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SUGAR ALCOHOL- XYLITOL PRODUCTION USING *SACCHAROMYCES* SPECIES ISOLATED FROM PALM WINE FOR SUSTAINABLE DEVELOPMENT IN FOOD INDUSTRY

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Abstract. Xylitol, a naturally occurring sugar alcohol, is produced chemically on a large scale. This study was carried out to locally produce xylitol using yeast isolates, which were isolated from palm wine. The yeast *Saccharomyces* species were identified using morphological characteristics and biochemical tests. Xylitol production was carried out at 30 °C using glucose and D-xylulose, which were added to the mineral media. From the results we obtained, the *Saccharomyces* species isolated can be used for biological production of xylitol.

Keywords: Saccharomyces, xylitol, glucose, D-xylulose, Palm- wine

1. INTRODUCTION

Over the past years, there have been increases in the consumption of sugars in foods. This has negatively impacted the health of consumers leading to a dramatic upsurge in health-related diseases such as obesity and diabetes. Hence there is a severe need to search for new categories of nutraceuticals and functional foods. Polyols or sugar alcohols are the best alternatives because when they are used as bulk sweeteners, they offer benefits such low-calorie in-take, prebiotic effects, and promotion of dental health that might be helpful in the control of diabetes, and weight control. Nutrients in foods and food additives reveal the health quality of foods. Food additives are regarded as substances added to foodstuff to perform certain technological functions such as give color, sweeten, or help in food preservation [1]. Food depends mainly on additives in foods that are allowed or tolerated only when they are harmless to one's health. Thus, advances in biological studies and re-orientation of people's lifestyles have resulted in sweeteners being found in almost all food products.

Sugar alcohols are low carbohydrates that are digestible and obtained by substituting an aldehyde group with hydroxyl groups. Examples of sugar alcohols are sorbitol, mannitol from the hydrogenated monosaccharide, isomalt, maltitol, lactitol from the hydrogenated disaccharide, and mixtures of hydrogenated mono, di, and oligosaccharides (starch hydrolysates). Polyols could occur naturally in small quantities in some fruits and vegetables. Xylitol, a form of a polyol with the chemical formula (C₅H₁₂O₅) erythro-pentitol, can be used as an emulsifier, humectants, stabilizer sweetener, and thickener. The Food and Drug Administration (FDA) has been approved xylitol as a dietary food additive since 1963. It has applications in foods, pharmaceuticals, and oral health products in more than 35 countries. Finland, as a nation, have national programs promoting the use of xylitol in chewing gum to reduce dental carries among children [2, 3]. In most fruits, vegetables, berries, oats, and mushrooms, xylitol occur at varying concentrations [3, 5, 6, 7, 8]. Xylitol is among the seven food additives identified by E number under European Union legislation xylitol (E967). Xylitol has been noted as the sweetest amongst all sugar alcohols. Evredilek, in 2012 stated that xylitol has the same sweetness and bulk as sucrose with no aftertaste [9]. Also, it dissolves with a cooling sensation in the mouth, and insulin is not required for its metabolism [10, 6, 7, 8]. Xylitol, because of its benefits, has diverse biomedical applications. These areas are in dental health: reduction in tooth decay, plaque formation and inhibition of *Streptococcus mutans* and *Streptococcus sobrinus* that are responsible for caries and plaque formation [5, 3, 11, 12, 13, 14]. Xylitol increases the pH values in the oral cavity that contributes to the complexing of Ca²⁺ and remineralizing of the tooth enamel [15, 16, 17]. It increases saliva production, which helps in treating xerostomia. It protects salivary proteins with a protein-stabilizing effect and improves breath odor. It reduces infections in nasopharynx by



exhibiting anti-bacterial activities on pneumococcal nasal colonization [5, 18, 19, 20]. Xylitol has a low calorie and low glycemic index [1]. Its antiketogenic effects decrease serum-free fatty acid levels and improve peripheral glucose utilization. Xylitol could serve as an energy source in infusion therapy [21], sanitizer [22], as a preventive factor of adrenocortical suppression during steroid therapy [21], and phenylenediamine-induced hepatotoxicity [23]. It favors the absorption of calcium and B-vitamins. Xylitol inhibits yeast growth including *Candida albicans*, decreases glycation of proteins and helps to maintain healthy gut [24, 10, 4, 25, 26]. Xylitol are produced from xylan, usually obtained from birch trees and hardwood. Its production is via metal-catalyzed hydrogenation reaction of a corresponding sugar such as D-xylose. This process is initiated when xylan is isolated from wood and hydrolyzed to xylose. The xylose solution is purified chromatographically, and subjected to hydrogenation in the presence of nickel catalyst at high temperature and pressure conditions.

As a result of burgeoning costs associated with the traditional industrial production, biotechnological production systems from corn cobs, waste of sugar cane, and other fibres were developed but not on a commercial scale [11]. The bioconversion of D-xylose from lignocellulosic residues into xylitol has gained increased attention due to its high efficiency and perceived cost-effectiveness [27]. The process is precise, and the microbial production could be improved by metabolic engineering [27]. Ortiz and co-workers in 2013, and Akinterinwa and co-workers 2008, reported that xylitol could be produced efficiently by yeasts such as the genus *Candida* species that naturally obtain xylitol as an intermediate product during D-xylose metabolism [28, 29]. However, there had been a drawback because of the pathogenic nature of the *Candida* species, which cannot be used in the food industry. Also, microalgae and *Candida tropicalis*, a yeast isolated from palm wine has been reported to accumulate xylitol as a result of high xylose assimilating activity [30].

Palm wine is an alcoholic beverage from the sap of various palm trees contains yeasts, which can be beneficial. Palmwine can be sourced by tapping oil palm tree *Elaies guineensis* or from the Raphia tree *Raphia hookeri* and *Raphia vinifera* [31]. Palm wine is consumed for its nutritional benefits such as nicotinic acid, vitamin C, thiamin, and riboflavin and protein [32] and probiotic content [33]. The yeasts occurring in palm wine are *Saccharomyces spp.*, *Candida spp.*, *Saccharomyces chevalieris* and *Kloechera apiculata* [34]. *Saccharomyces cerevisiae* is a species of budding yeast; it is perhaps the most useful organisms in baking and brewing [35]. Bacterial species that occur in palm wine include *Bacillus cereus*, *Enterococcus faecalis*, *Bacillus firmus*, *Lactobacillus*, *Acetobacter*, *Micrococcus*, *Serratia*, *Leuconostoc*, *Sarcina*, *Streptococcus*, *Bacillus*, *Brevibacterium*, *Pediococcus*, and *Klebsiella*. Based on the growing demand for xylitol, and efforts towards the reduction of production costs, authors explored an investigative study on alternative means of production of xylitol for sustainable development in the food industry. We isolated wild yeast (*Saccharomyces cerevisiae*) from palm wine and explored the making of xylitol from D-xylulose and glucose.

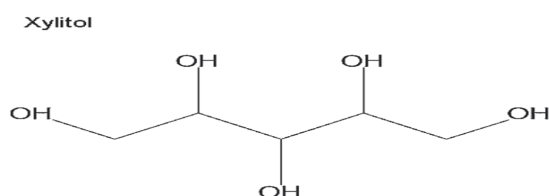


Figure 1.0: Chemical structure of xylitol adapted from [1].

2.0 MATERIALS AND METHODS

Chemicals and Reagents

The salts KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, CaCl_2 , Alkaline copper tartrate are of analytical grades and sourced from Merck Germany. Yeast extract was purchased from Micro Master India. The Microbiology laboratory, Covenant University, supplied glucose, phenol red, and agar-agar.

Media preparation

The enrichment experiments were performed using yeast peptone dextrose extract agar described by [36]. The medium consisted of grams (g) Yeast extract 10; glucose 10; peptone 20, distilled water 1000ml; and 50ug chloramphenicol/ml.

Sample collection

Fresh palm-wine was sourced from Iju, Ota, Ogun state and stored in sterile plastic container at 4 °C in the laboratory until further use.

Characterization of yeast from palm-wine.

The yeast strains were obtained after the fresh palm wine was left for 48 hrs to ferment. About 1 ml of the fermented palm wine was diluted using sterile distilled water to 10⁶ CFU/mL. Aliquots of the sample were inoculated on yeast peptone dextrose extract agar by the spread plate method [36]. Single colonies of the yeast grew after 48hrs. The discrete colonies were selected and sub-cultured on yeast peptone dextrose agar to obtain pure cultures. The yeasts' were identified using micro-examination, morphological and physiological tests. Morphological examinations were done using lactophenol blue under a bright field microscope. Features examined include colony color, shape, surface appearance, the opacity of colony, and arrangement; others include the presence of pseudohyphae and reproduction mode. The yeast capacity to use various sugars were assayed. The sugars tested are glucose, galactose, sucrose, maltose, lactose, and raffinose tests [37, 38]. Other tests carried out are growth at 30-37°C, 3.0% sodium chloride, 3.0% ethanol, and 50% glucose.

Preparation of Glucose and D- xylulose solutions

The screening for xylitol production by our isolate was performed as described by [39] with slight modification. The standard medium consisted of (w/v) Medium A: 10 g glucose, 0.1 KH₂PO₄, 0.05 MgSO₄·7H₂O, 0.01 CaCl₂·2H₂O, 0.01 NaCl, 0.1 yeast extract (Difco) and pH 5.0. The medium B had similar salts as medium A except for glucose that was substituted with (5 w/v) D-xylulose. Both media were autoclaved at 110 °C for 5 min.

Preparation of Nelson-Somogyi reagent

The xylitol was determined using the Somogyi-Nelson Assay'. The determination of reducing sugar was based on the absorbance at 540 nm of a colored complex between a copper oxidized sugar and arsenomolybdate. Carbohydrate presence was determined by comparison with a calibration curve generated during the standardization of the colorimeter. In this, Nelson's reagents (A, B, and C) were prepared differently. For reagent, A 2.5 g of Na₂CO₃ (anhydrous), 2.5 g potassium sodium tartrate, 2 g sodium bicarbonate, and 2 g sodium sulphate (anhydrous) were dissolved in 70ml water and diluted to 100 ml. The sulphate was added in small amounts. For the reagent B, 7.5 g of CuSO₄·5H₂O were added to 50 ml of water, and one drop of conc.H₂SO₄ added. For the reagent C, 25ml of Nelson's A and 1.0 ml of Nelson's B mixed with Nelson's color reagent :(Arsenomolybdate reagent) five (5g) ammonium molybdate was dissolved in 80 ml water, and 4.2 ml of Conc.H₂SO₄ added. 0.6g sodium arsenate was dissolved in 5ml water and added to acid molybdate. This mixture was then made up to 100 ml and stored.

Screening for xylitol production using the glucose and D-xylulose medium

A loopful of the two characterized yeast *Saccharomyces* species (A, B) were inoculated in each set-up of the 8 ml of sterilized medium. The tubes containing the cells were incubated on an incubator shaker (Model H2Q-X 300) at 300 revs/min at 30 °C for five days. The same set-up procedure was used for medium B except that 10ml of medium B was placed in a brown bottle for 24hrs at 37°C. The reduction of D-xylulose to xylitol was monitored. The reagent appeared yellow without a green tinge [39].

Determination of the reducing sugar in the glucose and D-xylulose medium using the Nelson-Somogyi method

To the series of tubes, different concentrations (0.2, 0.4, 0.6, 0.8) of glucose were added and made up to 1 ml with water. One (1ml) of Nelson's reagent C (Alkaline copper tartrate) was added to each tube vortexed and boiled for 20mins in a boiling water bath and allowed to cool for 5mins. One (1) ml of Nelson's color reagent

(Arsenomolybdate reagent) was added with a brief vortex and placed the whole test tube at 37°C for 5mins. These were diluted with 5ml distilled water and vortexed. The absorbance was measured at 540nm.

Calculation:

The standard curve of OD at 540nm Vs Glucose concentration ($\mu\text{g}/\mu\text{l}$) of the standards was plotted, and the amount of reduced sugar determined by calculating the glucose concentration of the sample using the equation $Y = ax + b$.

3.0 RESULTS

Six yeast strains were isolated and identified from the fermented palm wine. Different tests, which include morphological and physiological characterization, were used for the identification of the yeasts. These characteristics are represented in Table 1.0a. In all, the probable organisms suspected were *Saccharomyces cerevisiae*, where each showed growth after 48hrs and as well different vegetative increases.

Table 1.0b, shows the behavior of the yeast cells in the different sugar tests conducted. None of the yeasts strain was able to metabolize maltose while all grew in glucose, but all showed a positive reaction to the lactophenol blue test. Strains A1, A4, A5 A6 effectively utilized galactose while A1 and A3 used sucrose. Strain A3 could not metabolize galactose, raffinose, and fructose sugars. Since all the strains obtained fall within the *Saccharomyces cerevisiae* species, we selected two strains for further analysis for the production of xylitol. We named the *Saccharomyces* species as strain A and B.

Table 1.0a: Morphological characteristics of the isolated yeast

Sample	Colony color	Colony shape	Surface appearance	Opacity	Colony arrangement	Growth in 24 hrs	Growth in 48 hrs	Possible organism
A1	White to creamy	Circular	Raised	Opaque	Single budding	-	+	<i>Saccharomyces cerevisiae</i>
A2	Creamy	spherical	Entire	Opaque	Non	-	+	<i>Saccharomyces cerevisiae</i>
A3	Creamy white	spherical	Smooth and flat	Opaque	Unilateral budding	-	+	<i>Saccharomyces cerevisiae</i>
A4	creamy	Round punctiform	Smooth and flat	Opaque	Multilateral budding	-	+	<i>Saccharomyces cerevisiae</i>
A5	Creamy white	circular	Raised and smooth	Opaque		-	+	<i>Saccharomyces cerevisiae</i>
A6	Creamy white	circular	Raised and smooth	Opaque		-	+	<i>Saccharomyces cerevisiae</i>

- No growth occurred, +growth occurred.

Table 1.0b: shows the sugar test carried out for the biochemical identification of the yeast isolated.

Different Isolates	Galactose	Sucrose	Raffinose	Lactose	Glucose	Fructose	Maltose	Lactophenol stain
A1	+	+	+	-	+	+	-	+
A2	-	-	+	-	+	+	-	+
A3	-	+	-	+	+	-	-	+
A4	+	-	+	-	+	-	-	+
A5	+	-	+	-	+	+	-	+
A6	+	-	+	-	+	+	-	+

- + = positive - = negative

- The sugar test includes a glucose test, galactose test, sucrose test, fructose, maltose, lactose test, and lactophenol stain test.

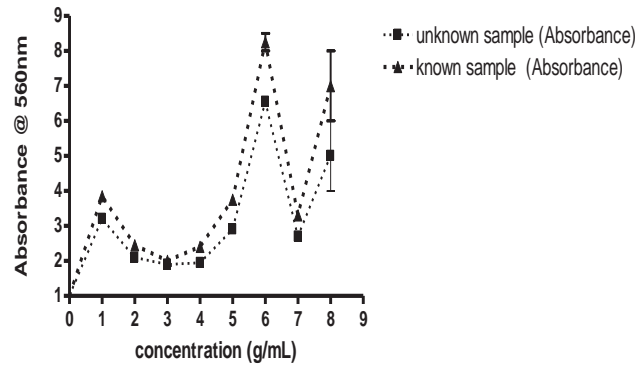


Figure 2.0a: Standardization experiment using D-xylulose

This graph shows the absorbance concentration of the sample after carrying out the reducing sugar test using the Nelson Somogyi method. It shows that the absorbance rate of sugar is higher than that present in the sample.

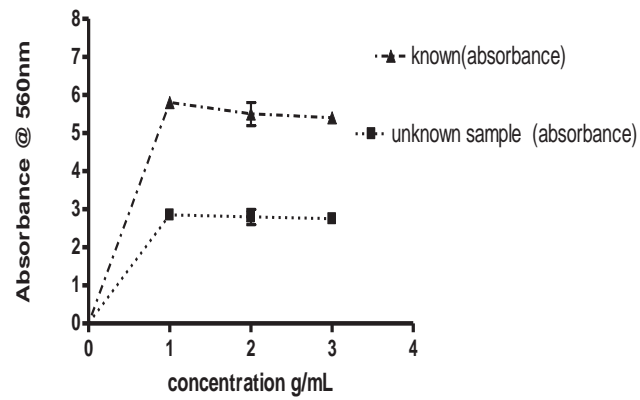


Figure 2.0b: Standard curve generated for glucose during the standardization of the spectrophotometer.

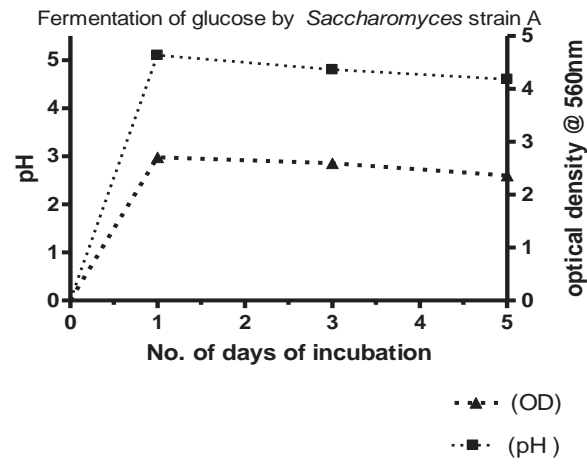


Figure 3.0a: Optical density and pH glucose by *Saccharomyces* species strain A

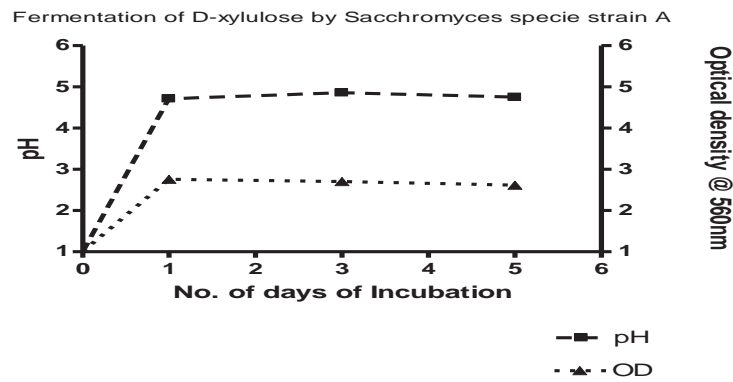


Figure 3.0b: Optical density and pH of the D-xylulose by *Saccharomyces* species strain A

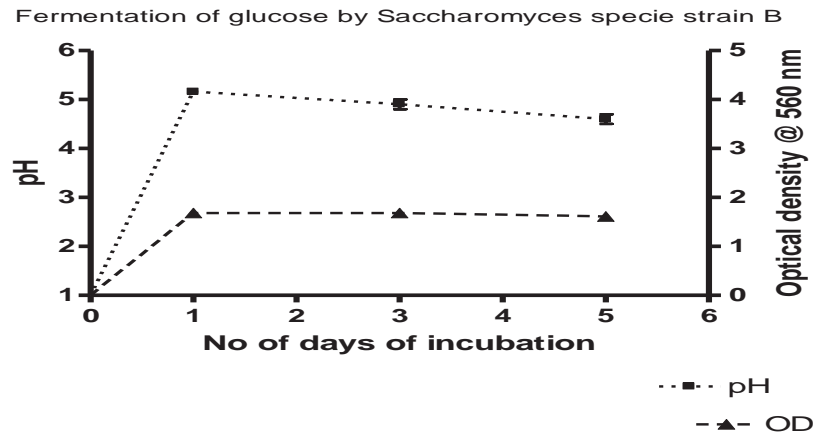


Figure 4.0a: Optical density and pH use of glucose by *Saccharomyces* strain B

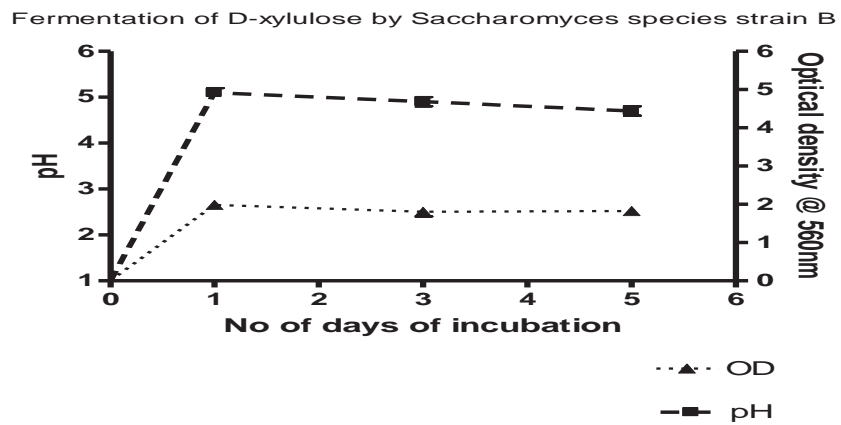


Figure 4.0b: Optical density and pH of the D-xylulose by *Saccharomyces* species strain B

During the glucose assay, there was a color change from light yellow to creamy yellow Plate1.



Plate 1.0: After Fermentation, Xylitol produced with Glucose and D-xylulose

4.0 DISCUSSION

The continuous screening and identification of microorganisms for the production of xylitol is the best approach to reducing the cost of production of xylitol. From previous studies, the use of yeast has been cited as a better alternative towards reducing the burgeoning cost of xylitol production. In this study, we isolated and characterized *Saccharomyces* species strains (Table 1.0 a and b), which can grow well on D-xylulose with consequent production of xylitol (See Figures 3.0a, 3.0b - 4.0a, 4.0b).

The pH and turbidity optical density (OD fluxes) followed similar trend in all the screening experiments (See Figures 3.0a,b - 4.0a,b). This may be due to the different potentials in the utilization of the amended sugars (D-xylulose and glucose). *Saccharomyces cerevisiae* could ferment D-xylulose through the pentose phosphate pathway. Thus the growth fluxes from this study suggest that our organisms may have followed such route. The *Saccharomyces* species strain A maintained similar trend in the pH while *Saccharomyces* strain B had a slightly decreased pH from 5.20 to 4.8 (See Figure 4.0a and 4.0b). The pH, temperature, and genetic nature have been found to influence yeast behavior during xylitol production. *Saccharomyces* species strains A and B grew well at 30°C. According to [28, 29], xylitol could be produced by *Candida* species. The *Saccharomyces* species obtained, when inoculated into different carbon source D-xylulose and the glucose medium, produced varying colors. During the glucose assay, there was a color change from light yellow to creamy yellow (See Plate 1.0). The reducing sugar test conducted according to the Nelson Somogyi absorbance rate test showed similar dynamics as the standards. This investigative data generated shows the practicability of xylitol production from D xylulose. Due to limited access to modern real-time techniques, further analyses of the xylitol to elucidate the chemical structures and properties of the produce sugar alcohol could not be achieved. However, the merit of characterizing and documenting wild strains of *Saccharomyces* species with the capacity of producing xylitol is a good development strategy for sustainable food production. In conclusion, the *Saccharomyces* species strains from palm wine could produce xylitol, especially for the overwhelming health benefits that xylitol provides.

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