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ISOLATION AND COMPARATIVE ASSESSMENT OF DOUGH-FERMENTING POTENTIALS OF PURIFIED YEAST CELLS FROM LOCALLY TAPPED PALM WINE

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Abstract: *Yeasts from palm wine can become suitable substitutes to their counterparts imported in Nigeria. In this study, total of eight Elaeis guineensis and Raphia hookeri palm wine samples were collected from Enugu and Imo States, Southeastern Nigeria. Their physicochemical analysis, isolation, identification, and purification of yeast isolates in the samples were conducted. After immobilization, both free and immobilized yeasts were used to ferment the dough. The effects of isolates on organoleptic properties of resulting dough were analyzed. However, S. cerevisiae was the most predominant, other yeast species isolated were S. uvarum, S. carlbengesis, and Schizosaccharomyces pombe. There was no significant difference in flavour and taste of dough fermented by thirteen free and thirteen immobilized yeast isolates, as well as baker's yeast, used. Dough fermented with immobilized S. carlbengesis isolated from E. guineensis palm wine from Orlu and baker's yeast had the best appearances, while the best texture was recorded in dough fermented with immobilized S. carlbengesis isolated from E. guineensis, from Enugu. The dough hardness recorded with baker's yeasts was not significantly different from those fermented by immobilized S. cerevisiae and S. carlbengesis (both from E. guineensis from Owerri) isolates, as well as free S. carlbengesis (E. guineensis from Enugu State) and S. cerevisiae (R. hookeri from Enugu State). Also, yeasts from E. guineensis performed better than those from R. hookeri, with immobilization, a great influence on the organoleptic properties of the resulting dough was observed. Consequently, some yeast isolated in locally tapped palm wine can be usefully applied in bread-making.*

Keywords*: Fermentation; Yeast immobilization; Dough; Palm wine*

1. Introduction

Palm wine is produced by fermentation of sap of some tropical plants from the family *Palmae,* such as *R. hookeri* and *E. guineensis* [1]. Palm sap serves as a suitable substrate for the growth of diverse species of microorganisms. When freshly tapped, and had not undergone fermentation, sap is sweet-tasting, thick, and consists of approximately 10-12% sugar, especially sucrose [2]. Fermentation of palm wine by indigenous strains of microorganisms reduce its sugar constituent, thereby increasing the concentrations of alcohol and other products of yeast fermentation. The presence of microbial suspension responsible for fermentation turns fermented sap into a milky, white liquid [2]. Palm sap can be tapped in different ways, including immature inflorescence tapping, usually practiced in Nigeria, Cote d'Ivoire and Benin, or stem tapping [3]. Tapping of raffia palm is undertaken when it begins to flower. It involves the removal of newly formed inflorescence and making deep cut into phloem vessel of palm tree [4].

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Studies have reported that the population of yeasts and other microorganisms found in palm wine are determined by its source and degree of fermentation [5]. Different microorganisms, such as aerobic mesophilic bacteria, acetic acid bacteria, coliforms, yeasts, and lactic acid bacteria have been isolated from palm wine samples [3]. Obi *et al*. [6] reported the isolation of eight (8) bacterial strains, including *Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Lactobacillus* spp, *Bacillus, Streptococcus* spp, *Serratia marcescens,* and *Acetobacter* spp from palm wine samples obtained from Umuariaga Community, Abia State, Nigeria. Similar reports had revealed that the presence of about 10^4 to 10^7 cfu/mL of yeast populations, 10^6 to 10^9 cfu/mL of total aerobic mesophiles, 10^5 to 10^8 cfu/mL of acetic acid bacteria, 10^3 to 10^7 cfu/mL of total coliforms, and 10^7 to 10^9 cfu/mL of lactic acid bacteria in palm wine samples [7-9].

Metabolic activities of yeasts and other microorganisms in palm wine have been shown to determine its physicochemical properties. Ukwuru and Awah [10] reported a value of pH 5 in palm wine samples after 24 h of collection. Thus, freshness of palm wine samples can be determined by assessing its pH. After 24 h of fermentation, the total dissolved solid recorded in palm wine samples obtained from Etche and Obio-Akpo Local Government Areas, Rivers State, Nigeria, increased from 600 mg/L and 400 mg/L to 1322 mg/L and 1406 mg/L, respectively. Also, during the period, pH of the samples decreased from 6.3 to 3.2 and 6.4 to 3.1, respectively. This suggests that shelf life of palm wine is only about 24 h, beyond which they turn into sour because of high concentrations of fermentation products [7].

S. cerevisiae has been reported to be the most prevalent yeast in palm wine, and possesses high fermenting potential, which enables it to convert sugar presents in palm wine into ethanol and carbon dioxide. *S. cerevisiae* is generally regarded as safe by the U.S. Food and Drug Administration (FDA), hence its wider applications in different studies [11]. Another strain of yeasts, *S. uvarum,* also known as "wine yeast", is also utilized in food processing industry due to its potential in wine production. Where malic acid constitutes a problem to wine making, *Schizosaccharomyces pombe* is found very useful [12].

In view of efforts by the Nigerian government to increase local sourcing of industrial raw materials, the massive fermenting potentials of yeasts from palm wine have not been fully exploited in local food industries. Hence, this study was aimed to isolate and comparatively assess dough-fermenting potential of purified crude yeast cells from locally tapped palm wine.

2. Materials and methods

2.1. Collection of palm wine samples

All plastic containers used for collection of palm wine samples were first surface sterilized according to the modified method of Wu *et al.* [13]. This was done by washing with detergent and repeatedly rinsing with sterilized distilled water. Then, they were washed with ethanol for 5 minutes, rinsed before washing with sodium hypochlorite solution for 15 minutes. Finally, they were rinsed four times with sterilized distilled water for 10 minutes in each case. Then, they were used to separately collect freshly tapped palm wine of *Raphia hookeri* and *Elaeis guineensis,* from palm wine tappers. A total of eight (8) palm wine samples were collected; four (4) of which were from each of *R. hookeri* and *E. guineensis*. Two (2) samples of each palm wine were collected

from Enugu and Imo States, Southeastern Nigeria. In Enugu State, samples of *R. hookeri* were collected from Orji River and Udi, and labelled RHE 1 and RHE 2, respectively. Those of *E. guineensis* were labelled from Enugu North and Udi, and labelled EGE 1 and EGE 2, respectively. On the other hand, in Imo State, *R. hookeri* palm wine samples were collected from Emeabiam (RHO 1) and Umuagwo (RHO 2). *E. guineensis* palm wine samples were collected from Mbaise (EGO 1) and Orlu (EGO 2). Each palm wine sample was collected in triplicates. Samples were preserved in ice packs and transported to the laboratory for further analysis.

2.2. Determination of physicochemical properties of fresh palm wine samples

The physicochemical properties of freshly tapped *R. hookeri* and *E. guineensis* palm wine samples were determined. The pH was measured using Orion Star A3210 series pH meter (Thermo Fisher Scientific Inc., Waltham, MA, USA), while total soluble solids (TSS) were determined using Vee Gee 43064 hand refractometer (VEE GEE Scientific, LLC, Vernon Hills USA). Specific gravity of each palm wine sample was measured using M100 hydrometer (LS Scientific Kent United Kingdom). Following the methods of Ejimofor *et al.* [14], total acidity was measured by titrating each sample with NaOH, using phenolphthalein as indicator, and calculating its concentration of lactic acid. Total sugars and reducing sugars contents were determined following Lane-Eynon volumetric method, by titrating each sample with Fehling reagents. Alcohol content was measured by fractional distillation of samples, followed by determination of distillate values, and then extrapolation of alcohol content from alcohol table. Percentage moisture content was determined by recording and substituting the weights of a given volume of palm wine sample, before and after complete evaporation of water, and was calculated by using Equation 1:

$$\%\, Moisture\ content = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100$$

Percentage total solid (%TS) was determined by weighing the remnant after complete evaporation of known volume palm wine sample, and substituting in the Equation 2:

$$\%\, TS = \frac{W_{final}}{W_{initial}} \times 100$$

2.3. Isolation of yeasts from palm wine

Yeasts were isolated from each of the palm wine samples following the method described by Cheesebough [15]. Initially, ten-fold serial dilution of each fresh palm wine sample was prepared. Then aliquots from 4^{th}, 5^{th} and 7^{th} dilutions were spread on different plates with Sabouraud Chloramphenicol Agar (SCA) (Sisco Research Laboratories (SRL), Maharashtra India). The SCA was earlier prepared under aseptic conditions, following the manufacturer's direction. The plates were incubated at room temperature, for 72 h, before observation for formation of yeast colonies was made.

2.4. Cultural identification and purification of yeast cells

Cultural identification of yeast cells was done based on colour, shapes, and the budding nature of cells. Following the method described by Ogbulie and Nwakanma [16], wet mounts of the isolates were prepared using Lugol's iodine. Then, the examination was made under low power (×10) and high power (×40) objectives. The identified yeast cells were purified as described by Martyniuk and Oron [17], with some modifications. Yeast broth was prepared and sterilized according to the

manufacturer's direction. Then, pure culture of each yeast isolate was prepared by aseptically sub-culturing isolates from the plates and incubating at 25 °C for 72 h. Following incubation, the broth cultures were centrifuged at 12,000 rpm for 10 minutes, and supernatant was discarded. Washing was done three times by adding sterilized distilled water to the pellets and centrifuging at 12,000 rpm for 10 minutes. Afterwards, each isolate of purified yeast cells was divided into two parts; one part was immobilized while the other was not.

2.5. Immobilization of yeast cell

Purified yeast cells were immobilized using sodium alginate and calcium chloride, according to Nevratil [18]. In one beaker, 1 g of sodium alginate was dissolved in 4 mL of sterilized distilled water, while in another beaker, 4 g of calcium chloride was dissolved in 15 mL of sterilized distilled water. Then, each purified yeast isolate was separately mixed with sodium alginate. Using sterilized syringe, the yeast and sodium alginate mixture was slowly dropped into the beaker containing calcium chloride, to form beads. After 10 minutes, the beads, containing immobilized yeast cells were separated using sieve.

2.6. Dough fermentation

Fermentation of dough was aseptically carried out using the method described by Bites [19]. One gramme (1 g) of each sugar and NaCl was mixed with 4 g of flour in each of 42 containers. The experiments were grouped as follows:

Treatment 1: Comprised of 13 containers, each mixed with beads containing each of the immobilized pure yeast isolates from each of the palm wines.

Treatment 2: Comprised of 13 containers, each mixed with each of the non-immobilized pure yeast isolates from each of the palm wines.

Treatment 3: Comprised of 1 container, mixed with commercially available baker's yeast, as positive control.

Treatment 4: Comprised of 1 container, mixed without any yeast added, as negative control.

Then, 15 mL of warm water was added to each of the mixtures in all the treatment, properly stirred, and covered for 45 minutes, to allow the dough to rise. The groundnut oil was heated, each of the dough was in turn dropped it, and fried until it turned brown. Then, the fried dough was transferred to different sieves. A questionnaire was designed and administered to 20 panelists who tasted the dough prepared with different rising agents, to assess their organoleptic properties. Six organoleptic properties of the dough, including flavour, appearance, texture, puffiness, taste, and hardness were considered. Range of scores on 5-point hedonic scale, with 5 representing "liked extremely" and 1 "dislike extremely" were used for the assessment [20].

2.7. Statistical analysis

Data generated from the questionnaire were analyzed with ANOVA, using MINITAB version 17 software (Minitab LLC, Witkoppen South Africa). Post hoc least significant difference (LSD) test ($p = 0.05$) was used to identify the rising agents that produced significant difference in their effect on the dough.

3. Results and discussion

3.1. Physicochemical compositions of *R. hookeri and E. guineensis palm wine*

Figure 1 shows the pH values and specific gravity of all palm wine samples. Generally, the palm wine samples were acidic, with samples from *R. hooheri* having higher pH values. The pH of the samples was in the following order: RHE 2 (3.81)>RHO

2(3.78)>RHE 1(3.70)>RHO 1(3.69)>EGO 1(3.62) <EGE 1(3.51)>EGO 2(3.49)>EGE 3(3.42)>EGE 2(3.39)>EGE 4(3.39). These lower pH values are essential for an improved shelf life of the palm wine. In another previous study of palm tree and raffia palm, the pH of *E. guineensis* was 6.0 with cloudy appearance, while pH of palm wine was 7.2, with milky colouration [14]. The pH values were higher than the pH obtained in the present study. It had been reported that pH of palm wine decreases with increasing storage time, such that pH at time 0 becomes higher than pH after 6 h [1]. This could be attributed to continuous fermentation activities of yeasts in the palm wine as observed in this study.

There was no significant difference of the specific gravity recorded in all studied palm wine samples. The specific gravity of all the palm wine samples were within the range of 1.0 to 1.02 which is in line with the findings of Biose *et al.* [24].

As presented in Fig. 2, it can be observed that the highest sugar content of palm wine was recorded for sample EGO 1 (12.8%), while the lowest was for RHE 1 and RHE 2 with 3% each. Palm wine from *E. guineensis* had higher sugar content. Also, moisture content was highest in EGO 1 sample, but least in EGE 1 sample.

The glucose content (g/100 mL) was 0.75 and 0.60; fructose content (g/100 mL) was 1.05 and 0.80; sucrose content (g/100 mL) was 2.90 and 2.50; maltose content (g/100 mL) was 1.80 and 0.09, and total sugar content (g/100 mL) was 6.50 and 3.99, respectively for palm wine from *E. guineensis* and *R. hookeri*. Fermentable sugar utilized by yeasts during bread making, arise from different sources. Some fermentable sugars, such as fructose, glucose, maltose, and sucrose occur naturally in flour. Fermentable sugars, including sucrose may also be added by the baker, which may be up to 25% *w/w*. Amylolytic breakdown of starch also supplies maltose to the dough during bread-making. In making sweet dough, the tolerance of yeast to high osmotic stress is essential to their industrial importance [22]. Plain dough is unique because yeasts completely consume pre-existing free sugars during fermentation, leaving only the starch-derived maltose for sustenance of fermentation. Here, the potential of yeasts to rapidly ferment maltose is a necessary industrial factor [22].

The moisture content of *R. hookeri* samples was higher than those of *E. guineensis*, with the highest value of 9.7%, recorded in RHE 1, and the least value of 8.5% in EGE 1. Percentage total solid content was higher in *E. guineensis* samples, with the highest value of 14.6% recorded in EGO 1, while the least of 3.1% was found sample RHE 2. The highest percentage alcoholic content was found in palm wine samples tapped from *E. guineensis*. There were 3.60, 3.74, 3.53, 3.60, 3.67, 3.13, 0.46, and 0.40 % alcoholic contents for EGE 1, EGE 2, RHE 1, RHE 2,1, and RHO 2 samples, respectively. The percentage titratable acidity of EGO 1, EGO 2, EGE 3, EGE 4, RHE 1, RHO 1, RHO 2, RHE 2, EGE 2, and EGE 1 palm wine samples were 5.29%, 5.23%, 5.17%, 4.1%, 2.8%, 2.73%, 2.67%, 2.61%, 2.32%, and 2.26 % respectively.

The alcohol content of palm wine samples in the present study was 4.3 g/100 mL and 4.0 g/100 mL for palm tree and raffia palm, respectively. Notably, alcohol content is an essential factor which makes palm wine safe for drinking due to its ability to eliminate opportunistic and pathogenic microorganisms that may be found in palm wine [21].

3.2. Cultural characteristics of yeast isolates

Table 1 shows that a total of thirteen (13) yeast isolates were found from all studied palm wine samples.

The strains identified were *Saccharomyces cerevisiae, S. uvarum, S. carlbengesis,* and *Schizosaccharomyces pombe.* The results showed that palm wine samples from *E. guineensis* had higher diversity of yeast isolates than samples from *R. hookeri*.

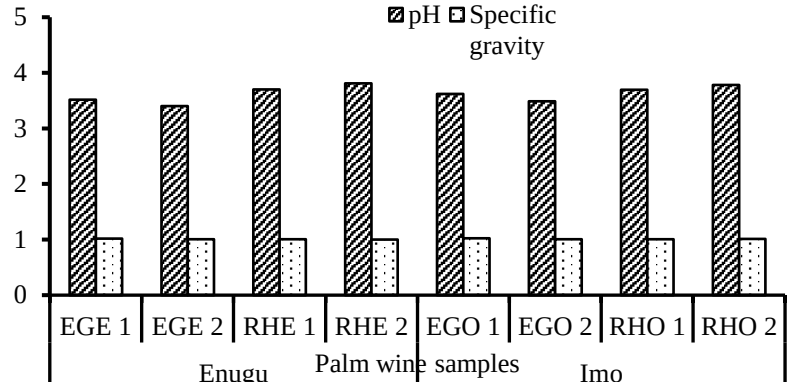

Fig. 1. Specific gravity and pH of *E. guineensis* (EG) and *R. hookeri* (RH) palm wine samples

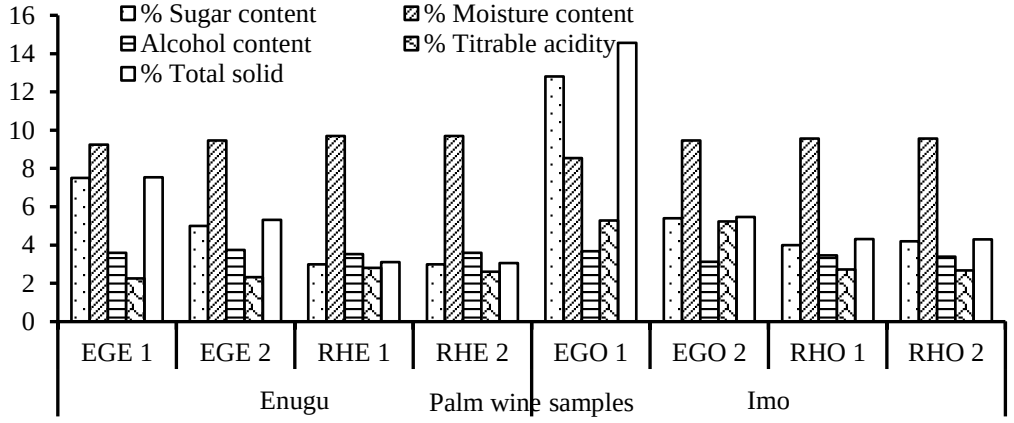

Fig. 2. **Physicochemical properties of *E. guineensis* (EG) and *R. hookeri* (RH) palm wine samples. Sample RHE 1 is from Oji River, RHE 2 from Udi, both in Enugu State; RHO 1 from Emeabiam and RHO 2 from Umuagwo, both in Imo State**

Yeasts are ubiquitous in their occurrence and can exploit diverse substrates for growth. The nutritional composition of fresh palm wine presents it as a suitable medium for growth of many other microorganisms. Microorganisms found in palm wine maybe introduced from the environment, tapping equipment, containers, and the plant surfaces. However, most of them are subsequently eliminated as alcohol content of palm wine increases with fermentation [21]. Besides palm wine, another study had reported isolation of four (4) different yeast species, including *Rhodotorula minuta, Rhodotorula mucilagnosa, Candida krusei,* and *Candida colliculosa,* from pineapple, sweet orange, and palm wine [23]. Similarly, *Candida* spp and *S. cerevisiae* were among the yeast isolates identified in palm wine samples studied by Olabisi [22].

Table 1.

Cultural morphology and microscopic identification of yeast isolates

Sample code	Isolate code	Cultural morphology	Microscopic description	Yeast identity
EGE 1	P1	Medium sized milky colony with smooth surface	Circular bud cells with smooth surface	*S. cerevisiae*
	P2	Pinpoint whitish colony	Circular bud cells with rough surface	*S. uvarum*
EGE 2	P3	Medium sized milky colony with smooth surface	Irregular shaped bud cells with smooth surface	*S. carlbengesis*
	P4	Large sized milky colony with smooth surface	Circular bud cells with smooth surface	*S. cerevisiae*
RHE 1	P5	Medium sized milky colony with smooth surface	Circular bud cells with smooth surface	*S. cerevisiae*
RHE 2	P6	Medium sized milky colony with smooth surface	Circular bud cells with smooth surface	*S. cerevisiae*
	P7	Pinpoint whitish colony	Rod like shape	*Schizosaccharo myces pombe*
EGO 1	P8	Medium sized milky colony with smooth surface	Circular bud cells with smooth surface	*S. cerevisiae*
EGO 2	P9	Medium sized milky colony with smooth surface	Circular bud cells with smooth surface	*S. cerevisiae*
	P10	Large sized milky colony with smooth surface	Rod like shape	*Schizosaccharo myces pombe*
	P11	Twin shaped milky colony with smooth surface	Irregular shaped bud cells with smooth surface	*S. carlbengesis*
RHO 1	P12	Large sized milky colony with smooth surface	Circular bud cells with smooth surface	*S. cerevisiae*
RHO 2	P13	Large sized irregular shaped colony with rough surface	Circular bud cells with rough surface	*S. uvarum*

***** EGO – *E. guineensis* palm wine from Owerri; EGE – *E. guineensis* palm wine from Enugu; RHO – *R. hookeri* palm wine from Owerri; RHE – R. hookeri palm wine from Owerri.

3.3 Percentage occurrence of yeast strains in palm wine

A total of thirteen yeast isolates were obtained from *R. hookeri* and *E. guineensis* palm wine samples, collected from two locations in Imo State, and two locations in Enugu State. Assessment of their identities revealed that there were four different strains, with their percentage occurrences presented in Fig. 3. Overall, 70% of the isolates were *Saccharomyces cerevisiae*, while each of *Saccharomyces uvarum*, *Sacharomyces carlbengesis*, and *Schizosaccharomyces pombe* was 10%. A previous study has established that out of 20 yeast isolates obtained from palm wine, most isolates were predominantly *Saccharomyces* spp. Other yeast genera found included members of *Candida* and *Pichia*, which indicated higher potential of *S.* spp. to withstand increasing ethanol concentrations of up to 10% (v/v) in aging palm wine [21].

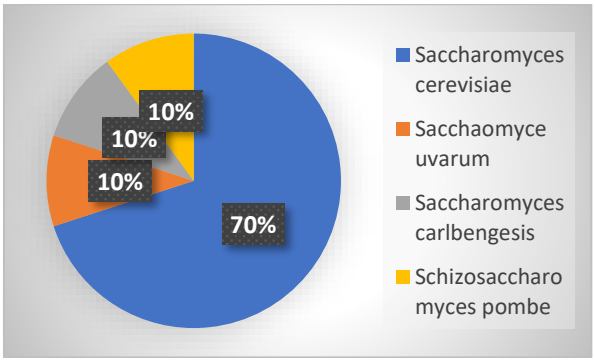

Fig. 3. Percentage occurrences of yeast isolates in palm wine samples

3.4. Dough rising potentials of yeast isolates.

Dough prepared with 13 free (P1-13) and 13 immobilized (PM1-13) yeast isolates from palm wine samples, as well as baker's yeast (BY) and baking powder without yeasts (BP), used as controls are presented in Fig. 4. Statistical analysis ($\alpha = 0.1$) of responses of the panelists' showed that significant difference was evident in the effect of various treatments on properties of resulting dough. Immobilized pure yeast isolates were recovered after been used for dough making. These results confirm that yeast isolates from locally available sources, such as palm wine can be useful in dough-making. Similarly, successful bread-making using isolated yeast had been widely reported. Ayoade *et al.* [20] had reported successful application of *S. cerevisiae* strain TSA66 obtained from burukutu (a locally fermented beverage) in composite bread production, with good loaf volume and loaf weight. Furthermore, the moisture content of bread samples fermented by yeast species isolated from pineapple, sweet orange, and palm wine, was found to range from 0.30 to 24.1; the protein content ranged from 3.33 to 5.42; fat content ranged from 4.17 to 6.80; fibre content ranged from 1.49 to 3.50, ash content ranged from 1.33 to 3.10; carbohydrate content ranged from 43.33 to 89.11, and energy content ranged from 256.20 to 407.23 [23].

3.5. Organoleptic properties of fried dough

The results of organoleptic properties of resulting dough obtained from their sensory assessment by panelists were as follows:

Flavour

The responses of panelists on the flavour of resulting fermented dough are shown in Fig. 5. The best flavour was observed in dough fermented in BP (baking powder without yeast) and PM6 (immobilized *S. cerevisiae* isolated from *R. hookeri* palm wine from Enugu State. They were followed by BY and PM4 (immobilized *S. cerevisiae* isolated from *E. guineensis* palm wine from Enugu State). The samples P10, P12, and PM12 were not that flavoured compared to other samples. Statistical analysis ($\alpha = 0.1$) indicated that there was no significant difference in the flavour of dough fermented by the rising agents. However, the flavour recorded in dough fermented with immobilized yeast cell was comparably higher than others, while it was least in unfermented sample (negative control).

Appearance

Fig. 6 presented the responses of panelists on appearance of resulting dough fermented with different rising agents. At ($\alpha = 0.1$), results showed that there were significant differences in the appearances of the dough. Generally, most of the dough fermented by immobilized cells had the better appearance.

Dough fermented with PM11 isolate (*S. carlbengesis* isolated from *E. guineensis* palm wine from Owerri and BY (baker's yeast) had best appearance, compared to other rising agents used. Related dough appearances were recorded in dough fermented with PM6, P11, PM9, P13, P12, P8, P9, P7, P6, P8, P3, P1, P4, BP, PM3, PM7, and PM1. However, others were significantly different from them.

Texture

The responses of the panelists on texture of the processed dough were analyzed, and results obtained are presented in Fig. 7. At ($\alpha = 0.1$), it was observed that there was significant difference in the effectives of the 13 different isolates: as both positive and negative controls, on texture of resulting dough.

Fig. 4. Dough fermented with different rising agents (BY and BP as control, P1-P13 (free yeasts) and PM1-PM12 (immobilized yeasts) from palm wine)

Thus, PM3 (*S. carlbengesis* isolated from *E. guineensis* from Enugu), which had the lowest mean value of 1.75, was the most effective rising agent in giving the dough a good texture. However, its texture was not significantly different from those of BY, PM6, PM10, P6, PM9, PM11, P7, PM13, P8, P9, P10, P11, P12, PM12, and PM4. They were all effective in giving the dough good texture, the other rising agents to include BP, P5. P4, PM7, PM2, PM8, P13, P3, PM1, P1, and PM1 were significantly different from PM8 as they are less effective in giving the dough good texture. The mean values of responses for PM2 (2.35), PM3 (1.75), PM4 (2.25), PM5 (2.35), PM9 (2.1), PM17 (2.0), PM11 (2.1), and PM13 (2.2) are lower than P2 (2.75), P3 (2.7), P4 (2.35), P5 (2.35), P9 (2.25), P10 (2.25), P11 (2.25), and P13 (2.7), respectively. These results indicated that a good number of the immobilized yeasts were more effective in giving the dough a good texture, than their purified free yeast counterparts.

Taste

The responses of panelists on taste of dough fermented with different yeast isolates, and their controls, are presented in Fig. 8. From the results of statistical analysis ($\alpha = 0.1$) of responses, it was observed that there was no significant difference in the effectiveness of the rising agents on the taste of resulting dough. However, the best taste was recorded in dough fermented with P2 (*S. uvarum* from *E. guineensis* from Enugu State) and PM3 (immobilized *S. carlbengesis* from *E. guineensis* from Enugu State). They were closely related to the taste recorded in BP. The dough samples fermented with BY and PM5, P6, P7, PM12, and PM13 had relatively similar taste.

Hardness

In Fig. 9, the responses of the panelists on hardness of fermented dough were presented. This indicated that the best hardness was obtained from fermentation with BY, while the least was observed in P2 (free *S. uvarum* isolated from *E. guineensis* from Enugu State) fermented sample. Statistical analysis of results ($\alpha = 0.1$) revealed that there was significant difference in the effectiveness of the different yeasts isolates on hardness of resulting dough. The hardness of dough fermented by BY was closely only related to those fermented by PM11, P5, P3, P12,

and PM9. Unlike in many previous organoleptic properties studied, these results imply that more free yeast samples were able to produce dough with better hardness, than immobilized yeast samples.

Puffiness

Figure 10 indicated the response of panelists on puffiness of dough fermented with different yeast isolates, and their controls. These showed that dough resulting from BY had the best mean puffiness. However, the puffiness of its dough was not significantly different from those of samples fermented by P3, P10, P11, P12, PM3, PM8, P9, P5, PM6, PM9, PM10, PM11, and PM12. This revealed that puffiness of dough fermented by six free yeast isolates and seven immobilized yeast isolates were related, and not significantly different from those of bakers' yeast, BY.

Earlier, Balarabe *et al.* [23] did not find any significant difference ($P > 0.05$) between different isolates applied in bread-making, in terms of perception of aroma, leavening, taste, texture, appearance, and acceptability. This partly differs from the findings of the present study, which recorded significant differences in some organoleptic properties of dough fermented, including texture, puffiness, hardness, and appearance, by some isolates.

However, the commercially available bakers' yeast produced the best organoleptic properties in almost all the parameters studied, the similarity observed between its effects and those of some of the isolate's points to their possible industrial usefulness in bakery.

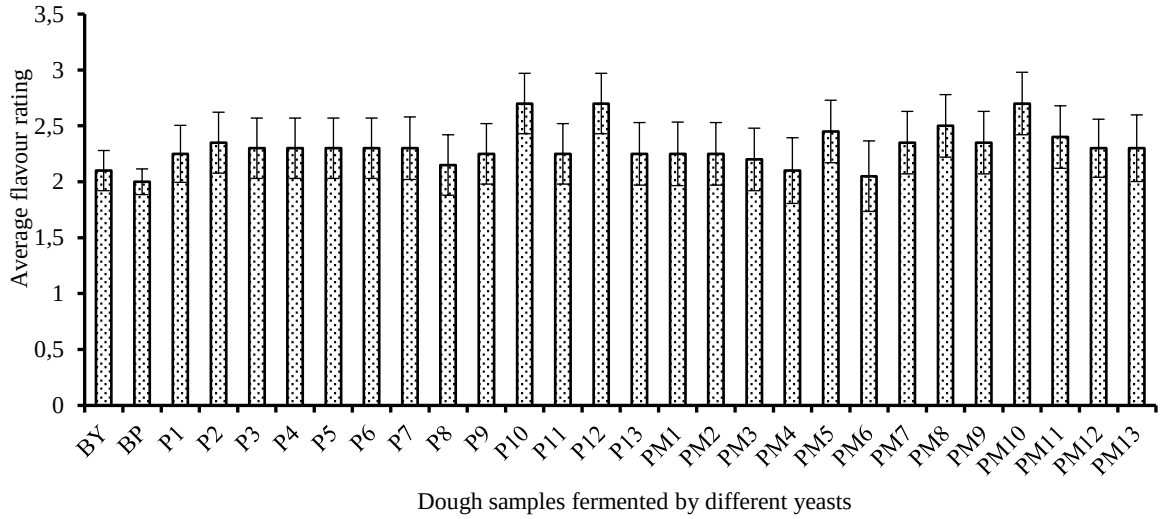

Fig. 5. Flavour dough fermented with different rising agents

It was observed that immobilization of yeasts enhanced their ability to produce dough with better organoleptic properties. The reasons responsible for this would require further studies for better understanding.

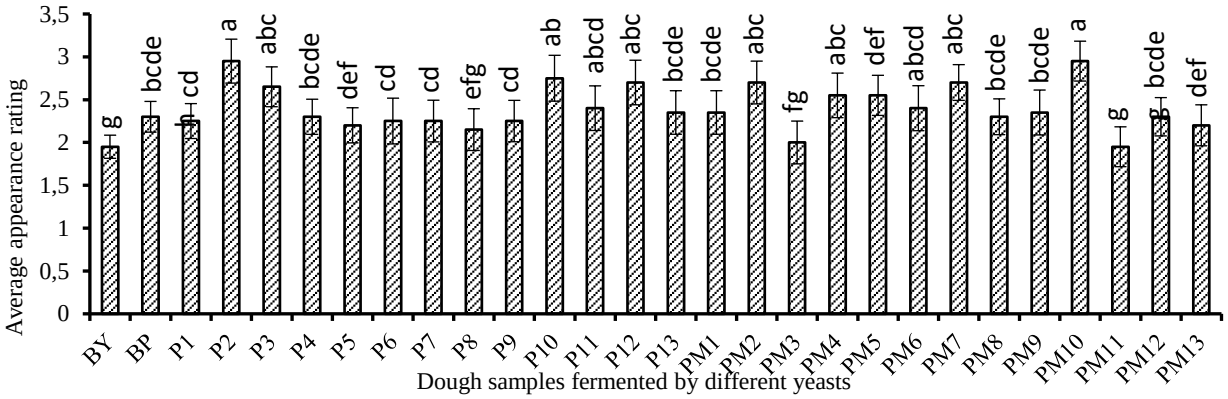

Fig. 6. Appearance of dough fermented with different yeast isolates.

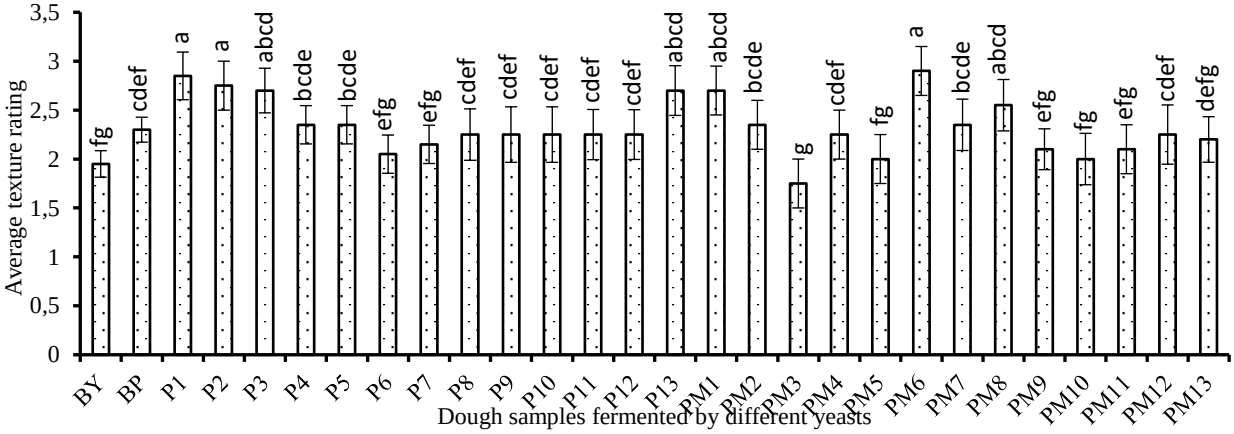

Fig. 7. Texture of dough fermented with different isolates

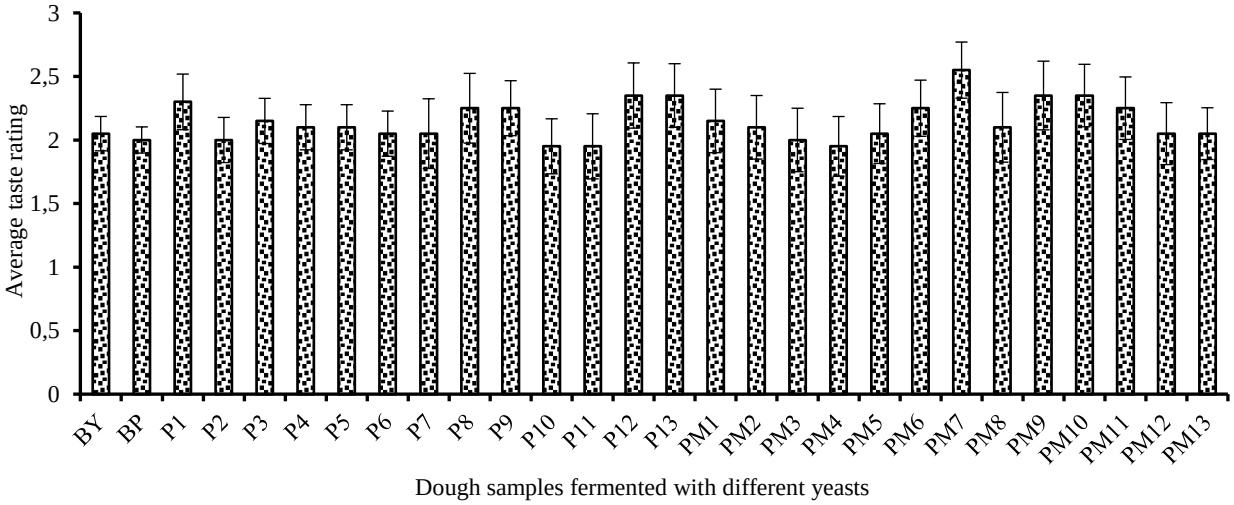

Fig. 8. **Taste of dough fermented with different isolates of yeasts.**

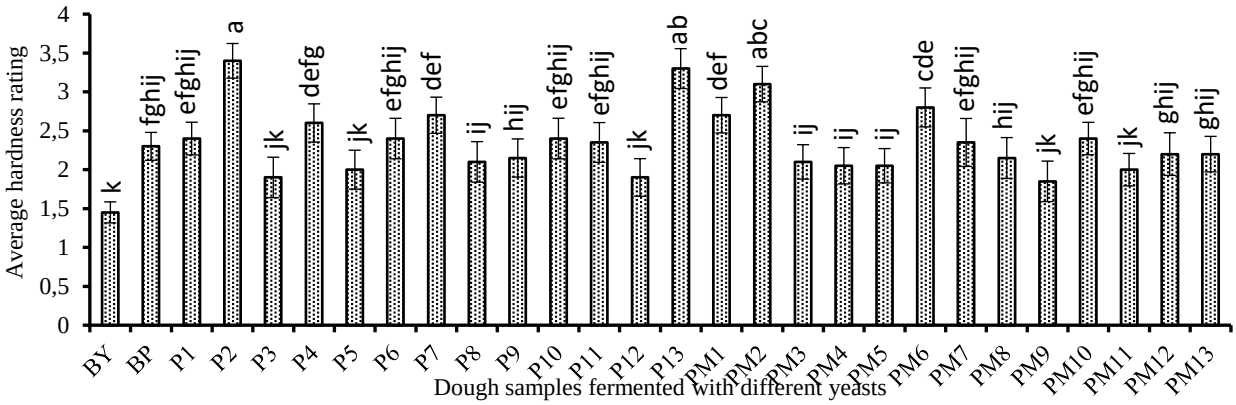

Fig. 9. The interval plot of hardness for the processed dough

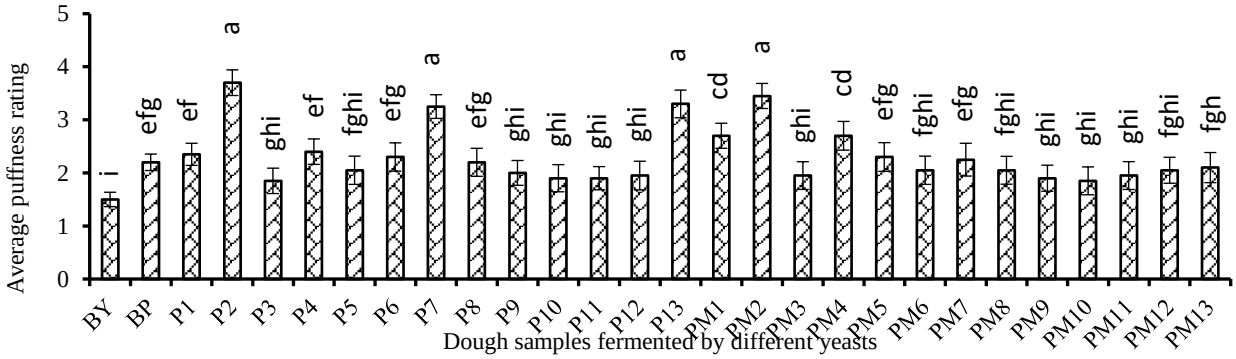

Fig. 10. Puffiness of dough fermented with different rising agents

4. Conclusion

In conclusion, the results of this study revealed the presence of diverse yeast strains, including *S. cerevisiae, S. uvarum, S. carlbengesis, and Schizosaccharomyces pombe,* in the palm wine samples. However, *S. cerevisiae* was the most predominant. Apart from flavour and taste, there were significant differences in the effect of the 13 yeast isolates and their controls, on other organoleptic properties of resulting dough, such as hardness, puffiness, and appearance. Immobilization of yeasts considerably enhanced effects of yeast on organoleptic properties of dough. All the immobilized pure yeast cell used in dough preparation were recovered and the cells could be recycled and reused in further processing of food. Comparatively, the sugar content was highest in palm wine from *E. guineensis* than *R. hookeri* which depicts the high alcohol content of the beverage due to sugar fermentation. Indeed, the potential of yeasts to rapidly ferment maltose is a necessary industrial factor. Hence, these results showed that yeast from palm wine can be used to prepare an acceptable dough and the indigenous yeasts is relevant in food industry.

5. References

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