of chemicals, including carbon dioxide, ethanol, and glycerol. However, during wine

fermentation, yeasts have a little stronger affinity for glucose than for fructose, which results in variations in the consumption of both sugars during the fermentative process (Berthels et al. 2004, Wang et al. 2004, Guillaume et al. 2007, Berthels et al. 2008). The rate of sugar uptake, especially the uptake of fructose, is the main factor limiting the rate of alcohol synthesis by yeast (Wang et al. 2004), which is unacceptable in the production of dry wines.

Table 1: Changes in fructose concentrations (g/L) during storage in different conditions

	Source 1		Source 2		Source 3	
Days	Chilling storage	Room storage	Chilling storage	Room storage	Chilling storage	Room storage
1	52.96 ± 0.210^{Aa}	52.96 ± 0.317^{Aa}	53.35 ± 0.450^{Aa}	53.42 ± 0.555^{Aa}	53.57 ± 0.354^{Aa}	53.49 ± 0.490^{Aa}
2	50.43 ± 0.437^{Ab}	45.12 ± 0.279^{Bb}	50.76 ± 0.943^{Ab}	45.61 ± 0.279^{Bb}	51.09 ± 0.314^{Ab}	45.62 ± 0.579^{Bb}
3	49.39 ± 0.411^{Ab}	3.24 ± 0.097^{Bc}	49.75 ± 0.082^{Ab}	3.32 ± 0.104^{Bc}	50.02 ± 0.241^{Ab}	3.16 ± 0.055^{Bc}
4	49.69 ± 0.300^{Ab}	1.89 ± 0.032^{Bd}	49.76 ± 0.184^{Ab}	1.69 ± 0.038^{Bd}	49.62 ± 0.394^{Ac}	1.69 ± 0.140^{Bcd}
5	49.71 ± 0.297^{Ab}	1.35 ± 0.063^{Bd}	50.04 ± 0.331^{Ab}	1.37 ± 0.028^{Bd}	49.38 ± 0.219^{Ac}	1.30 ± 0.045^{Bde}
6	49.76 ± 0.256^{Ab}	0.01 ± 0.000^{Be}	49.66 ± 0.417^{Ab}	0.01 ± 0.000^{Be}	49.56 ± 0.369^{Ac}	0.01 ± 0.000^{Be}

Mean concentration ± SEM (N = 3) followed by different statistical superscript capital and small letters across the rows and columns, respectively, indicates differences in mean concentrations according to Turkey's HSD at $p < 0.05$.

Table 2: Changes in glucose concentrations (g/L) during storage in different conditions

	Source 1		Source 2		Source 3	
Days	Chilling storage	Room storage	Chilling storage	Room storage	Chilling storage	Room storage
1	47.55 ± 0.372^{Aa}	47.93 ± 0.440^{Aa}	47.82 ± 0.501^{Aa}	47.23 ± 0.476^{Aa}	47.40 ± 0.187^{Aa}	47.61 ± 0.230^{Aa}
2	44.06 ± 0.270^{Ab}	40.53 ± 0.321^{Cb}	43.99 ± 0.276^{Ab}	41.27 ± 0.892^{BCb}	43.95 ± 0.475^{ABb}	40.51 ± 0.625^{Cb}
3	41.40 ± 0.243^{Ac}	15.81 ± 0.125^{Bc}	41.53 ± 0.198^{Ac}	15.06 ± 0.310^{Bc}	41.44 ± 0.392^{Acd}	15.60 ± 0.293^{Bc}
4	42.54 ± 0.155^{Ac}	13.77 ± 0.047^{Bd}	42.15 ± 0.368^{Ac}	13.49 ± 0.204^{Bc}	42.51 ± 0.242^{Abc}	12.90 ± 0.129^{Bd}
5	41.04 ± 0.032^{Ac}	13.81 ± 0.533^{Bd}	41.58 ± 0.281^{Ac}	13.83 ± 0.253^{Bc}	41.57 ± 0.215^{Acd}	14.07 ± 0.370^{Bcd}
6	39.41 ± 0.431^{Ad}	14.78 ± 0.163^{Bcd}	39.17 ± 0.315^{Ad}	14.91 ± 0.098^{Bc}	39.98 ± 0.617^{Ad}	14.77 ± 0.189^{Bc}

Mean concentration ± SEM (N = 3) followed by different statistical superscript capital and small letters across the rows and columns, respectively, indicates differences in mean concentrations according to Turkey's HSD at $p < 0.05$.

But in this work, fructose content was substantially higher and fermented more quickly than glucose (Tables 1 and 2). On days 3 and 5, respectively, of storage at room temperature, residual fructose levels were 5 times lower and 10 times lower than residual

glucose levels (Tables 1 and 2). These findings also conflict with those of earlier research, which claimed that the amount of residual glucose in stuck wines was 10 times lower than the residual fructose (Emmerich and Radler 1983, Wang et al. 2004, Bisson 2005, Guillaume et al. 2007). From day 2 to day 5 of storage at room temperature, the fructose-glucose ratio (FGR) varied from 1.1 to 0.1, respectively. The addition of must to wine after fermentation can be determined using the fructose-glucose ratio in the unfermented residual sugar. The fact that fructose was zero on day 6 and glucose ranged from 14.77 to 14.91 g/L suggests that there was no fructose left in the bamboo beverage at that time (Tables 1 and 2). Additionally, results demonstrated that fructose peak was missing during analysis on day five of storage (Figure 4).

Table 3: Changes in sucrose concentration (g/L) during storage in different conditions

	Source 1		Source 2		Source 3	
Days	Chilling storage	Room storage	Chilling storage	Room storage	Chilling storage	Room storage
1	0.38 ± 0.009^{Aa}	0.40 ± 0.032^{Aa}	0.38 ± 0.012^{Aa}	0.36 ± 0.009^{Aa}	0.38 ± 0.009^{Aa}	0.37 ± 0.029^{Aa}
2	0.36 ± 0.004^{Aa}	0.18 ± 0.003^{Bb}	0.36 ± 0.010^{Aa}	0.18 ± 0.003^{Bb}	0.36 ± 0.005^{Aa}	0.18 ± 0.003^{Bb}
3	0.37 ± 0.001^{Aa}	0.13 ± 0.000^{Bc}	0.38 ± 0.006^{Aa}	0.13 ± 0.003^{Bc}	0.36 ± 0.002^{Aa}	0.13 ± 0.005^{Bbc}
4	0.37 ± 0.004^{Aa}	0.09 ± 0.005^{Bc}	0.38 ± 0.001^{Aa}	0.10 ± 0.008^{Bd}	0.37 ± 0.006^{Aa}	0.11 ± 0.007^{Bc}
5	0.36 ± 0.002^{Aa}	0.03 ± 0.000^{Bd}	0.36 ± 0.007^{Aa}	0.03 ± 0.001^{Be}	0.36 ± 0.006^{Aa}	0.03 ± 0.001^{Bd}
6	0.36 ± 0.006^{Aa}	0.00 ± 0.000^{Bd}	0.38 ± 0.005^{Aa}	0.00 ± 0.000^{Bf}	0.37 ± 0.006^{Aa}	0.00 ± 0.000^{Bd}

Mean concentration ± SEM (N = 3) followed by different statistical superscript capital and small letters across the rows and columns, respectively, indicates differences in mean concentrations according to Turkey's HSD at $p < 0.05$.

Given that the fermentation process was not under control in this investigation, it is difficult to explain why there was higher concentration of glucose than fructose. Instead, it was largely dependent on exogenous microorganisms entering the system in uncontrolled patterns (spontaneous), a process also known as open fermentation. In this situation, the fermentation process can be contributed by both *Saccharomyces* and non-*Saccharomyces* yeasts. According to Maicas (2020), some non-*Saccharomyces* yeasts can survive and carry out their metabolic functions until the spontaneous fermentation is complete. This has a good impact on the sensory quality of wines. While the majority of non-*Saccharomyces* yeasts exhibit some technological limitations compared to *S. cerevisiae*, such as reduced fermentative power and ethanol production, non-*Saccharomyces* yeasts also exhibit traits that are lacking in *S. cerevisiae*, such as the production of high levels of aromatic compounds like esters, higher alcohols, and fatty acids (Maicas 2020).

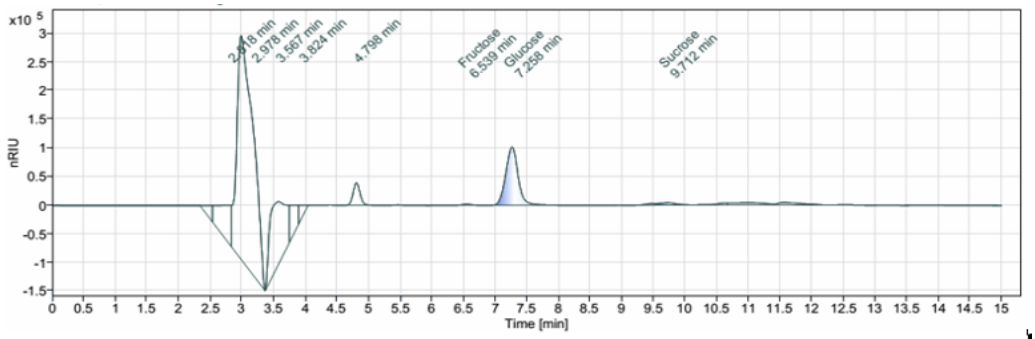

Figure 4: High-performance liquid chromatography bamboo beverage peaks for fructose, glucose and sucrose on day five (5) stored at room temperature.

Although some yeasts from the species *Saccharomyces rouxii* and *Saccharomyces bailii* preferentially ferment fructose, most yeasts, including the normal wine yeasts, ferment glucose more quickly than fructose (fructophilic yeasts). According to the research done by Emmerich and Radler in 1983, *Saccharomyces bailii* ferments fructose before glucose.

Quality control for amino acids

The correlation coefficients for amino acids ranged from 0.9114 to 0.9909, with glutamic acid having the lowest value (0.9114), followed by aspartic acid (0.9251) and tryosine (0.9546). The rest of the amino acids had correlation coefficients greater than 0.96. Similar findings were reported by Vakili et al. (2022) in their analysis of several free amino acids in newborn formula and medicinal food items for inborn metabolic abnormalities. Tyrosine, serine, and alanine exhibited significantly lower values of recoveries, with values of 62.511.743, 67.383.182, and 67.951.689, respectively. The recoveries ranged from 62.51 to 115.49 percent. Arginine (114.32 ± 4.063%), histidine (115.49 ± 1.239%), glutamic acid (112.05 ± 8.797%), and phenylalanine (108.63 ± 3.376%) all exhibited recoveries above 100%, while glycine (99.12 ± 3.865%), methionine (95.25 ± 0.751%), and leucine (95.27 ± 1.255%) had recoveries between 90% and 100%. The recoveries for the remaining amino acids ranged from 70 to 90%. The recovery values were within the acceptable recovery range (60–115%, for a method level of 10 ppb) range as specified by FDA Foods Program Regulatory Science Steering Committee (2019).

Determination of amino acids in bamboo juice and *ulanzi*

Table 4 shows amino acids for bamboo beverage (at day one) and after 90 days of storage. In this study, a total of 15 L-amino acids were established (Table 4). Some amino acids are partially or metabolized by yeast cells during their growth phase when amino acids are used as carbon or nitrogen sources (Carnevillier et al. 1999). Amino acids are the major sources of nitrogen for brewing yeast. The concentration and type of available nitrogen impacts two aspects of yeast cell function: fermentation rate and biomass generation. These two factors determine how well yeast ferments (Mas et al. 2014). The yeasts convert the sugars into ethyl alcohol and carbon dioxide by using the amino acids as nitrogen sources (Ali et al. 2009). A number of amino acids in the bamboo beverage were identified in this study, with tyrosine being the most prevalent (597.68 mg/L for source 1, 599.44 mg/L for source 2 and 597.83 mg/L for source 3), followed by valine (261.13 mg/L for source 1, 261.24 mg/L for source 2 and 262.54 mg/L for source 3), L-threonine (76.69 mg/L for source 1, 76.91 mg/L for source 2 and 77.13 mg/L for source 3), and L-serine (66.37 mg/L for source 1, 67.23 mg/L for source 2 and 66.68 mg/L for source 3). Additionally, amino acids are crucial building blocks for flavouring agents and biogenic amines (Ali et

al. 2009, Mas et al. 2014). Total free amino nitrogen is typically used to determine the quality of musts since low rates suggest fermentation problems caused by insufficient nutritional reserves of yeasts, which may result in sluggish/prolonged fermentation (Vilanova et al. 2007, Ali et al. 2009, Agustini et al. 2014, Mas et al. 2014, Kemsawasd et al. 2015).

Table 4: Amino acids for bamboo beverage (initial) and after fermentation (after 90 days of storage) (mg/L)

Amino acid	Storage time	Source 1	Source 2	Source 3
Alanine	Day 1	8.02 ± 0.052	8.10 ± 0.058	8.07 ± 0.059
	Day 90	20.91 ± 0.193	20.71 ± 0.363	21.07 ± 0.168
Arginine	Day 1	0.51 ± 0.007	0.50 ± 0.002	0.51 ± 0.014
	Day 90	nd	nd	nd
Aspartic acid	Day 1	nd	nd	nd
	Day 90	6.16 ± 0.072	6.16 ± 0.028	6.24 ± 0.070
Cysteine	Day 1	1.96 ± 0.036	1.97 ± 0.039	1.92 ± 0.081
	Day 90	54.37 ± 0.734	54.54 ±0 .381	54.84 ± 0.703
Glutanic acid	Day 1	26.45 ± 0.664	26.08 ± 0.489	25.50 ± 0.639
	Day 90	1.56 ± 0.016	1.54 ± 0.027	1.56 ± 0.015
Glycine	Day 1	24.21 ± 0.494	24.37 ± 0.660	23.58 ± 1.117
	Day 90	40.68 ± 0.288	40.89 ± 0.521	40.73 ± 0.405
Histidine	Day 1	nd	nd	nd
	Day 90	nd	nd	nd
Isoleucine	Day 1	12.45 ± 0.264	12.53 ± 0.289	12.45 ± 0.209
	Day 90	nd	nd	nd
Leucine	Day 1	5.14 ± 0.089	5.26 ± 0.074	5.13 ± 0.069
	Day 90	nd	nd	nd
Lysine	Day 1	nd	nd	nd
	Day 90	nd	nd	nd
Methionine	Day 1	1.07 ± 0.037	1.02 ± 0.031	1.09 ± 0.035
	Day 90	0.27 ± 0.006	0.26 ± 0.007	0.28 ± 0.013
Phenylalanine	Day 1	0.59 ± 0.039	0.55 ± 0.038	0.61 ± 0.023
	Day 90	nd	nd	nd
Proline	Day 1	2.10 ± 0.075	2.18 ± 0.100	2.07 ± 0.028
	Day 90	0.27 ± 0.009	0.28 ± 0.007	0.28 ± 0.006
Serine	Day 1	66.37 ± 0.485	67.23 ± 0.182	66.68 ± 0.745
	Day 90	2.73 ± 0.020	2.73 ± 0.025	2.75 ± 0.061
Threonine	Day 1	76.69 ± 0.284	76.91 ± 0.568	77.13 ± 0.079
	Day 90	77.52 ± 0.336	77.34 ± 0.339	76.77 ± 0.300
Tryptophan	Day 1	1.93 ± 0.040	1.89 ± 0.035	1.88 ± 0.037
	Day 90	nd	nd	nd
Tyrosine	Day 1	597.68 ± 1.778	599.44 ± 0.925	597.83 ± 0.717
	Day 90	nd	nd	nd
Valine	Day 1	261.13 ± 0.856	261.24 ± 1.007	262.54 ± 1.830
	Day 90	nd	nd	nd

nd = Not detected.

The total amino acid content (TAAC) of bamboo beverage was 1086.30 mg/L, 1089.27 mg/L and 1086,99 mg/L, for source 1, source 2 and source 3, respectively (before fermentation) and 204.47 mg/L, 204.45 mg/L and 204.52 mg/L for source 1, source 2 and

source 3, respectively after 90 days of storage at room temperature. After 90 days of storage the TAAC was significantly decreased. It was, however, noticed that there was a significant increase of some amino acids for example cysteine, glycine and alanine (Table 4). The concentrations of the amino acids found in this study such as isoleucine, valine, phenylalanine, glycine, alanine, tyrosine, arginine and leucine, are considered important, as these are important parts of the complex system regulating the biosynthesis of flavour-active compounds formed by yeast (Ferreira and Guido 2018).

Determination of alcohol content in bamboo juice and *ulanzi*

Alcohol influences various components, including sensory perceptions, making it a significant component in alcoholic beverages like wines. Additionally, it interacts with other elements of wine, such as scents and tannins, which affect the viscosity and body of the wine as well as our perceptions of astringency, sourness, sweetness, aroma, and flavour (Jordão et al. 2015). The presence of alcohol along with sugars, amino acids, and phenols defines the balance of the wine when organoleptic properties are taken into account. In this regard, the law stipulates that the wines' actual alcohol content must not be less than 15% by volume and that the total alcoholic strength should not be less than 17.5% by volume (Reboredo-Rodríguez et al. 2015) with the exception of wines with a designation of origin or a geographical indication, which have specific rules. In this study, alcohol content ranged from 3.11 to 9.05% v/v at room storage temperature. The alcohol content is within the wine's range reported in the literature of 9 to 13% (Ozturk and Anli 2014, Jordão et al. 2015). The alcohol content was significantly increased ($p < 0.05$) at room temperature and slightly increased at chilled temperature (Table 5).

On the other hand, high alcohol content has negative effects on human health and is not palatable for most consumers who drink responsibly (Ozturk and Anli 2014). As a result, several nations develop innovative techniques for reducing the alcohol content of wines while retaining their quality and sensory sensations. According to Jordão et al. (2015), the demands for alcoholic beverages with lower alcohol (9–13% v/v) are driven by social and health concerns for many consumers in various nations.

Table 5: Alcohol content (% v/v) in bamboo beverage during storage

Days	Chilling storage			Room storage		
	Source 1	Source 2	Source 3	Source 1	Source 2	Source 3
1	3.10 ± 0.041^{Da}	3.14 ± 0.038^{Da}	3.14 ± 0.034^{Da}	3.16 ± 0.040^{Ea}	3.11 ± 0.013^{Fa}	3.11 ± 0.069^{Fa}
2	3.44 ± 0.018^{Cb}	3.42 ± 0.012^{Cb}	3.46 ± 0.018^{BCb}	4.14 ± 0.041^{Da}	4.14 ± 0.034^{Ea}	4.14 ± 0.037^{Ea}
3	3.44 ± 0.018^{Cb}	3.48 ± 0.009^{Cb}	3.52 ± 0.078^{BCb}	7.11 ± 0.076^{Ca}	7.10 ± 0.066^{Da}	7.00 ± 0.012^{Da}
4	3.42 ± 0.022^{Cb}	3.42 ± 0.017^{Cb}	3.37 ± 0.033^{CDb}	7.33 ± 0.062^{Ca}	7.32 ± 0.032^{Ca}	7.42 ± 0.017^{Ca}
5	3.68 ± 0.013^{Bb}	3.65 ± 0.025^{Bb}	3.71 ± 0.075^{Bb}	8.16 ± 0.060^{Ba}	8.17 ± 0.046^{Ba}	8.23 ± 0.026^{Ba}
6	4.16 ± 0.083^{Ab}	4.24 ± 0.036^{Ab}	4.20 ± 0.056^{Ab}	9.04 ± 0.041^{Aa}	9.05 ± 0.050^{Aa}	9.00 ± 0.023^{Aa}

Based on the reported data on grape wine, i.e. the sugar content of the juice of ripe grapes varies from 150 to 250 g/L and the alcohol content of wine is 9–15% v/v (Ozturk and Anli 2014). This study demonstrated that *ulanzi*, which is fermented from bamboo juice, is a wine with a sugar content of 100.89 g/L, 101.55 g/L and 101.35 g/L for source 1, source 2 and source 3, respectively. While alcohol content was 9.04, 9.05 and 9.00% v/v

for source 1, source 2 and source 3, respectively. Overall, alcoholic content of the bamboo juice was significantly increased during storage ($p < 0.05$) (Table 5). Implicitly, fermentation was taking place as sugars were being consumed by the yeast to produce alcohol and carbon dioxide. The early stages of wine fermentation transform must sugar into ethanol, carbon dioxide, and other important secondary metabolites (Vilela 2019).

pH values of the bamboo juice and *ulanzi*

It is crucial to determine the wine's pH since it affects several aspects of winemaking, including the biological stability, colour, rate of oxidation, and protein stability. Due to the importance of pH in quantifying the final product (bamboo juice) and it is being an indicator in the fermentation process, the pH was measured throughout the storage time, both at the room and cold temperatures.

The pH levels were not significant ($p > 0.05$) between days 1 and 2, but there was a significant decrease ($p < 0.05$) of pH levels from day 2 to day 6 (Table 6) at room temperature storage. There was no significant difference in pH ($p > 0.05$) during chilled storage (except on day 6 which showed significant difference, $p < 0.05$), implying that the fermentation was not taking place at this temperature or there was a very slow fermentation process at chilling temperature.

Table 6: pH values in bamboo beverage during storage

Days	Chilling storage			Room storage		
	source 1	source 2	source 3	source 1	source 2	source 3
1	4.05 ± 0.003^{Aa}	4.04 ± 0.003^{Aa}	4.05 ± 0.003^{Aa}	4.05 ± 0.000^{Aa}	4.04 ± 0.003^{Aa}	4.06 ± 0.007^{Aa}
2	4.04 ± 0.003^{Abc}	4.03 ± 0.003^{Ac}	4.04 ± 0.007^{Abc}	4.06 ± 0.003^{Aa}	4.05 ± 0.003^{Aab}	4.06 ± 0.003^{Aa}
3	4.04 ± 0.007^{Aa}	4.04 ± 0.006^{Aa}	4.04 ± 0.012^{Aa}	3.87 ± 0.003^{Bb}	3.88 ± 0.003^{Cb}	3.88 ± 0.007^{Bb}
4	4.02 ± 0.006^{Aa}	4.03 ± 0.003^{Aa}	4.01 ± 0.003^{Aa}	3.90 ± 0.006^{Bb}	3.91 ± 0.006^{Bb}	3.89 ± 0.012^{Bb}
5	4.03 ± 0.003^{Aa}	4.03 ± 0.006^{Aa}	4.03 ± 0.009^{Aa}	3.59 ± 0.030^{Cb}	3.59 ± 0.007^{Eb}	3.58 ± 0.003^{Db}
6	3.97 ± 0.035^{Ba}	3.96 ± 0.027^{Ba}	4.07 ± 0.033^{Aa}	3.63 ± 0.009^{Cb}	3.63 ± 0.006^{Db}	3.63 ± 0.007^{Cb}

Mean value ± SEM (N = 3), values with different statistical superscript capital and small letters across the columns and rows, respectively, indicate differences in pH content according to Turkey's HSD at $p < 0.05$.

Conclusion

The bamboo beverage has been successfully described in this study in terms of sugars, amino acids, alcohol content, and pH, all of which are significant parameters in the alcoholic beverages industry. The amino acids, alcohol content and pH analysed in bamboo beverage fall within the values reported in grapes and other wines, which hints at *ulanzi* being classified as a wine. As such, there is a need to analyse all other important parameters such as vitamins, minerals, and toxins to position accordingly the bamboo beverage in the local and international markets. Furthermore, given that certain tourists who travel to Ruaha National Park (RNP) and the surrounding area appreciate this beverage, the inclusion of its scientific justification may enhance the tourism sector. Besides, it is important to consider value addition for the drink in order to manufacture different types of bamboo wines (table, sparkling, red, and white wines). In addition, to effectively commercialize this beverage, a methodical (holistic) approach (mainly good manufacturing practices) must be used at every stage of its value chain, including

production, processing, packaging, and marketing.

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Conflict of interest

There is no conflict of interest regarding this work.

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