Journal of Medicinal Plants Studies

www.PlantsJournal.com

Chidi Edbert Duru
Surface Chemistry and
Environmental Technology
(SCENT) Research Unit,
Department of Chemistry, Imo
State University, Owerri, PMB,
Imo, Nigeria

Ijeoma Akunna Duru
Federal University of
Technology Owerri, Department
of Chemistry, PMB 1526, Imo,
Nigeria

Ali Bilar
Federal University of
Technology Owerri, Department
of Chemistry, PMB 1526, Imo,
Nigeria

Computational investigation of sugar fermentation inhibition by bergenin at the pyruvate decarboxylate isoenzyme 1 target of *Scharomyces cervisiae*

Chidi Edbert Duru, Ijeoma Akunna Duru and Ali Bilar

Abstract
The powdered bark of *Sacoglottis gabonensis* has been used over the years to improve the shelve life of palm wine. The inhibition of sugar fermentation by bergenin a major phytochemical extract from the bark of this plant at the pyruvate decarboxylate isoenzyme 1 of *Saccharomyces cerevisiae* was studied using in silico methods. The binding affinity of glucose, fructose, sucrose, maltose, and the natural product bergenin were –3.5 Kcal/mol, –3.4 Kcal/mol, –4.0 Kcal/mol, –4.6 Kcal/mol, and –4.5 Kcal/mol, respectively. Maltose fermentation cannot occur without its hydrolysis to glucose molecules. Since the binding affinity of bergenin is much higher than those of glucose and fructose, then the saturation of the fermentative active sites in pyruvate decarboxylate isoenzyme 1 by this molecule would prevent the occurrence of this reaction. The findings from this study support the age-long practice of increasing the shelf life of freshly tapped palm wine by adding the pulverized bark of *S. gabonensis* into it.

Keywords: Fermentation, *Sacoglottis gabonensis*, Glucose, Bergenin

Introduction
Palm wine, one of humanity's oldest beverages, is the phloem exudates from the palm tree [1]. It is the most prevalent naturally fermented alcoholic beverage in West Africa, especially Nigeria. It is known under various names in West Africa, such as 'mimbo' in Cameroon, 'nsafufuo' in Ghana, and 'bandji' in Côte d'Ivoire [2]. In Nigeria, it is called emu or oguro in Yoruba, mmanya ngwo in Igbo, and gya in Hausa dialect [3]. The wine is obtained by tapping, which involves removing leaves around an immature male inflorescence and making triangular incisions near its apex until the palm juice begins to flow out. It is a sweet, nearly neutral, whitish effervescent liquid when freshly tapped, containing 10 – 12 % of sugar, mainly sucrose [4]. The major problem facing palm wine distribution after tapping is acquiring a sour taste from the metabolic activities of numerous microorganisms contained in it. This process called fermentation is mediated by *Saccharomyces cerevisiae* and *Saccharomyces carlsbergenesis*, top and bottom-fermenting yeasts, respectively [5]. The use of chemical preservatives and other modern preservation methods to increase the shelf life of palm wine have been explored by palm wine distributors. The earliest attempt made in this regard was to use refrigeration and pasteurization to preserve the wine. Refrigeration only retarded microbial growth while the wine gets sour over time. Besides, these methods are costly, and cannot be afforded by low-income palm wine tappers who often reside in rural African communities without electricity. The unavailability of modern preservation methods, coupled with palm wine is a traditional beverage mostly tapped in rural areas, the traditional method of preserving the wine has been sought after and applied. The preservative effect of *Sacoglottis gabonensis* stem bark in palm wine has been widely reported [6, 7]. The tree bark is known as 'Nche' in Igbo, and 'Edat' or 'Mkpaeto' in Akwa Ibom [7]. It is pulverized and placed inside the gourd before being hung for collection of the palm wine, or the dust can be added to the freshly tapped wine. This imparts an amber colour and slightly bitter taste to the wine. A biologically active compound bergenin has been reported to be a significant constituent of this plant's bark [8]. In this study, the inhibitory efficiency of bergenin against sugar fermentation by yeast was determined using in silico methods. Computational simulations were used to obtain the binding affinity of bergenin, and the constituent sugars in palm wine on the active site of pyruvate decarboxylate isoenzyme 1 of *S. cerevisiae*.

Corresponding Author:
Chidi Edbert Duru
Surface Chemistry and
Environmental Technology
(SCENT) Research Unit,
Department of Chemistry, Imo
State University, Owerri, PMB,
Imo, Nigeria

Computational Methods
Identification and preparation of molecular target
Pyruvate decarboxylase isoenzyme 1 (PDI-1) (ID: 1PYD) from *Saccharomyces cerevisiae* with resolution 2.40 Å was identified from literature and used as a target in this study. The protein was retrieved from the Protein Data Bank (PDB) database. It consisted of two chains, A and B. Chain A of the protein was used for the docking studies in other to improve the accuracy of the ligand binding [9]. The interfering crystallographic water molecules and minimization of the protein were done using UCSF Chimera 1.14 [10].
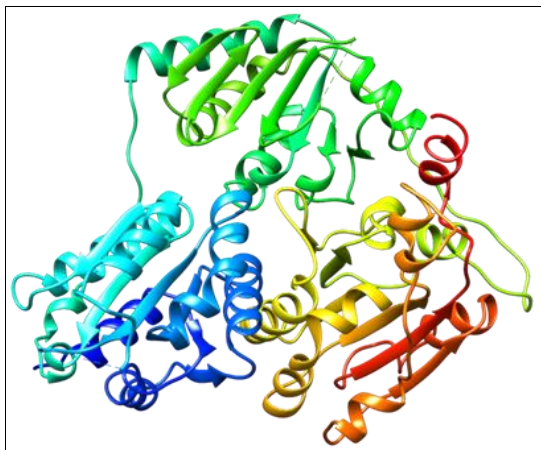

Fig 1: Minimized protein of pyruvate decarboxylase isoenzyme 1

Determination of active site on protein
The largest active site of the protein was viewed, and the amino acid residues at this site were selected using the Computed Atlas for Surface Topography of Proteins (CASTp) [11].
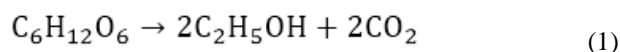
Docking studies
The binding affinity studies of bergenin and the sugar molecules were performed by site-directed docking on a specified PDI-1 binding pocket. The multiple docking of the ligands and protein was done with Autodock Vina in PyRx software version 0.8 [12]. The center grid box sizes were x center: -17.09, y center: -7.27, and z center: 19.79. The results in terms of binding energy for each compound were obtained.

Analysis of protein-ligand interactions
Post docking interactions of the molecules at the PDI-1 active site were visualized, and the post docking analysis was performed using Biovia Discovery studio 4.5 [13].

Results and discussion
Fermentation is a natural chemical process by which living cells convert starch or sugar into ethanol and organic acids anaerobically [14, 15].

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \qquad (1)$$

During the process, naturally available yeast *S. cerevisiae* converts pyruvate generated from glucose metabolism into acetaldehyde, which then produces ethanol and carbon dioxide [16] (Figure 2).
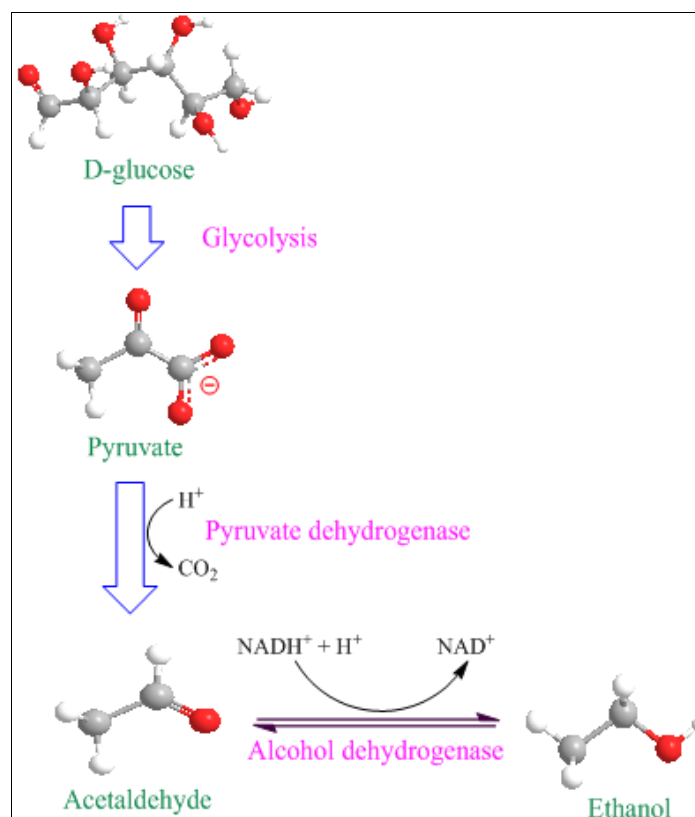

Fig 2: Metabolism of fermentation in *Saccharomyces cerevisiae*

The glycolytic pathway comprises ten reactions which produce two ATP molecules, two NADH molecules, and two pyruvate molecules. Pyruvate can be directed to oxygen-dependent and oxygen-independent pathways. If oxygen is present, pyruvate can be transported to the mitochondrial matrix and converted to Acetyl-CoA which proceeds to the tricarboxylic acid cycle. In the absence of oxygen, pyruvate decarboxylase catalyzes pyruvate conversion to acetaldehyde with the release of carbon dioxide. The acetaldehyde is then converted to ethanol through alcohol dehydrogenase [17].

The binding of berginin, glucose, fructose, sucrose, maltose, and the cocrystallized ligand of the protein thiamine diphosphate on the active site of PDI-1 from *S. cerevisiae* is shown in Figure 3.
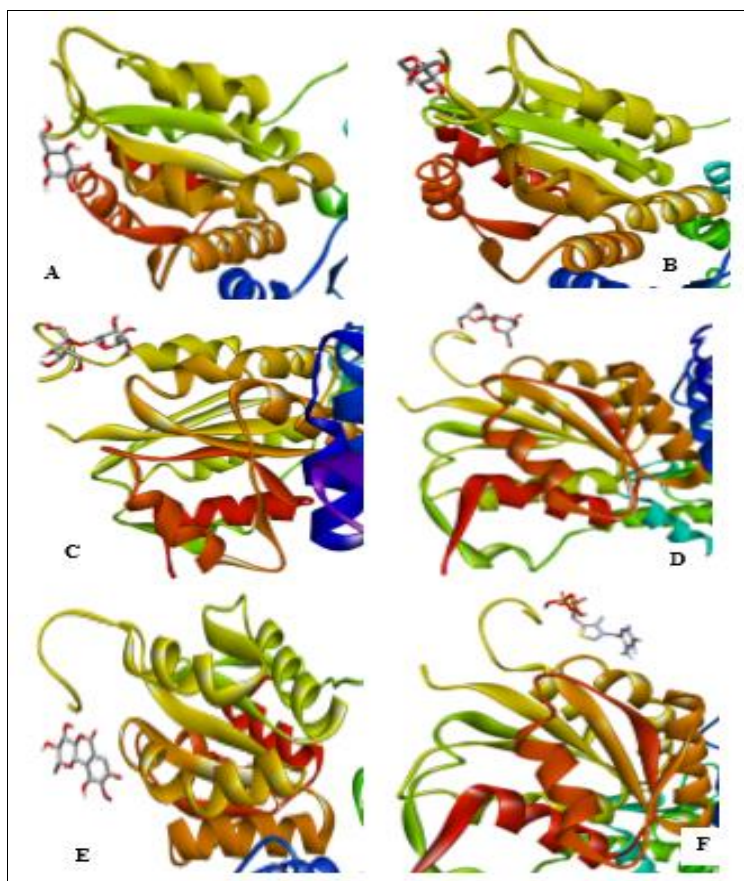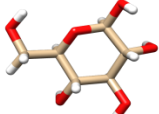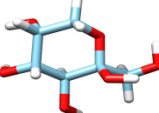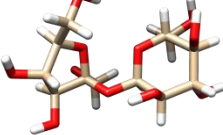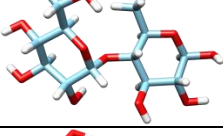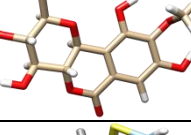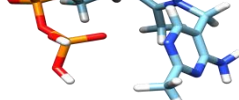

Fig 3: Binding of A. glucose B. fructose C. sucrose D. maltose E. bergenin F. thiamine diphosphate at the active site of PCI-1 The binding affinity values of the molecules at this active site are shown in Table 1.

Table 1: Binding affinities of the studied molecules on PDI-1 of *S. cerevisiae*

Compound	PubChem ID	Structure	ΔG Energy (Kcal/mol)
Glucose	5793		-3.5
Fructose	2723872		-3.4
Sucrose	5988		-4.0
Maltose	6255		-4.6
Bergenin	66065		-4.5
Thiamine diphosphate	1132		-5.1

The inhibition of sugar fermentation in palm wine can only occur if the binding affinity of bergenin from the bark of *S. gabonensis* on the enzyme target is higher than those of all the sugars in the juice. The binding affinity of bergenin and the studied sugar molecules on the active site of the target increased in the order maltose > bergenin > sucrose > glucose > fructose with values –4.6 Kcal/mol, –4.5 Kcal/mol, –4.0 Kcal/mol, –3.5 Kcal/mol, and –3.4 Kcal/mol respectively. The binding affinity of maltose was slightly higher than the value obtained for bergenin, suggesting that this compound may not efficiently inhibit the fermentation of this sugar by the yeast.

Maltose, however, cannot be fermented without been hydrolyzed to its basic units, which are two molecules of glucose [18]. At this stage, their fermentation can then be inhibited by bergenin. Since the binding affinity of bergenin is higher than those of glucose and fructose, which are the maximum sugar units that can be fermented by the yeast, it can inhibit their conversion to ethanol when used to preserve palm wine.

The 3D and 2D protein-ligand interaction images of the cocrystallized ligand form PCI-1 and those from the studied molecules are shown in Figure 4.
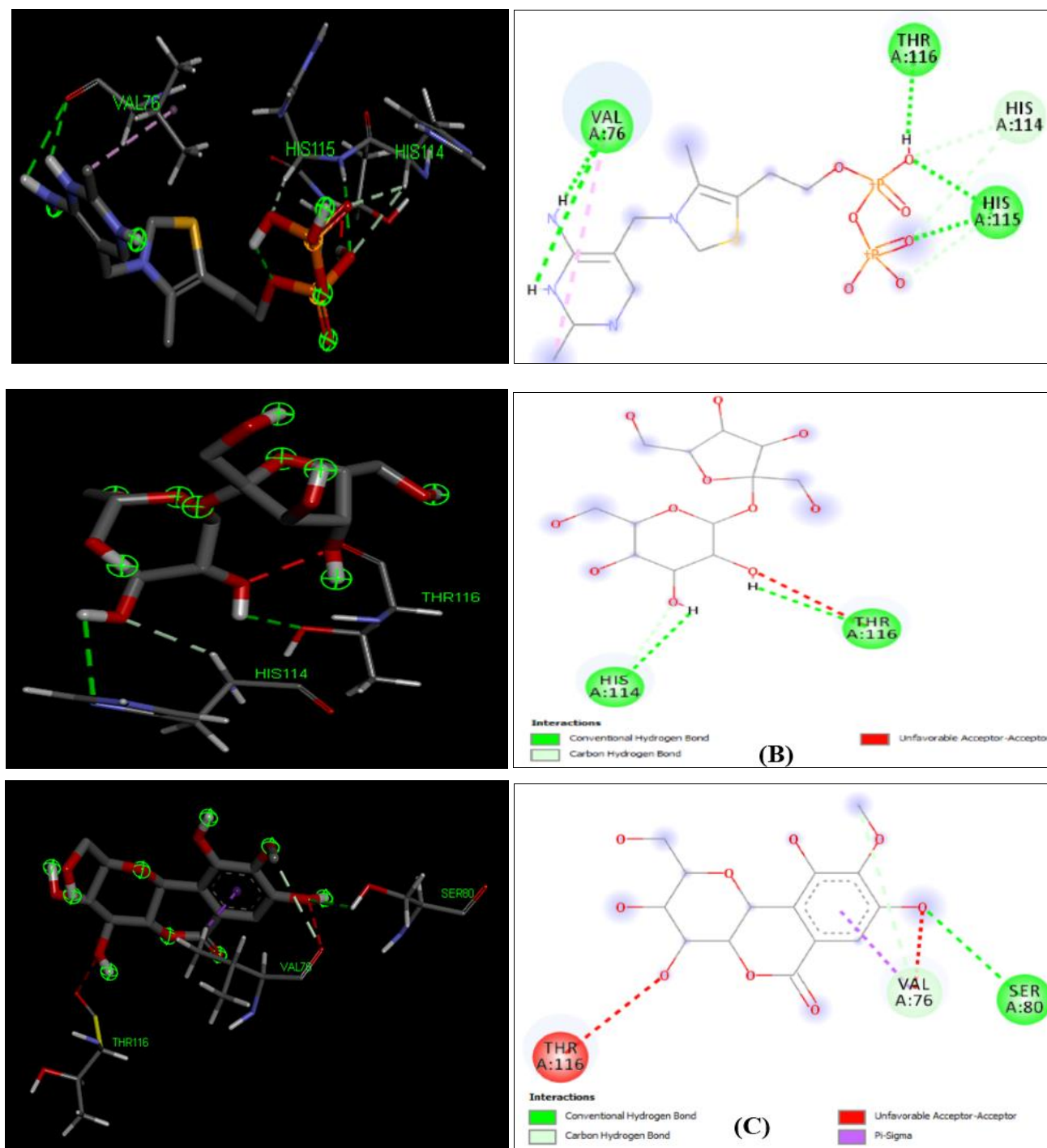

Fig 4: 3D (left) and 2D (right) views of molecular interactions of (A) Thiamine diphosphate (B) Maltose (C) Bergenin

The protein residues that interacted with the molecules at the enzyme active site are summarized in Table 2.

Table 2: Protein residue interactions with thiamine diphosphate, maltose, and bergenin

Compound	Hydrogen bond	Carbon-Hydrogen bond	Pi-Sigma	Unfavorable acceptor-acceptor	Alkyl
Thiamine diphosphate	VAL76(2); HIS115(2); THR116(1)	HIS114(2); HIS115(1)	-	-	VAL76(1)
Maltose	HIS114(1); THR116(1)	HIS114(1)	-	THR116(1)	-
Bergenin	SER80(1)	VAL76(1)	VAL76(1)	THR116(1); VAL76(1)	-

The modes of interaction of bergenin with the protein residues were different from those of maltose and thiamine diphosphate. Thiamine diphosphate, maltose, and bergenin were held at the target's active site by three, two, and one hydrogen bond respectively. The hydrogen bond interactions occurred at VAL76, HIS115, and THR116 in thiamine diphosphate, HIS114, and THR 116 in maltose, while bergenin had a single interaction at SER80. Since hydrogen bonds are the major interactive forces in the protein-ligand binding of molecules, its presence in bergenin interaction with PCI-1 indicated that this molecule has good stability in the protein pocket.

Conclusions
The inhibitory efficiency of sugar fermentation by berginin on the pyruvate decarboxylate enzyme from *S. cerevisiae* was studied using computational simulation. The binding affinity value of bergenin on the active site of the yeast protein was higher than those of glucose, fructose, and sucrose. Though the binding affinity of maltose was the highest of all the studied sugars, its fermentation to ethanol is not likely to be achieved in the presence of bergenin, since maltose must be hydrolyzed to glucose before fermentation can occur. The hydrogen bond interaction between bergenin and PCI-1 enzyme indicated that the protein-ligand complex formed was very stable. This study corroborates the claim that the bark of *S. gabonensis* could increase the shelf life of palm wine, which contains the studied sugars.

Conflict of interest
We declare that no conflict of interest with the data contained in this manuscript.

References
1. Ojimelukwe PC. Effects of preservation with *Saccoglottis gabonensis* on the microbiology of fermenting palm wine. Journal of Innovation in Life Science 2002;6:19-27.
2. Karamoko D, Djeni NT, N'guessan KF, Bouatenin KMJ-P, Dje KM. The biochemical and microbiological quality of palm wine samples produced at different periods during tapping and changes which occurred during their storage. Food Control 2012;26:504-511.
3. Jespersen L. Occurrence and taxonomic characteristics of strains *of Saccharomyces cerevisiae* predominant in African indigenous fermented foods and beverages. *FEMS* Yeast Research 2003;3:191-200.
4. Agu RC, Okenchi MU, Ude CM, Onyia AI, Onwumelu AH, Ajiwe VIE. Fermentation kinetic studies of Nigerian palm wines; *Elaeis guinensis* and *Raphia hookeri* for preservation by bottling. Journal of Food Science and Technology 1999;36(3):205-209.
5. Ezeronye, OU, Okerentugba PO. Production of genetic recombinations of yeast for the treatment of effluent from a Nigerian paper recycling plant. Journal of Agriculture, Biotechnology and Environment 1999;1:53-59.
6. Ojimelukwe PC. Effect of preservation with *Saccoglottis gabonensis* on the biochemistry and sensory attributes of fermenting palm wine. Journal of Food Biochemistry 2000;25:411-424.
7. Maduka HCC, Okoye ZSC, Ladeji O, Egbe PE. The Protective role of *Sacoglottis gabonensis* stem bark extract against peroxidation reactions *in vivo* and *in vitro*. Nigerian Journal of Biotechnology 1999;1(10):1-8.
8. Tchouya GRF, Obiang GDN, Bongui J, Lebibi J. Phytochemical study of *Sacoglottis gabonensis* (Baill.) Urb. Isolation of bioactive compounds from the stem bark. Chemical Science International Journal 2015;11(4):1-5.
9. Sasikala RP, Meena KS. Molecular docking studies and ADMET properties of compounds from *Physalis minima L.* leaves root and fruit. Innov J Life Sci 2016; 4:21-25.
10. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin, TE. UCSF Chimera- a visualization system for exploratory research and analysis. Journal of Computational Chemistry 2004;25(13):1605-1612. http://sts.bioe.uic.edu/castp/index.html?j_5f45dd381f58d
11. Tsao YC, Chang YJ, Wang CH, Chen L. Discovery of isoplumbagin as a novel NQO1 substrate and anti-cancer quinone. International Journal of Molecular Sciences 2020;21(12):4378.
12. BIOVIA DS. Discovery studio modeling environment. San Diego, Dassault Systemes, Release, 4, 2015.
13. Walker GM, Stewart GG. Saccharomyces cerevisiae in the production of fermented beverages. Beverages 2016;2:30.
14. Duru CE. Time series model for glucose conversion to ethanol, in relation to ethanol loss during fermentation. Journal of Chemical Society of Nigeria 2014;30(2):37-39.
15. Maicas S. The role of yeasts in fermentation processes. Microorganisms 2020;8(8), 1142.
16. Wills C. Regulation of sugar and ethanol metabolism in *Saccharomyces cerevisiae*. Critical Reviews in Biochemistry and Molecular Bioloy 1990;25(4):245-280.
17. Duru CE, Duru IA. Comparative study of the efficiency of mineral acid catalysts in the conversion of starch to glucose. Futo Journal Series 2016;2(2):136-145.