Original Research Article

Proximate composition of banana and pineapple wine fermented by *Meyerozyma guilliermondii* and *Pichia guilliermondii*

Abstract

Wine is an alcoholic beverage produced from juices of a variety of fruits by the fermentative action of microorganisms. There is the quest for alternative sources of must for wine fermentation, however the proximate composition should be known to ascertain how nutritious it is. The study was thus aimed at determining the proximate composition of the wines fermented by *Meyerozyma guilliermondii* and *Pichia guilliermondii*. Two isolates identified as *Meyerozyma guilliermondii* strain 1621 and *Pichia guilliermondii* strain PAX-PAT 18S were used for the fermentation of the substrates obtained from a mixture of pineapple and banana pulp. The fermentation process was for a period of 28 days, followed by a series of racking, clarification, and aging process which was for 2 months. The fermentation process comprised two setups: one was fermented by *Meyerozyma guilliermondii* strain 1621 and the other by *Pichia guilliermondii* strain PAX-PAT 18S. The process was monitored and the proximate analysis of the wines was ascertained. The wine produced by *Meyerozyma guilliermondii* strain 1621 had a moisture content of 82.56 %, ash content of 1.41 %, fat content of 0.08 %, protein content of 1.43%, and carbohydrate content of 9.77%. The wine produced by *Pichia guilliermondii* strain PAX-PAT 18S had a moisture content of 79.51%, ash content of 1.19%, fat content of 0.15%, protein content of 0.49%, and carbohydrate content of 10.49%. Although this study is not exhaustive, it shows that wines with acceptable nutritional composition can be successfully produced using *Meyerozyma guilliermondii* strain 1621 and *Pichia guilliermondii* strain PAX-PAT 18S.

Keywords: Wine production, fermentation, banana and pineapple substrate, *Meyerozyma guilliermondii, Pichia guilliermondii*, proximate composition.

1.0 Introduction

Wine is an alcoholic drink made from fermented fruit juice, and any fruit with a good proportion of sugar may be used for wine production. The wines are named after the fruit involved [1]. Banana (*Musa acuminata*) is a staple food that is perishable, non-seasonal, and is readily available in Nigeria. Ripe bananas are consumed raw as a dessert fruit and it has a sugar content of 20 % while an unripe banana has a sugar content of only 2 %. It contains eleven (11) vitamins which include Vitamin B1 (thiamine), B2 (riboflavin), B6 (pyridoxine), B9 (folic acid), Vitamin A, and Vitamin C. It is also rich in phosphorus, calcium, and fiber materials, although its fat and protein contents are low [2]. Following the high nutritional content of banana, it is consumed in large quantity in a variety of ways in Africa. It can be cooked (deep fried, baked, dehydrated), processed into flour, or fermented for the production of beverages such as banana juice, beer, vinegar, and wine. Banana can also be eaten raw [3,4]. Banana has a short shelf-life because of

the temperature and humidity conditions in tropical countries like Nigeria; fermenting banana juice into wine is considered to be a good means of utilizing excess banana since banana wine provides a rich source of vitamins and ensures harnessing the fruits into a useful by-product [5].

Pineapple (*Ananas comosus*) is a tropical fruit that has excellent juiciness, a vibrant tropical flavour, and immense health benefits. It contains a considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, water, and different minerals that are ideal for the digestive system. The fresh ones (pineapples) are rich in bromelain which is anti-inflammatory to reduce swelling in inflammatory conditions (such as arthritis, sore throat, and sinusitis). It can be used to produce some food items like pickles, jelly, and jam [6]. It contains minimal fat and sodium [7]. The edible part of pineapple contains 81.2-86.2 % moisture and 13-19 % total solids (fructose, sucrose, and glucose are the main components). The total solids consist of about 85 % carbohydrates and 2-3 % fibre. The pineapple pulp has very low ash content, nitrogenous compounds, and 0. 1% lipids, of which 25-30 % of the nitrogenous compounds are proteins. It contains ascorbic acid and is a good source of vitamin C which helps fight bacterial and viral infections, and is an effective antioxidant that helps the body to absorb iron. It also contains copper which assists in the absorption of iron and regulates heart rate and blood pressure [8]. Alternative substrates for wine production are needed to reduce the pressure on the regular ones used such as grapes. Also, there is much dependence on *Saccharomyces cerevisiae* for fermentation while there is the possibility that other species of yeast could ferment "must" to produce nutritious wine. This study was thus aimed at determining the proximate composition of wine produced by the fermentative action of *Meyerozyma guilliermondii* strain 1621 and *Pichia guilliermondii* on pineapple and banana must in comparison with commercial wine.

2.0 Materials and Methods

2.1 Sample collection

Ripe Queen pineapple (*Ananas comosus*) and Cavendish banana (*Musa acuminata*) fruits were purchased from Mile 3 market, Port Harcourt, Rivers State, Nigeria, and used to prepare the must using the method described by Ogodo *et al.* [9]. The must was in the ratio of 3:1 for pineapple and banana, respectively. The must was prepared for two different set ups: one being fermented by *Meyerozyma guillermondii* strain 1621 and the other by *Pichia guilliermondii* strain PAX-PAT 18S, both isolated from palm wine. The must was analysed for the proximate parameters. The starter culture was prepared by developing the inocula as described by Ogodo *et al.* [9].

2.2 Fermentation of the Wine

The fermentation of the must to wine was carried out as described by Ogodo *et al.* (2015) with modifications. The inoculum and yeast nutrients were added to the two (2) fermentation tanks and the fermentation process lasted for 28 days. At the end of the fermentation, the wines were racked with minimum exposure to air and clarified. The filtrate was allowed to age for a period of two (2) months after which the wines were bottled and the proximate analysis of the wines was carried out.

2.3 Determination of Moisture Content (%)

The method described by Moronkola *et al.* was used to ascertain the moisture content of the substrates [10]. Five grams (5 g) of the sample was weighed into a Petri dish and placed in an air draught oven at a temperature of 100^0C for 1 hour. The Petri dish was weighed after cooling. The same process was repeated thrice until a constant weight was obtained.

$$\% \text{ Moisture} = \frac{\text{loss in weight due to dryness}}{\text{Weight of the sample used}} \times 100$$

$$= \frac{W2 - W3}{W2 - W1} \times 100\%$$

Where W1 = Weight of empty crucibles

W2 = Weight of crucible + Sample before drying

W3 = Weight of crucible + Sample after attaining constant weight on drying.

2.4 Determination of Lipid Content (%)

Two (2) g of sample was introduced into a filter paper and placed in a Soxhlet extractor which was positioned in a dry pre-weighed distillation flask. The solvent (acetone) was introduced into the flask through the condenser end which was connected to the extractor. The setup was held in place with a retort stand clamp. The temperature of the condenser was regulated using a cold-water jet. The hot solvent was refluxed continuously to extract the entire lipid in the solvent chamber. To concentrate the lipid the solvent was evaporated and the extractor was finally disconnected. The flask was dried in an air oven to a constant weight and the percentage lipid weight was obtained after re-weighing [10].

$$\% \text{ lipid} = \frac{\text{Weight of flask and extract} - \text{Weight of dried sample}}{\text{Weight of sample extracted}} \times 100\ \%$$

2.5 Determination of Ash Content (%)

Porcelain crucible with the lid was ignited in a hot Bunsen burner flame and transferred into a desiccator to cool and the crucible was weighed as W1. Five (5) g of the sample was accurately weighed into a crucible as W2. This was gently placed in the muffle furnace set at 600^0C for 4 hours. The crucible was placed in a desiccator and allowed to cool. The sample that had become ash was put in the crucible and weighed after cooling as W3 without the lid; this process was repeated thrice [10].

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} \times 100\%$$

2.6 Determination of Crude Protein (%)

Crude protein was determined using the Micro Kjeldhal method [11]. One gram (1 g) of dry milled sample (w) and a catalyst mixture containing $HgSO_4$ and K_2SO_4 in the ratio of 1:9 was digested using concentrated H_2SO_4. The digested sample was diluted with water to a volume of 250 ml, and a 10 ml aliquot of the diluted sample was mixed with 10 ml NaOH solution (40 %)

to an excess alkaline reaction. The mixture was diluted with steam in the presence of 50 mg zinc using a Micro Kjeldahl distillation apparatus. Ammonia liberated during the process was collected into a 2 % boric acid solution containing a few drops of methylene blue. The distillate thus obtained was titrated against 0.01 N H_2SO_4. A blank was also run under the same conditions. From the actual volume of 0.01 N H_2SO_4 used, the amount of % nitrogen was calculated by equating 1 ml of 0.01 $(NH_3)_2SO_4$ to 0.00014g of nitrogen. The percentage of crude protein was obtained by multiplying the % nitrogen by 6.25.

$$\text{Nitrogen (\%)} = \frac{X \times 0.00014 \times 250 \times 100}{10 \times W} \times 100\%$$

Crude protein (%) = Nitrogen % × 6.25

X = ml of 0.01 N H_2SO_4 used

1ml of 0.01 N H_2SO_4 = 0.00014g of NH_3 nitrogen

W = weight of the sample in grams

250 = dilution factor

6.25 = protein conversion factor

2.7 Determination of Total Carbohydrate Content (%)

Total carbohydrate was determined using the Clegg Anthrone method [11]. One ml of the sample was transferred into a 25 ml flask. Precisely, 62 % of perchloric acid was added and shaken for 20 minutes for complete homogenization. The flask was filled with 25ml of distilled water and the solution formed was allowed to sediment and decanted. A pipette was used to transfer 1 ml of the filtrate into a 10 ml volumetric flask. One (1) ml of the solution was transferred into a test tube containing 5 ml of Anthrone reagent and mixed thoroughly. The mixture was read at 630 nm wavelength and distilled water was used as blank. A standard phase of 0.1 mg/ mol was prepared and treated with Anthrone and the absorbance was read.

The total carbohydrate was calculated as:

$$\text{\% Carbohydrate (glucose)} = \frac{25 \times \text{absorbance of sample}}{\text{Absorbance of standard glucose x volume of sample}}$$

3.0 Results

Table 1 presents the results of the proximate composition of the wine "must" before fermentation and the wines produced. The wine produced by *Meyerozyma guilliermondii* strain 1621 had a moisture content of 82.56 %, ash content of 1.41 %, fat content of 0.08 %, protein content of 1.43 %, and carbohydrate content of 9.77 %. The wine produced by *Pichia guilliermondii* strain PAX-PAT 18S had a moisture content of 79.51 %, ash content of 1.19 %, fat content of 0.15 %, protein content of 0.49 %, and carbohydrate content of 10.49 %. Statistically, there was no significant difference in the protein content between the wine "must", the laboratory-produced wines and the commercial wine fermented by *Saccharomyces cerevisiae* ($P \leq 0.05$). There was no significant difference in protein content between the wine "must" and the laboratory-produced wines but there was a significant difference between the wine "must', the laboratory-produced wines, and the commercial wine fermented by *Saccharomyces cerevisiae.*

For moisture content, there was no significant difference between the wine "must", the wine fermented by *Meyerozyma guilliermondii*, and the commercial wine fermented by *Saccharomyces cerevisiae* but there was a significant difference between the wine "must", the wine fermented by *Meyerozyma guilliermondii*, the commercial wine fermented by *Saccharomyces cerevisiae* and the wine fermented by *Pichia guilliermondii* ($P \leq 0.05$). For ash content, there was no significant difference between the wine "must" and the commercial fruit wine fermented by *Saccharomyces cerevisiae* but there was a significant between the wine "must", the wine fermented by *Meyerozyma guilliermondii*, the wine fermented by *Pichia guilliermondii* and the commercial fruit wine fermented by *Saccharomyces cerevisiae* ($P \leq 0.05$). There was a significant between carbohydrate contents of the wine "must", and the laboratory produced wines but there was no significant difference between the wine "must" and the commercial fruit wine fermented by *Saccharomyces cerevisiae* ($P \leq 0.05$).

Table 1: Mean Proximate Composition of the Must before Fermentation and the Wines Produced

Wines	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
Composition of must before fermentation	84.44 ± 0.37^{b}	3.52 ± 0.02^{c}	0.09 ± 0.00^{a}	1.45 ± 0.00^{a}	13.47 ± 0.16^{b}
Wine fermented by *M. guilliermondii*	82.56 ± 1.44^{b}	1.41 ± 0.03^{b}	0.08 ± 0.00^{a}	1.43 ± 0.00^{a}	9.77 ± 0.71^{a}
Wine fermented by *P. guilliermondii*	79.51 ± 0.03^{a}	1.19 ± 0.01^{a}	0.15 ± 0.00^{a}	0.49 ± 0.00^{a}	10.49 ± 0.33^{a}
Commercial fruit wine	82.43 ± 0.00^{b}	2.27 ± 0.00^{c}	0.54 ± 0.00^{b}	0.53 ± 0.00^{a}	14.23 ± 0.00^{b}

Means with the same alphabets across the group are not significantly different ($P \leq 0.05$)

4.0 Discussion

Fermentation for the production of beverages like wine depends on the ability and performance of the yeast to convert the sugar content of the substrates to alcohol and esters. Blends of pineapple and banana were the substrates used for wine production using *M. guilliermondii* and *P. guilliermondii* isolates obtained from palm wine as the fermenting organisms. The mean value of the moisture content of the wine "must", the wine fermented by *Meyerozyma guilliermondii* and *Pichia guilliermondii* recorded was 84.4, 82.56, and 69.51 %, respectively. This accounts for their highly perishable nature and short shelf life under normal storage conditions [12]. This high moisture content makes the beverage suitable as a refreshing and thirst-quenching product which

qualifies it as a good beverage [13]. This high moisture content was similar to the results obtained by Chilaka *et al.* in their study which recorded a moisture content of 72-84%. Aminu *et al.* (2018), also recorded a moisture content of 70.94% in their study [14].

The ash content of the fruits was minimal and indicates the presence of mineral components in the fruits. The mean value of the ash content of the wine "must", the wine fermented by *Meyerozyma guilliermondii* and *Pichia guilliermondii* recorded was 3.52, 1.41, and 1.19 %, respectively. This agrees with study by Chilaka *et al.* who reported an ash content of 3.83 % in pineapple fruit but in contrast with the results obtained by Aminu *et al.* who recorded an ash content of 0.34 % [14,15].

There was a minimal amount of fat in the fruits and this indicates that the wine could provide protection against excess body lipids (cholesterol) and it demonstrates the desirable nutritive quality of the fruit wine produced. The fat content of the wine "must", the wine fermented by *Meyerozyma guilliermondii* and *Pichia guilliermondii* observed was 0.09, 0.08, and 0.15 %, respectively. This corresponded with the findings of Aminu *et al.* (2019) who reported the fat content of their fruits as 0.04 % and Chilaka *et al.* that recorded a fat content of 0.24 % in watermelon and 0.17 % in pineapple fruits [14].

The protein content was low and this could be an indication that there will not be accumulation of protein due to consumption of the fruits as reported by Okegbile and Taiwo [16]. The protein content of the wine "must", the wine fermented by *Meyerozyma guilliermondii* and *Pichia guilliermondii* observed was 1.45, 1.43 and 0.49 %, respectively. This was similar to the findings of Chilaka *et al.* who recorded a protein content of 2.57% in passion fruits [14].

Sucrose (sugar) was added to the must in order to supplement the sugar content in the fruits. The fruits contained a reasonable amount of carbohydrate which gives an account of their high caloric value. The carbohydrate content for the wine "must", the wine fermented by *Meyerozyma guilliermondii* and *Pichia guilliermondii* was observed to be 13.47, 9.77 and 10.49 %, respectively. This was in consonance with the findings of Chilaka *et al.* who reported a carbohydrate content of 10.87 % for pineapple fruit but in contrast with the carbohydrate content of 17.55 % for passion fruit [14]. The result was also in contrast with the findings of Aminu *et al.* who recorded a carbohydrate content of 27.81 % [15].

5.0 Conclusion

This study was carried out to determine the proximate composition of the banana and pineapple musts and the wines fermented by *Meyerozyma guilliermondii* strain 1621and *Pichia guilliermondii* strain PAX-PAT 18S. It shows that banana and pineapple are suitable substrates for wine production because the wine produced by *Meyerozyma guilliermondii* strain 1621 had a moisture content of 82.56 %, ash content of 1.41 %, fat content of 0.08 %, protein content of 1.43 %, and carbohydrate content of 82.56 % while the wine produced by *Pichia guilliermondii*

strain PAX-PAT 18S had a moisture content of 79.51 %, ash content of 1.19 %, fat content of 0.15 %, protein content of 0.49 %, and carbohydrate content of 10.49 %.

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