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Some Properties of Two Forms of Yeast Isolated from Naturally Fermented Pawpaw (*Carica papaya*)

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Carica papaya **(pawpaw) was naturally fermented for 14 days at the room temperature range of 27°C to 31°C. The pH of the pawpaw changed from 3.73 to 3.01. During the fermentation, 'Bol 1' (Oval) and 'Bol 2' (elongated) forms of yeast were isolated and their properties namely: fermentation of carbohydrates, formation of pseudomycelium and spore, reduction of nitrate, optimum growth temperature and qualities of bread loaves made with the isolates were determined. The 'Bol 1' yeast fermented more carbohydrates faster than the elongated one. It fermented glucose, fructose, sucrose, mannose, trehalose, galactose, maltose and cellobiose but assimilated arabinose and lactose. The 'Bol 2' isolate could breakdown the carbohydrates with the exception of galactose, arabinose, lactose, trehalose and cellobiose. The two yeasts did not form spore nor pseudomycelium neither reduce nitrate. They grew optimally at 37°C. Relative best quality bread loaves were obtained with mixture of the two yeasts followed by the 'Bol 1' isolate, then commercial yeast (Fermipan or Saf-instant) and 'Bol 2' type with total scores of 82%, 80%, 72% and 51% respectively. Also, the mix isolates produced higher amount of alcohol from pawpaw than the 'Bol 1' while the 'Bol 2' yeast synthesized the least quantity.**

Keywords: *Carica papaya* (Pawpaw), yeasts, fermentation.

Carica papaya (pawpaw or papaya or tree melon), a common tropical berry fruit belongs to the family Caricaceae (Dutta,1989). Many varieties are known. The pear shaped type is common in Nigeria. The fruit is composed mainly of sugars such as sucrose (48.3%), glucose (29%) and fructose (21%) (Chan and Kwok, 1976; Rice *et al*., 1978). Rice *et al.* (1978) reported the presence of small quantity of sedoheptulose in pawpaw. It also contains some minerals and vitamins A, B and C (Nagy and Shaw 1980; Mayhew and Penny, 1988; Dutta, 1989).

Yeasts are mostly unicellular fungi lacking locomotion organ with cells measuring 4 to 5mm in diameter. Yeasts occur in substances rich in sugar such as pineapple, pawpaw, sugar cane, flower parts and others. Yeasts are capable of carrying out fermentation which is important in industrial biochemical processes including brewing, production of alcohol, bread, wine and single cell protein. Palm wine contains *Saccharomyces* and *Schizosaccharomyces* species (Obisanya *et al*., 1987). The sap of oil plam (*Elaeis guineensis*) is known to contain *S. cerevisiae, S. chevalieri and Candida* species (Semiari and Udoh, 1995). Okafor in 1987 reported that *Klockera apiculata, Candida* species*, Saccharomyces cerevisiae, Zygosaccharomyces mrakii, S. chevalieri* var. chevalieri. *S. ellipsoideus* var. ellipsoideus*, S. pastorianus, Schizosaccharomyces pombe, S. vafer, Saccharomycodes vini, Saccharomyces ludwigii*

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and *Pichia* species were isolated during the fermentation of palm wine. Cane juice is rich in *Saccharomyces cerevisiae, S. carlsbergensis, Candida krusei, C. guillermondi, Torulaspora, Pichia membranaefaciens* and *P. fermentans* (Ayes and Sandine, 1987). Also, *P. membranaefaciens, S. bayanus* and *Torulaspora delbrueckii* were identified in tunas fermented to produce Colonche beverage. *Saccharomyces cerevisiae* and *Klockera apiculata* were obtained from sap of the stem and inflorescence of coconut palm (*Cocos nucifera* L.) used to produce Tuba beverage. Natural yeast flora of *Candida incospicua*, *C. quertiana* and *S. cerevisiae* were associated with Tepache wine made with juices of fruits including pineapple, orange and apple (Uloa and Herrera, 1979).

In order to study the yeasts which exist naturally in Nigerian pawpaw, this work was designed to isolate yeasts associated with natural fermentation of pawpaw and preliminarily investigate some properties of the isolates.

MATERIALS AND METHODS

Materials

Pawpaw was obtained from "Oba" market, Akure, Ondo State, Nigeria. All chemicals used were of analytical grades except otherwise stated. The pawpaw was peeled, the inner pulp (330.20 g) was manually diced and mashed using a blender which had been previously surface sterilized with alcohol.

Methods

Natural fermentation of pawpaw

The mashed pawpaw was transferred into a sterile vat and fermentation was allowed to set in. Temperature and pH were measured and, samples required for yeast isolation were collected at the beginning and every 24 h of the experiment until 15th day. The day the experiment was set up was regarded as 1st day of fermentation.

Isolation of yeast

Half of a millilitre of the fermenting pawpaw was added to 4.5 ml of sterile distilled water and 0.2 ml of it was spread on peptone water agar (PWGA) made up of peptone water (1.5% w/v), glucose (2% w/v) and agar (2% w/v) acidified with lactic acid to 0.02% (v/v). Incubation was carried out at 28°C for 24 h after which the grown culture was streaked out on fresh plates containing the same medium. Single colonies were restreaked, stained with lactophenol-in-cotton blue and examined under a light microscope with oil immersion lens (×100). This step was repeated for four times to purify the culture. Pure isolates were kept on PWGA slants for further studies.

Determination of optimum growth temperature

Five millilitres of PWG (PWGA without agar) was inoculated with 1/10th loopful of the yeast and incubated at 28°C for 24 h. Optical densities of the cultures were read at 600 nanometers. Number of cells was determined by the method of Cruickshank *et al*. (1972). An aliquot containing 96×10^6 cells of each yeast was added to separate 5 ml of fresh PWG broth. Uninoculated medium served as control. Test cultures and control medium were incubated at 4°C, 15°C, 28°C, 37°C, 50°C and 60°C for 24 h. Grown cultures were diluted and control medium were spread on plates of PWGA. They were incubated at 28°C for 24 h and colonies were counted.

Test for formation of pseudomycelium

The method of Onions *et al*. (1981) was used with little modifications. Sterile PWGA was poured into petri dish and allowed to solidify. A sterile glass slide was placed on the agar, layered with the same medium, allowed to solidify and streaked lengthwise with a loopful of 24 h old culture of each yeast. Sterile cover slip was placed at the middle of the streak, incubated at 28°C for 5 days and examined under a light microscope (×40).

Test for formation of spore

Smear of the yeasts (previously grown in minmal medium containing $CaCl_2$ (1000 mM), KH_2PO_4 (500 mM), K_2HPO_4 (500 mM), Ferric citrate (10 mM), $MgSO_4$ (250 mM), K_2SO_4 (1500 mM), $MnSO_4$ (1 mM), H_3BO_3 (2 mM), $ZnSO_4$ (0.5 mM), $CuSO_4$ (0.2 mM), $CoSO_4$ (0.1 mM) and Na_2MoO_4 (0.1 mM)) were prepared on glass slides, flooded with malachite green while it was steamed for 10 min. They were rinsed in water and counterstained with safranin, rinsed in water, dried and observed under the light microscope.

Test for reduction of nitrate

A 24 h old culture was used to inoculate a minimal medium containing 0.2% (w/v) of potassium nitrate and glucose at 1% (w/v). It was

incubated at 28°C for 72 h, nitrate reduction reagent (containing sulphalinic acid in acetic acid) was added, left and observed up till about 3 h.

Test for fermentation of carbohydrates

Starter cultures were prepared with 5 ml of acidified PWG containing glucose. It was inoculated with 1/10th loopful of each of the yeasts in separate tubes and grown at 28°C for 24 h. Fermentation of the carbohydrates was carried out by inoculating 39 x 10^3 cells of the yeast into sterile 5 ml of PW containing phenol red, durham tube and 1% (w/v) of each of arabinose, lactose, glucose, fructose, sucrose, mannose, trehalose, galactose, maltose and cellobiose substituted for glucose. They were incubated at 28°C for 1 to 7 days.

Preparation of bread

Hundred millilitres of acidified PWG was inoculated with 24 h starter culture and incubated at 28°C for 24 h. Cells were centrifuged at maximum centrifugal force of 12,168 × 10^3 g for 20 minutes (MSE Minor 35 Centrifuge), washed and resuspended in sterile distilled water. Commercial baker's yeast (Fermipan or Saf-instant Ltd.) was suspended in separate sterile distilled water. Bread dough was made according to the method of Pyler (1988) with slight modification. The wheat flour (25 g), sodium chloride (0.25 g), sterile distilled water (16.25 ml), sugar (2.25 g), fat (10 g) were mixed with 8 x 10^9 cells (OD_{470} = 1.90) of each yeast, mixture of the oval and elongated isolates, and the commercial yeast. The doughs were leavened at 37°C for 1 h and 50°C for 3 h. Baking was carried out at about 200°C for 10 min.

Production of alcohol

Pawpaw was peeled, the inner pulp was mashed and pasteurized at 87°C for 45 min to kill the indigenous yeasts. It was allowed to cool and inoculated with 100 ml of 24 h old culture of the oval, elongated, mixture of the isolates separately. Unpasteurized, uninoculated pawpaw served as the control. Fermentation was carried out at room temperature of 28 to 31°C for 14 days. The samples were sieved with sieve number 60 (Gallemkamp Griffin SIH=310-V) and the extracted liquids were distilled.

Comparative analysis

Bread

Bread loaves were compared as follows. A six-man panel was set up to score the loaves of bread made based on the physical properties: texture, appearance, colour, taste, leavening (raising) and aroma. The score grades used were bad=0, fair=1, satisfactory=2 good=3.

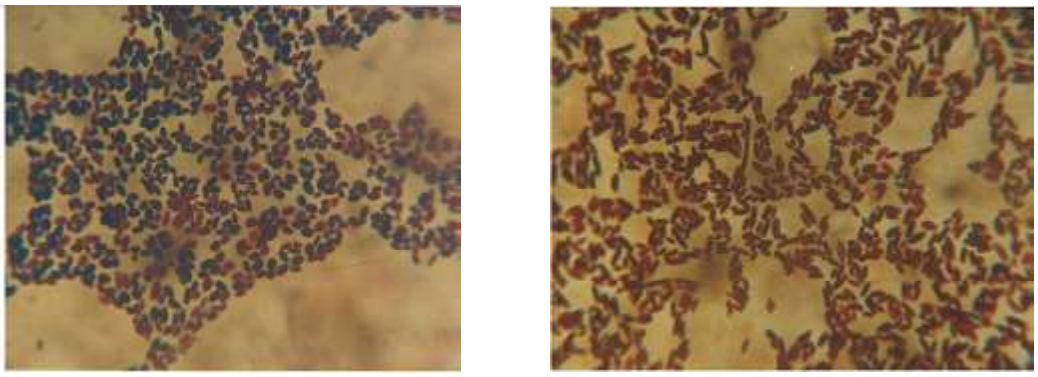
Distillates

Amount of alcohol obtained from the fermented pawpaw was expressed in volume per gram of the pawpaw. Presence of alcohol in the distillates was determined by flame test. Flame was applied to each sample and the ignition ability was noted in minute.

RESULTS AND DISCUSSION

Isolates

Two yeasts isolated from the naturally fermented pawpaw were oval and elongated forms (Fig. 1). They were coded as 'Bol 1' (Fig. 1a) and



(a) 'Bol 1' (Oval form) (b) 'Bol 2' (Elongated form)

Fig. 1. Microscopic morphology of two forms of yeast isolated from naturally fermented pawpaw. (a) 'Bol 1' (Oval form) (b) 'Bol 2' (Elongated form)

'Bol 2' (Fig. 1b) respectively. On agar, the 'Bol 1' yeast appeared cream-coloured and larger than the latter which was white and tiny. Both isolates were elevated with smooth surfaces and edges on agar.

Temperature and pH

Temperature of the naturally fermented pawpaw ranged between 27°C and 31°C (Fig. 2). This increase could be associated with the fact that the fermentation was carried out at room temperature which could be influenced by the ambient temperature (data not shown) which was not constant during the natural fermentation. The pawpaw medium was acidic with initial pH of 3.73 which changed to 3.01 during the experiment (Fig. 2). This indicates that the fermented products were acidic. The isolates appeared to produce acidic molecules during the fermentation. The two yeasts showed similar growth pattern with increase in number of colonies from 4°C to 37°C after which there was a decrease (Fig. 3). The optimum growth temperature was 37°C. The isolates appeared to be mesophiles. Temperature and hydrogen (H^+) ion concentration of a medium in which an organism flourishes greatly influence its growth rate. The isolates appeared to be mesophiles. The pH of the pawpaw was favourable to the isolates since yeasts generally show good growth in low pH than neutral or alkaline media.

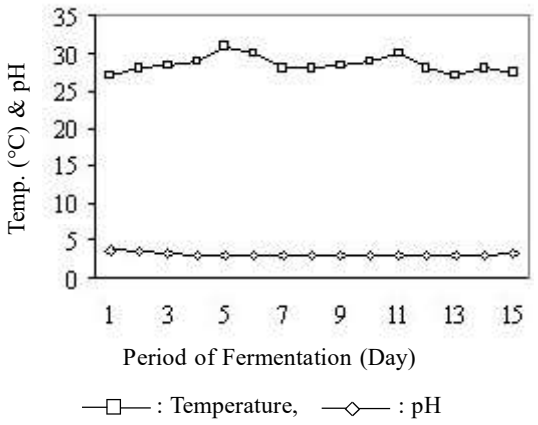


Fig. 2. Temperature and pH of pawpaw during natural fermentation

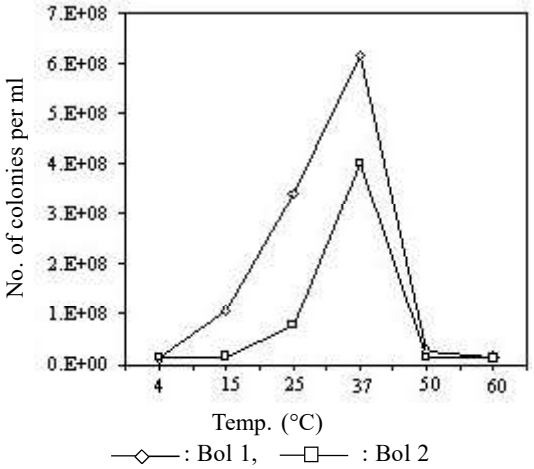


Fig. 3. Effect of temperature on the growth of isolated yeast

Table 1. Fermentation of Carbohydrates

Carbohydrates	Yeasts	
	Oval	Elongated
Galactose	F	-
Glucose	F	F
Fructose	F	F
Mannose	F	F
Sucrose	F	F
Trehalose	F	-
Cellobiose	F	-
Maltose	F	F
Arabinose	AS	-
Lactose	AS	-

F: fermentation, - : no fermentation AS: assimilation

Formation of pseudomycelium and spore, reduction of nitrate and fermentation of carbohydrates

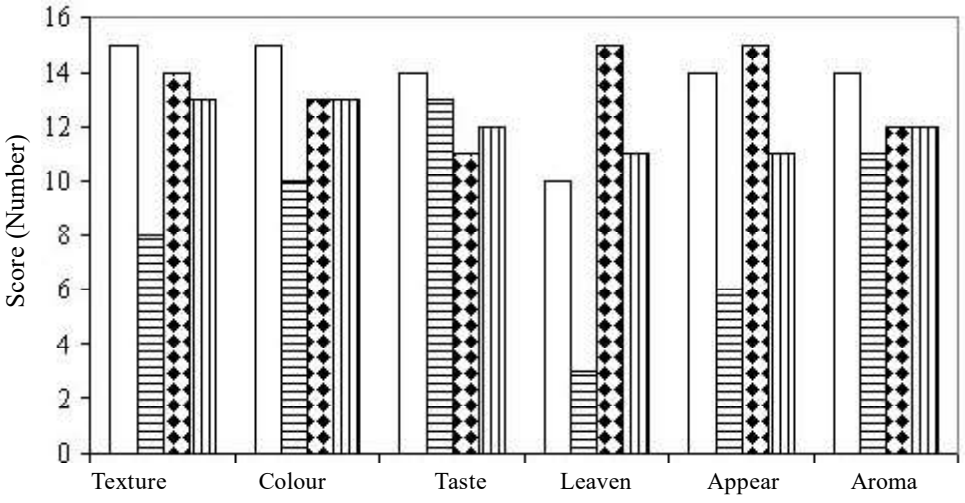
Both yeasts did not produce pseudomycelium neither reduce nitrate nor form spore. Thus, they are not filamentous and non-spore formers. The 'Bol 1' yeast was able to use all the carbohydrates tested within 48 h of incubation (Table 1). It digested galactose, glucose, fructose, mannose, sucrose and trehalose with the evolution of gas but no gas was released during the fermentation of maltose. Cellobiose was weakly broken down without gas release.

Lactose and arabinose were not fermented but assimilated. In contrast, the 'Bol 2' isolate catabolized only glucose, fructose, mannose, sucrose and maltose but there was

no gas released from the last two sugars after 7 days. It did not ferment nor grew in cellobiose, galactose, trehalose, lactose and arabinose.

Physical characteristics of bread loaves made with the yeasts

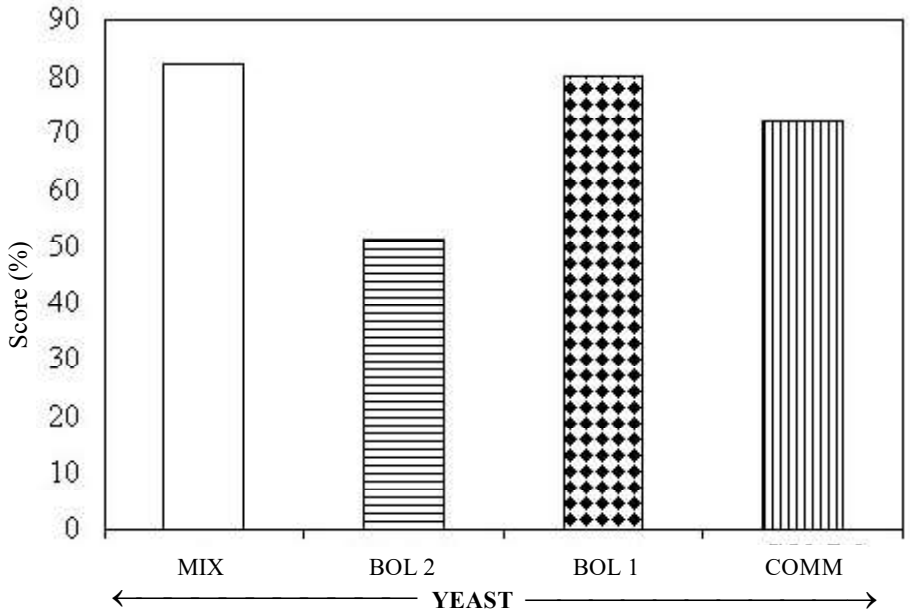
Individual characteristics of the bread loaves produced with the various yeasts are shown in Fig. 4a. Bread made with the 'Bol 1' isolate showed the best texture, colour and taste followed by the mix, commercial and lastly the 'Bol 2' yeast which was the sweetest out of all. Loaves produced with mix isolates scored highest in leavening and appearance followed by the 'Bol 1', commercial and 'Bol 2' yeasts. Overall, the bread made with mix isolate rated best (82%) followed by the 'Bol 1' form (80%), then commercial yeast (Fermipan



APPEAR: APPEARANCE

: 'Bol 1' yeast -: 'Bol 2' yeast ♦ : Mix yeasts |: Commercial yeast

(a) Various properties of the bread loaves



COMM: COMMERCIAL YEAST

(b) Overall grades of qualities of the bread loaves

Fig. 4. Physical properties of bread loaves made with different yeasts.

or Saf-instant, 72%) and the 'Bol 2' type (51%) (Figs. 4b). The high leavening activity of the 'Bol 1' isolate is related to its good fermentative ability. The 'Bol 2' form could not ferment sucrose which was digested by the 'Bol 1' yeast with gas release hence the poor raising ability associated with former and very sweet taste that was observed in its bread. A mixture of the two isolated yeasts will be suitable in bread making because of the very good leavening property when compared with the individual yeasts.

Alcohol

In general, alcohol obtained with the use of all the isolates was low in volume (5 to 10 ml) with the highest quantity obtained from the mix isolates (3.12%) followed by the 'Bol 1' (2.19%) and the 'Bol 2' (1.56%). This could be because acidic molecules were synthesized more than the alcohol or that the alcohol produced during early days of the fermentation was converted to acid due to long period of the process. However, alcohol made with the use of the mix isolates ignited best followed by that from oval type and lastly the elongated yeast.

CONCLUSION

The two yeast isolates differ in morphology, cultural and fermentation properties. Relatively the isolate 'Bol 1' has a very good fermentative quality which can be harnessed in relevant processes. The properties of 'Bol 2' yeast can be improved on for fermentation purposes. The features of the two yeasts are similar to those of *Candida versatalis* (formerly called *Torulopsis versatalis*) having spherical to oval shape, able to ferment glucose, galactose, sucrose, maltose and produce ethanol. Thus, they are called as *Candida versatalis* variety 'Bol 1' and *Candida versatalis* variety 'Bol 2'. Some members of the genus *Candida* such as *C. apicola* and few others are different in certain respects.

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