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Original Research Article

Production and proximate composition of bread produced using yeast species Isolated from Palm wine

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Abstract
Isolation and sensory evaluation of palm wine yeast from palm wine gotten from Ndoro market was analyzed on bread fermentation. The ability to get quality bread depends on its ingredients, skill and a very good fermentative agent. In this study, palm wine was gotten from Ndoro market, together with commercial yeast, which serves as the control yeast and they were cultured at 30^0C to isolate different yeast species from palm wine using the appropriate identification techniques like the colony morphology, microscopic observations and fermentative ability. The yeast isolates were then used to bake bread at 180^oC for 8 minutes and the sensory properties of the bread like volume, crust, color, internal color, structure, texture, flavor, aroma, crumb clarity and elasticity were analyzed. Bread sample B5 showed the highest mean when compared to volume (4.4), crust (4.0), color (4.3), internal color (4.4), structure (3.3), texture (3.6), flavor (3.7), aroma (4.1) and crumb clarity (3.4) more than the control which had volume (4.3), crust (3.5), color (3.7), internal color (4.0), structure (2.5), texture (3.1), flavor (3.5), aroma (4.0) and crumb clarity (3.4),. Although some of the isolates did not have high leavening ability like the control, they influenced the aroma and flavor more than the control. Thus, these isolates may be combined to give varieties of sensory properties on bread and with the right industrial facilities would become an indigenous baker's yeast.

Keywords: bread, fermentation, palm wine, production, yeast

INTRODUCTION

Bread is traditionally made from flour, water, salt and yeast. Bread in all its form is an important part of the human diet, but for many people, it is more than that (Adeleke and Odedeji, 2010). It has a honey comb structure and may be regarded as solid foam with a multitude of pockets of carbon dioxide distributed uniformly throughout it bulk (Lean, 2006). Bread is a staple food from flour or meal mixed with other dry and liquid ingredients, usually combined with a leavening agent and kneaded, shaped into loaves and baked. Bread alone with cereals, rice and pasta make up the foundation of a healthy diet. Derived from grains, these foods are rich sources of energy (Carbohydrate), they provide some protein, are economical and naturally low in fat (Burrier and Legras, 2014). Not only is it an important source of carbohydrate, it's also portable and compact, which helps to explain why it has been an integral part of our diet for thousands of years (Loham, 2012).

The outer hard portion of bread is called the crust. The crumbs texture is greatly determined by the quality of the pores texture in the bread (Daobread, 2012). The bread ingredients are formulated to give bread its taste, structure, aroma, texture and nutrients. These ingredients includes flour, liquids, leavening agent, salt, sweeteners, fats or oil and additive (optional) (Burrier and Lucas, 2014; Ashton, 2009).

Yeasts are living unicellular, eukaryotic, polyphyletic and ubiquitous micro-organisms commonly found on fruits, vegetables and other plant materials. They are facultative anaerobes and can respire and survive under both aerobic and anaerobic conditions. In the absence of oxygen, they can ferment sugar into alcohol (Ethanol) and carbon dioxide and enough energy and convert sugar into high biomass (Kevin, 2005).

Palm wine is an alcohol beverage produced from the sap of various palm tree species and usually consumed in parts of African, Asia and South America (Chandraskhar et al., 2012). In Africa, the sap is most often taken from wild data palms such as Phoenix sylvestris (The palmyra) and caryotaurens, from oil palms such as Elasia guineensis or from raphia, kithul or nipa palm. The liquid collected is a cloudy whitish beverage with a sweet alcoholic taste and very short shelf life of only one day. The wine is consumed in a variety of flavors varying from sweet unfermented to sour, fermented and vinegary (Chandraskhar et al., 2012). Palm wine contains good amount of microorganisms, the types and number of organisms encountered vary widely even from tree to tree (Theivendiravagah et al., 1987). From microbial analysis of palm wine, it includes both yeast and bacteria. Ezeronye and Okerentugba (2001) reported the genetically and physiologically different isolated yeasts from palm wine. Yeast populations have been reported in the palm wine in concentration of about 104 to 107 Cfu/ml. Palm wine yeast isolated from freshly tapped palm wine are mainly Saccharomyces and Candida from different trees. The Saccharomyces species identified are as follows: Schizosaccharomyces pombe, Saccharomyces cerevisiae, Debaromyces hensenii, Zygosaccharomyces rouxii (Chandraskhar et al., 2012).

Elasia guineensis harbours many yeast species. Although considerable amount of work has been done in characterizing the yeast strains present in palm wine, there is only little information available on the leavening ability of the yeast. Even though palm wine is consumed and used around the world and also plays an important role in the economic and social life of the people, palm wine has not been comprehensively evaluated for quality improvement and possible exploitation of the biological and chemical constituents or by products (Ovoba *et al.,* 2012). The main aim of this research work was to produce bread using yeast isolated from palm wine and determining the proximate composition of the bread.

2.0 MATERIALS AND METHODS

2.1 Sample collection

The palm wine samples were sourced from Ndoro Oboro Market in Ikwuano L.G.A, Abia State. The commercial yeast used as control was obtained from Umuahia market. The freshly tapped oil palm tree palm wine (Nkwu enu) was purchased from sellers at the sites stated above. 10 different fresh palm wine samples were obtained using sterile containers and were immediately transported to the laboratory in iced coolers. The samples were stored in the refrigerator at 4^{o}C until used.

2.2 Isolation of yeast species

The fresh palm wine samples were serially diluted according to Cheesbrough (2005); Ezigbo *et al.* (2014). 10g of the instant dry yeast were dissolved in 200ml of water to get the stock. The stock was then serially diluted and 1ml of suitable dilution was inoculated aseptically by pour plate method in triplicate on Potato Dextrose Agar (PDA) (Harigan, 1998). The plates were incubated at $30^{o}C\pm2^{o}C$ for 48 hours for the colonies to develop.

2.3 Characterization of yeast species

Yeast colonies were inoculated into Yeast Fermentation broth (YFB) containing Brothymol Blue inside test tubes containing Durham tubes to which different carbon sources (glucose, sucrose, fructose and maltose) were added. The tubes were incubated at 30^{o}C for 72 hours. Colour changes from green to yellow indicated sugar utilization by yeast. The results were compared with positive and negative controls (Maaruf, 2011).

2.4 Cultivation of yeast isolates for dough fermentation

Yeast isolates from the slant cultures were cultured on sterile PDA. The isolates were cultured separately at $30^{o}C\pm2^{o}C$ in peptone broth machine containing 25% (w/v) glucose in 100ml of conical flask equipped with air locks. The set up was agitated continuously for 72 hours in rotary shaker regulated at 150 rpm. After good growth was observed, the biomass concentrate for each isolate was obtained by centrifuging it in an MSE Centrifuge Machine at 10,000 for 15 minutes. The resulting cells were then washed with sterile distilled water after which it was re-suspended in 10ml sterile distilled water in readiness for further use.

2.5 Dough fermentation

All the yeast isolates and the control yeast were used to ferment dough by baking bread in order to test for their fermentative ability. Sample of the dough was prepared according to the basic method described by Young and Cauvign, (2007). The sample was left at 30^{o}C for about $1\frac{1}{4}$ hours and baked in an oven at 180^{o}C for 8 minutes.

2.6 Determination of physical and sensory properties of breads

Various properties of the bread samples which include volume, crust, color, internal color, structure, texture, flavor, aroma and crumb clarity were determined by a 20 enlightened judges. Five points grade was use in the analysis starting with Excellent = 5, very good = 4, Good = 3, satisfactory = 2 and poor = 1.

2.7 Proximate analyses of bread samples

2.7.1 Moisture Content

The Moisture content was determined by method of AOAC (2000). Two grams of bread sample was weighed into a pre-dried and weighed crucible and placed in thermostatically regulated hot air oven (MIDO/SS/F UK) at 105^{o}C for 8 hrs. The crucible was removed, cooled in a desiccation and weighted. The process was repeated until a constant weight was obtained.

$$\text{Moisture} = \frac{\text{Initial mass sample - mass after drying}}{\text{Initial mass of sample used}} \times 100$$

2.7.2 Protein content

The protein content was determined using a foss Tescator protein digestor and KJECTEC distillation 2200 distillation apparatus (Kjeldahl method) according to the procedure of AOAC, (2000), concentration H_2SO_4 12ml and 2 tablets of catalysts was put into a Kjeldahl digestion flask containing 1g of the sample. The flask would be placed in the digestor in a fume cupboard and switched on and digestion was done for 45 minutes to obtain a clear colourless solution. The digest was distilled with 4% boric acid, 20% sodium hydroxide solution will be automatically melted into it in the KJECTEC 220 distillation equipment until distillation was completed. The distillate was then titrated with 0.1m HCL until a violet color formation indicating the end point. A blank was run under the same condition as with the sample. Total nitrogen content was then calculated.

2.7.3 Fat content

The fat content was measured by the method of AOAC (2000). Two grams of dry bread sample was weighed into a cellulose thimble and plugged with glass wool. The extraction was carried out in a soxwet apparatus for 16 hours with 150ml of petroleum ether. The flask was removed and the silvered evaporated on stem bath in a hood. The flask and its contents were dried in the hot air oven at 103^{o}C for 30 minutes cooled in a desiccator and weight of crude fat extracted was measured and the percentage fat calculated.

$$\%\ \text{Crude Fat} = \frac{\text{Mass of fat obtained}}{\text{Dry mass of sample used}} \times 100$$

2.7.4 Fibre content

This was carried out by the method of AOAC (2000). Two grams of bread sample was transferred into 750ml Erlenmeyer flask and the flask and 0.5g asbestos added. 200 ml of boiling 1.25% H_2SO_4 was added to the flask and the flask was connected to cold finger condenser and immediately brought to boil on a hot plate for 30 minutes. The flask was removed and the content filtered through a linen cloth in a funnel and washed with boiling water until 1.25% NaOH solution. The flask was again connected to a condenser boiling for 30 minutes, filtered through linen cloth and thoroughly washed with boiling water.

The content was transferred into a gooch crucible, washed with 15ml of 95 ignited in pre-heated muffle furnace (Gallenkamp muffle furnace England) at 600^{o}C for 30 minutes. The flask was again cooled and reviewed. The weight difference was recorded and percent crude fibre content calculates.

$$\%\ \text{Fibre} = \frac{\text{Mass of fibre x 100}}{\text{Dry mass of sample used}}$$

2.7.5 Ash content

This was carried out by the method of AOAC (2000). Two grams of bread was weighed into a previously and weighed crucible. The crucible and content were ignited in a pre-heated furnace to 600^{o}C for 2 hours. The crucible was cooled in a desicator, weighed, and percent ash content was calculated.

$$\%\ \text{Ash} = \frac{\text{Mass of Ash x 100}}{\text{Dry mass of sample used}}$$

2.8 STATISTICAL ANALYSIS

The data were analyzed using the SPSS Statistical software. Comparison of means was done using the one-way analysis of variance (ANOVA). All statistical analysis was carried at 95% confidence interval.

3.0 RESULTS

Table 1 shows the morphological and biochemical characteristics of the yeast isolates from palm wine. They include *Schizosaccharomyces pombe, Debaryomyces hansenii, Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii.*

In Table 2 is presented the organoleptic properties of the bread samples. Bread 4 has the highest volume (4.4±0.22), crust (4.0±0.26) colour (4.3±0.21), texture (3.6±0.22) and internal colour (4.4 ±0.22) than other bread samples. Together with bread 2, both have best aroma (4.1±0.28).

The proximate composition of the bread samples is presented in Table 3. Bread 2 has the highest protein content (5.43±0.45); Bread 4 has the highest fat content (7.45 ±0.50); Breads 3 and 5 have the highest fibre contents (3.50±0.03 and 3.55±0.02) respectively while Bread 3 has the highest carbohydrate content (8.50 ± 0.30). Breads 1, 2, 3 and 5 have statistically same ash content which are higher than that of Bread 4.

Table 1: Morphological and biochemical characteristics of the yeast isolated from palm wine

S/N	Cell Shape	Glucose	Galactose	Sucrose	Lactose	Maltose	Fructose	Xylose	Mannose	Isolates
A	Cylindrical	A/G	A/-	A/G	-	A/-	A/G	A/-	A/G	*Schizosaccharomyces pombe*
B	Oval	A/G	A/G	A/G	A/G	A/-	A/G	-	A/-	*Debaryomyces hansenii*
C	Oval	A/G	A/G	A/G	-	A/G	A/G	A/-	A/-	*Saccharomyces cerevisiae*
D	Spherical	A/G	A/G	A/G	-	A/-	A/G	-	A/G	*Zygosaccharomyces rouxii*

Keys: A/G; Acid and gas production

Table 2: Physical and organoleptic analysis of Bread Samples produced using yeast isolates

Bread samples	Bread 1	Bread 2	Bread 3	Bread 4	Bread 5
Volume	2.9 ± 0.18^{ab}	2.1 ± 0.23^{ab}	1.2 ± 0.13^{b}	4.4 ± 0.22^{c}	3.3 ± 0.26^{b}
Crust	2.9 ± 0.23^{c}	3.5 ± 0.27^{b}	3.2 ± 0.25^{c}	4.0 ± 0.26^{a}	3.5 ± 0.17^{b}
Colour	3.6 ± 0.22^{b}	3.7 ± 0.26^{b}	3.7 ± 0.21^{b}	4.3 ± 0.21^{a}	3.7 ± 0.3^{b}
Internal colour	3.4 ± 0.27^{c}	3.4 ± 0.34^{c}	3.8 ± 0.20^{c}	4.4 ± 0.22^{a}	4.0 ± 0.3^{b}
Structure	2.5 ± 0.34^{a}	2.8 ± 0.20^{a}	2.4 ± 0.27^{a}	3.3 ± 0.21^{ab}	2.5 ± 0.7^{ab}
Texture	2.8 ± 0.25^{b}	3.0 ± 0.26^{b}	3.0 ± 0.30^{b}	3.6 ± 0.22^{a}	3.1 ± 0.23^{b}
Flavor	3.8 ± 0.20^{a}	3.7 ± 0.34^{a}	3.6 ± 0.22^{a}	3.7 ± 0.21^{a}	3.5 ± 0.31^{a}
Aroma	3.9 ± 0.23^{c}	4.1 ± 0.28^{a}	4.0 ± 0.30^{b}	4.1 ± 0.28^{a}	4.0 ± 0.26^{b}
Crumb clarity	2.7 ± 0.30^{b}	3.2 ± 0.36^{a}	2.7 ± 0.26^{b}	3.4 ± 0.22^{a}	3.4 ± 0.22^{a}
Elasticity	2.5 ± 0.37^{c}	3.2 ± 0.47^{b}	3.2 ± 0.42^{b}	3.8 ± 0.20^{a}	3.9 ± 0.23^{a}

Values are mean± SD; values with different superscripts across the rowa are significantly different (P≤0.05).

Key:
Bread 1 - Produced with *schizosaccharomyces pombe*
Bread 2 - Produced *Debaromyces hansenii*
Bread 3 - Produced with *Saccharomyces cerevisiae*
Bread 4 - Produced with *Zygosaccharomyces cerevisiae*
Bread 5 - Produced with commercial yeast (Positive control)

Table 3: Proximate composition of bread produced using yeasts isolated from palm wine

Bread samples	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
1	0.30 ± 0.01^{a}	3.33 ± 0.70^{a}	4.17 ± 0.60^{a}	2.55 ± 0.10^{c}	3.10 ± 0.10^{b}	2.55 ± 0.10^{a}
2	24.00 ± 1.20^{c}	5.43 ± 0.45^{c}	6.80 ± 0.40^{ac}	2.60 ± 0.20^{c}	2.45 ± 1.00^{b}	2.60 ± 0.20^{c}
3	$20.40+2.40^{c}$	3.85 ± 0.30^{bc}	6.80 ± 0.60^{ac}	3.50 ± 0.03^{c}	3.34 ± 0.30^{b}	8.50 ± 0.30^{a}
4	24.10 ± 1.40^{c}	4.90 ± 0.67^{bc}	7.45 ± 0.50^{c}	2.00 ± 0.20^{a}	1.33 ± 0.02^{a}	2.00 ± 0.20^{c}
5	24.02 ± 0.84^{c}	3.76 ± 0.85^{ab}	6.46 ± 0.20^{c}	3.55 ± 0.02^{c}	3.21 ± 0.76^{b}	1.49 ± 0.02^{a}

Values are mean ± SD; values with different superscript along the columns significantly different (P≤0.05).

Key:
Bread 1 - Produced with *Schizosaccharomyces pombe*
Bread 2 - Produced *Debaromyces hansenii*
Bread 3 - Produced with *Saccharomyces cerevisiae*
Bread 4 - Produced with *Zygosaccharomyces cerevisiae*
Bread 5 - Produced with commercial yeast (Positive control)

4.0 DISCUSSION

The present research was the production and proximate composition of bread using yeast species isolated from palm wine. Four different yeasts species namely *Schizosccharomyces Pombe*, *Debaryomyces hansenii*, *Saccharomyces cerevisae* and *Zygosaccharomyces rouxii* were obtained from freshly tapped palm wine samples. This result is in agreement with the findings of Ezeronye and Okerentugba, (2001). The variation in species of yeasts isolated is related to the fact that the yeast originated from several sources such as the tapping container, air, body of the palm wine tapper and the handlers. Palm wine contains a high level of sucrose (10-12%). The high sugar level tends to give support to the general belief that yeasts are bound in natural sugary substances like palm wine and ripped fruits.

Yeast isolates increased the volume of the bread even more than the commercial yeast which is due to its ability to produce carbon dioxide. This finding agrees with Cofalec (2014) and Francisca *et al.,* (1999) who stated that yeast produces carbon dioxide that results in dough leavening and contributes to the flavor and crumb structure of bread. Breads 1 and 2 were significantly different from the positive control. This means that they were not able to yield good results like the positive control. This agrees with Chukwuka, (2013), who stated that different strains of *S. cerevisiae* produces different proportions of carbon dioxide and alcohol. Therefore it implies that those isolates did not produce enough Carbon (iv) oxide for the quantity of flour used. Bread 3 was very similar to Bread 5 due to the fact that it produced little or no Carbon (iv) oxide. The crust, color and internal color of the breads were checked to get the overall acceptability of the bread since it could imply the yeasts' interaction with the ingredients of the bread. The bread ingredients are formulated to give bread its taste, structure, aroma, texture and nutrients. These ingredients include: flour, liquids, leavening agent, salt, sweeteners, fats or oil and additives (optional) (Ashton, 2009, Burrier, 2014).

The structures of the different breads showed that the volumes of Breads 3 and 4 were significantly different from Bread 5 as they have a good bread structure because of sufficient Carbon (iv) oxide produced by the yeasts which helped in the development of the gluten network. Thus, the doughs were fattest and biggest. This gave rise to bigger, lighter and airy loaf with tiny holes formed by the gas and sponge-like look. The structure of bread depends on dough ingredients, yeast activity, fermentation temperature and gas bubble formation (Lassoued *et al.,* 2007).

Fermentation of the dough affected the flavor and aroma all the breads including that made using the control yeast. This agrees with Chukwuka, (2013) who reported that during dough fermentation, yeast produces secondary by-products like ketones, higher alcohols, organic acids, aldehydes and esters. Most of the alcohols are cooked off during baking while the other metabolites react with one another and other elements contained in the fermenting dough to form new and more complexed flavored compound (BRIT, 2014).

Crumb clarity and the elasticity of the breads were better than Bread 1. This could be due to the ability of the yeasts to leaven the dough and production of Carbon (iv) oxide. This affects the strength of the dough to hold the gases produced and its ability to be stable enough to hold its shape and cell structure. The values of the pH are within the standard pH limits (5.30-6.0).

Bread 3 has the highest value of carbohydrate while Bread 4 has the highest value of moisture and fat. The protein content of bread produced by *Debaromyces hansenii* was higher than that produced using Bakers' Yeast. This means that Bread 1 can give more energy than bread produced using the Bakers' Yeast. It also proves that using *Saccharomyces cerevisiae* isolated from palm wine as a substitute for baker's yeast will give a better product in terms of the bread quality. The higher the presence of fat in bread derived from bran and germ inclusion, the higher the source of fat soluble vitamins and precursors such as vitamin A and provitamin A carotenoids as well as total energy content as reported by Khalting *et al.* (2014). The high proximate values recorded from this work suggest that bread samples produced from palm wine yeast are capable of meeting nutritional provisions as a staple food. These findings are in agreement with the findings of Joel *et al.* (2013).

CONCLUSION

This research has shown that *Saccharomyces cerevisiae* and *Zygosaccharomyces cerevisiae* isolated from palm wine performed better that Bakers' Yeast in terms of the proximate composition, physical and sensory properties of the bread

samples. The other yeast isolates may not be as good as isolate 3 in leavening but they were able to produce nice flavor and aroma in the breads. We also conclude that palm wine is a good source of yeasts that can e used in bread baking.

Recommendation

We therefore recommend that *Saccharomyces cerevisiae* and *Zygosaccharomyces cerevisiae* isolated from palm wine be used in bread baking due to better properties they possess over the conventional Bakers' Yeast.

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