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Physiochemical properties and Enzymatic profile of indigenous yeast isolated from palm wine in Nigeria

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Abstract

This study was aimed at investigating the physiochemical properties of fresh palm wine and enzymatic activities of indigenous yeast strains isolated from palm wine sample (Elaeis guineensis). The physiochemical properties was evaluated after 1h of tapping giving the following values 7.29^{a}±0.02%, 2.25^{a}±0.03%, 476.58^{d}±0.04 mg/L and 5.2^{c}±0.06 for ethanol, TTA, reducing sugar and pH respectively. The yeast strains was isolated and characterized based on their morphological, physiological, and biochemical characteristics. Two yeast cells were identified as *Saccharomyces cerevisiae* and *Geotrichum candidum.* They were screened for their ability to synthesize amylase and cellulase. Results revealed significant variation in the enzymatic profiles of the indigenous yeast strains. Cellulase activity was present suggesting the ability to break down cellulose; and a starch degrading ability indicated by amylase activity. All the yeast stains had good cellulase and amylase enzyme profile but YT3 which was identified as *Geotrichum candidum* exhibited the highest enzyme activity of 0.719U/ml (amylase) and 0.182U/ml (cellulase), suggesting their potential for efficient sugar utilization during fermentation. The findings from this study proved that indigenous microorganisms in palm wine can act as a source of enzymes especially cellulase and amylase; and the enzyme yield will most likely improve after optimization. The identification of yeast strains with enhanced enzymatic potential offers opportunities for the large-scale and industrial production of the enzyme.

Keyword: Palm wine; Indigenous yeast; physiochemical properties; Enzyme profile

1. Introduction

Palm wine, a fermented sap from the tropical plant of the palmae family produced and consumed widely in West Africa as an alcoholic drink in large quantities, is a nutritionally rich medium that promotes the growth of several organisms. Its sap is a sugary clear liquid that undergoes spontaneous fermentation by the natural microflora which reduces the sugar by converting it to alcohol as well as several other metabolites making the sap milky and effervescent due to the growth of the fermenting organisms [1,2]. In Nigeria, the two most commonly tapped palm trees are the oil palm (*Elaeis guineensis*) and raffia palm (*Raphia hookeri* and *Raphia vinifera*). The palm wine collection involves two methods, first the felling of the tree and tapping the wine from an incision made at the meristemic region and the second which is the inflorescent tapping via an incision made at the base [3, 4]. The latter is considered more acceptable since it does not involve the destruction of the palm tree and ultimately reduction in the population due to continuous tapping. The spontaneous fermentation in palm wine undergoes three stages, first the lactic acid fermentation, the alcohol fermentation then lastly the acetic acid fermentation; these involves three major microbial groups namely the yeast, lactic acid bacteria and acetic acid bacteria which play very significant roles in the overall quality of the palm wine [5, 6]. These organisms present in palm wine can alter the properties of the product as a result of their ability to produce a range of metabolites during fermentation.

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Yeasts are unicellular eukaryotes that are widely distributed in nature and are known for their fermentative ability and can also grow and metabolize various kinds of substrate. These yeast which acts as the primary fermenter, converts the sap into alcohol and other minor metabolites such as higher alcohols, polyols, esters, organic acids etc [7].

Several researches have shown that enzymes like lipase, amylase and cellulase have been found in yeast [8, 9]. Due to the high productivity, low cost, availability and survival in adverse conditions, various industries prefer microbial enzyme produced as intra or extra cellular metabolites for their processes [9, 10]. This has lead to fresh investigation by researcher into the extracellular enzyme profile of food grade yeast [11]. Amylase enzyme, a starch degrading enzyme that have numerous applications in the industrial sector is gaining importance in ~~with~~* the use of microorganisms for its production due to short incubation period, secretion of enzyme extracellularly, utilization of cheap medium constituents and cost effectiveness [12, 13, 14]. Cellulase also has its applications in various industries such as pharmaceuticals, textile, food and medicine [15]. The aim of this study is to investigate the physiochemical properties of the palm wine and the enzyme potential of the yeast isolated from the fermenting sap isolated from palm oil tree for use in various industrial applications

2. Materials and Methods

2.1. Sampling and Isolation of Microorganisms

Sample Collection: Fresh palm wine was collected from Amansea town located within Awka city in Anambra state, Nigeria. The freshly tapped palm wine sample was collected using pre-sterilized, labeled 100 ml capacity universal sample bottles. The sample was transported to the laboratory in a cooler equipped with packs of the freezing mixture of salt and ice block for microbial analysis within 1h of collection.

Isolation and enumeration: Sample dilution was carried out using the standard method. Palm wine sample was serially diluted to 10^{-3} by adding 1mL of the sample into test tubes containing 9 mL of distilled water. Then 0.1ml was inoculated in the plates containing SDA prepared with 0.1 g/L chloramphenicol and incubated at room temperature (27 ± 2°C) for 72 h.

Following the enumeration of the total yeast count, colonies were picked at random and subcultured for purification onto SDA under aseptic conditions using the streaking method and incubated at 30°C for 24-48 hours. Purified isolates were stocked in appropriate media at 4°C (refrigerated) on an agar slant for further use.

2.2. Physicochemical analysis

Ethanol: The ethanol content of the palm wine was determined using the method of [16]. To an aliquot (1ml) of the palm wine, 1 mL of sodium dichromate solution,1 mL of acetate buffer (pH 4.3) and 5 mL of 1N sulfuric acid was added in a test tube. The mixture was shaken gently for 1 min and allowed to stand for 120 mins as incubation period at room temperature resulted in formation of green colored reaction product. Following incubation period the absorbance at 578 nm was read on Axiom 752 spectrophotometer. The ethanol content was calculated using standard ethanol (100mg/ml).

Reducing Sugar: The reducing sugar content of the palm wine was estimated using the DinitroSalicyclic acid method described by Wood [17]. The palm wine (3ml) was mixed with 1ml of DNS in test tube. It was boiled in a water bath for 5mins and cooled down in running water. The absorbance was recorded at 540nm using Axiom 752 spectrophotometer. The reducing sugar content was calculated using glucose standard.

Determination of pH and Titratable Acidity: The pH of the palm wine was measured directly using a pH meter after calibration with standard buffers. Total titratable acid was determined according to the method of Afolabi and Owoola, [18]. The palm wine sample was titrated against 0.1M Sodium Hydroxide (NaOH) and 1% phenolphthalein was used as an indicator. It was calculated as percentage of lactic acid.

2.3. Characterization and identification of the yeast isolates

Microscopy: The morphology of the isolates was observed and then further identified microscopically by the type of spores and hyphae. The microscopic observations were carried out using a high-powered microscope of the objective lens (x400) and the photomicrographs were digitally recorded.

Substrate assimilation and sugar fermentation: The ability of selected yeast isolate to grow solely on different carbon source was studied. The carbon sources tested were D-glucose, D-galactose, fructose, maltose, sucrose. Fermentation

was performed using 0.1 mL 24-h old cultures to inoculateDurham tubes containing 6 mL medium. The medium was made up of 1% (w/v) yeast potato broth (YPD) with 2% sugar (glucose, galactose, fructose, maltose and sucrose). Cultures in 1% yeast extract served as thecontrol. The tubes were incubated at 25ºC for up to 14 days and inspectedperiodically for gas production and color change. Phenol red served as anindicator [19].

2.4. Enzyme assay

Screening: To screen for enzyme production, the method of Nwagu et al; [9] was used with slight modification. The medium containing 1% yeast extract, 0.5% MgSO4, 0.3% NaCl, 0.2% K_2HPO_4, and 0.3% KH_2PO_4 was prepared. To test for α-amylase, cassava starch (1%) was used as a substrate. CMC (1%) was used as carbon sources for cellulase production. After preparation, the media was sterilized in an autoclave (121ºC, 15 min), cooled, and the isolates inoculated and incubated at 30 ± 1ºC for 3 days. After incubation, the crude extract was collected by centrifugation and used for enzyme assay.

Cellulase activity: Cellulase activity was determined using dinitrosalicylic acid as described by Nwagu et al., [9] with some modification. Carboxymethyl cellulose (CMC, 1 %) solution was prepared in 0.2 M potassium acetate buffer (pH 5.5). One milliliter (1 ml) of the CMC solution was incubated with 1 ml of crude enzymatic extract at 60°C for 30mins. Afterward, the enzyme reaction was stopped using 1.0 mL dinitro salicylic acid (DNSA) and boiled for 5 min, diluted and optical density was determined at a wavelength of 540 nm using a spectrophotometer(Miller, 1959). One unit of enzyme activity (U) is the amount of enzyme releasing one mmol of glucose per minute.

Amylase activity: Alpha Amylase activity was determined using dinitrosalicylic acid as described by Nwagu et al., [9] with some modification.The enzyme extract (0.5 ml) was added to a test tube containing 0.5 ml of 0.5% soluble starch solution. The mixture is then incubated at 60°C for 30mins using a water bath. Then 1.0 ml of dinitrosalicylic acid reagent (DNSA) was added to each test tube to stop the reaction. The tubes were then placed in boiling water for 5 min for color development and cooled at room temperature. The contents of tubes were diluted up to 5 ml with distilled water. The absorbance was determined at 540 nm using a spectrophotometer and converted to mg of maltose using the standard prepared.

2.5. Statistical analysis

Statistical Package for Social Sciences (SPSS) was used to analyze all the data. One-way analysis of variance (ANOVA) was used to compare the means.Differences between the means were significant when $P < .05$.

Table 1 Physicochemical characteristics and yeast count of the palm wine

Properties	Values
Ethanol (%)	7.29^{a}±0.02
TTA (%)	2.25^{a}±0.03
RS (mg/L)	476.58^{d}±0.04
pH	5.2^{c}±0.06
Yeast count (CFU/ml)	2.1×10^{6}

Key: TTA: Total titratable acid, RS: Reducing sugar

3. Results and Discussion

The physicochemical characteristics and yeast count of the palm wine was evaluated as shown in Table 1. The percentage ethanol content gave 7.29^{a}±0.02 with a total titratable acid of 2.25^{a}±0.03, while the reducing sugar showed 476.58^{d}±0.04 mg/L and a pH of 5.2^{c}±0.06 after 1h of collection. The pH result is similar to that obtained by karamoko et al, [20] were they got 5.23 ± 0.18 from the Dura variety of oil palm. The values for alcohol, reducing sugar and titratable acids share no similarity to the mean values reported by other authors [5, 3]. The total titratable acid content of 2.25% was high compared to those of [3] that gave 0.25% probably due to different palm tree varieties or an early build up of organic acids leading to a decrease in the pH values.

The high ethanol content could be due to the fact that it was tapped in the morning, and according to Amoa-Awua*et al* [5], palm wine collected in the morning gave very high alcohol concentrations probably because of the accumulation that occurs at night.

A total number of 15 yeast cells were isolated and 10 were discarded based on their inability to produce cellulase and amylase as well as their colony and cell morphology. A total of 5 isolates were selected and further identified by their assimilation and fermentation abilities of 5 sugars and microscopic features. PT2, PT4 and PT5 fermented all sugars except fructose and assimilated mainly glucose, sucrosemaltose and galactose while PT1 did not ferment any of the sugars and PT3 showed weak fermentation for glucose and galactose. They both assimilated glucose, fructose and galactose. Two different yeasts were identified in the oil palm wine. Of all the isolates, three were identified as *Saccharomyces cerevisiae* and two as *Geotrichum candidum.* This makes *Saccharomyces cerevisiae* the dominant microorganism present in palm wine during fermentation, this is in agreement with previous studies carried out [4, 21, 5]. The non- *Saccharomyces* present in the palm wine was much lower. The presence of *Geotrichum candidum* in oil palm wine have also been reported previously [21], which was stated could be that the area the sample was collected is a niche for the growth of the specie. However, it has been involved in other food product like cheese during the ripening [22].

Table 2 Morphology and identity of the Yeast isolates

Isolates	**Colonial Morphology**	**Cellular Morphology**	**Identity**
PT1	Cream colored, flat, membranous	Arthrosporous conidia, dichotomously branched hyphae	*Geotrichum candidum*
PT2	Cream colored, Smooth, opaque, round	Globose ascospore, Pseudohyphae	*Saccharomyces cerevisiae*
PT3	Whitish colored, flat, smooth, slimy	Arthrosporous conidia, dichotomously branched hyphae	*Geotrichum candidum*
PT4	Creamy colored, Smooth, flat, opaque, round	Globose ascospore, Pseudohyphae	*Saccharomyces cerevisiae*
PT5	Cream-yellowish flat, round, opaque	Globose ascospore, Pseudohyphae	*Saccharomyces cerevisiae*

Table 3 Characteristics of yeast isolates from fresh oil palm wine

Characteristics	**PT1**	**PT2**	**PT3**	**PT4**	**PT5**
Sugar fermentation					
d-Glucose	-	+	w	+	+
Sucrose	-	+	-	+	+
Maltose	-	+	-	+	+
Fructose	-	-	-	-	-
d-Galactose	-	+	w	+	+
Sugar Assimilation					
d-Glucose	+	+	+	+	+
Sucrose	-	w	--	+	+
Maltose	-	w	--	w	+
Fructose	+	-	+	+	-
d-Galactose	+	+	+	+	-

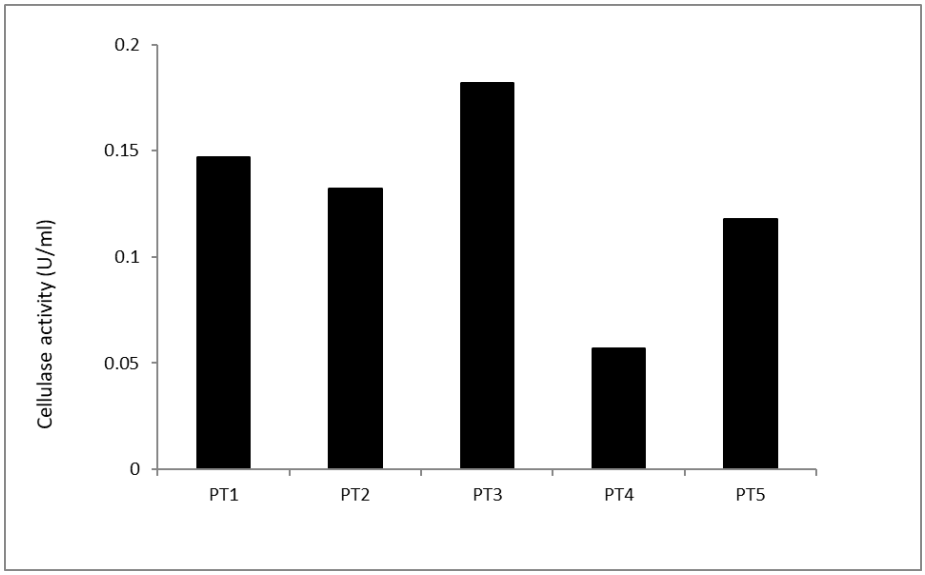

Figure 1 Cellulase activity of the yeast isolates

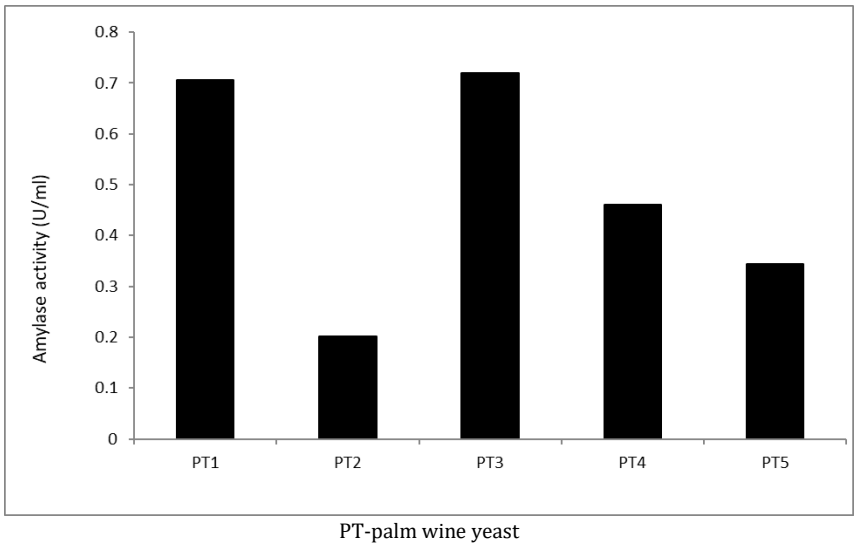

Figure 2 Amylase activity of the yeast isolates

The extracellular enzymatic profile of the indigenous yeast was conducted to determine their potential as seen in fig 1 and 2. Interestingly, the isolates showed potential for both cellulase and amylase production, although same strains of yeast showed variation in the enzyme activity obtained. The pt2, pt4 and pt5 which were identified as *Saccharomyces cerevisiae* gave enzyme activity of 0.132U/ml, 0.057U/ml and 0.118U/ml for cellulase and 0.201U/ml, 0.460U/ml and 0.344U/ml for amylase respectively while pt1 and pt3 identified as *Geotrichum candidum* gave 0.147U/ml and 0.182U/ml for cellulase with amylase activity of 0.705U/ml and 0.719U/ml respectively. Amoikon et al., [21],also reported similar study, were diverse sets of enzyme activity were observed for strains of same species with near identical genomic content. Maximum amylase and cellulase production was observed for pt3 at 0.719 and 0.182 respectively.

Amylase enzyme breaks down starch by attacking the 1,4-glycosidic bonds releasing a combination of glucose, maltose, and malto-oligosaccharide [23]. Many amylase producing bacteria have been isolated from palm wine [12, 24] but only very few yeast have been reported from palm wine [9]. Cellulase production from yeast strains have also been reported previously [25; 8; 9]. Omojasola and Jilani, [8], reported a cellulase activity of 1.11 U/mL from *Saccharomyces cerevisae* isolated from palm wine. A study conducted by Amaeze et al. [25], also showed *Saccharomyces cerevisae* from palm wine as a good cellulase producing organism with activity of 0.269 mg/ml.

According to Nwagu et al. [9], yeast strains isolated from palm wine gave very high amylase activities in the range of 84.21 – 146.39 U/ml and for cellulase 0.82 – 1.66 U/ml. Major cellulase producing organisms are known to be fungi due to their ability to degrade cellulose, hemicelluloses and lignin, although production of cellulase by microorganisms depends on the type of substrate and the strains used [26, 27].

4. Conclusion

The indigenous microbiota present in palm wine play very important functions in the biochemical properties, and the quality of the product as well as the presence of different metabolites produced. Enzyme profile gave different activities between the strains, however the yeast cells showed great cellulase and amylase activity with YT3 identified as *Geotrichum candidum* giving the highest performance. With the proper conditions in place, these fermenting yeasts present in palm wine have the potential to produce different extracellular industrial enzymes which can be used to improve the quality of various industrial products.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Obire O. Activity of Zymomonas species in palm sap obtained from three areas in Edo state, Nigeria. J Appl Sci and Environmental Management. 2005, 9: 25-30.

[2] Remison A, Odion-Owase AO. Comparative Assessment of the Microbiology of Palm SAP from Life and Felled Oil Palm (Elaeis guinensis) Tree. Direct Res J Biology Biotechnol. 2022, 8 (1): 1-6.

[3] 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. http://dx.doi.org/10.1016/j.foodcont.2012.02.018

[4] Djeni TN, Kouame KH, Ake FDM, Amoikon LST, Dje MK, Jeyaram K. Microbial Diversity and Metabolite Profiles of Palm Wine Produced From Three Different Palm Tree Species in Côte d'Ivoire. Scientific Reports. 2020, 10:1715. https://doi.org/10.1038/s41598-020-58587-2

[5] Amoa-Awua WK, Sampson E, Tano-Debrah K. Growth of Yeasts, Lactic and Acetic Bacteria in Palm Wine during Tapping and Fermentation from Felled Oil Palm (Elaeis guineensis) in Ghana. J Appl Microbiol. 2007, 102, 599-606. http://dx.doi.org/10.1111/j.1365-2672.2006.03074.x

[6] Santiago-Urbina JA, Ruíz-Terán F. Microbiology and biochemistry of traditional palm wine produced around the world. Int Food Res J. 2014, 21(4): 1261-1269.

[7] Walker GM, Hill AE. Saccharomyces cerevisiae in the production of whisky. Beverages. 2016, 2, 30-42.

[8] Omojasola PF, Jilani OP. Cellulase production by Trichoderma longi, Aspergillus niger and Saccharomyces cerevisiae cultured on plantain peel. Res j microbiol. 2009, 4(2): 67-74.

[9] Nwagu TNT, Osilo C, Arinze MN, Okpala GN, Amadi O, Ndubuisi IA, Okolo B, Moneke A, Agu R. A novel strain of Yarrowia phangngaensis producing a multienzyme complex, a source of enzyme additives for baking high cassava-wheat composite bread. Food biotechnol. 2021, 35 (2): 158-177. https://doi.org/10.1080/08905436.2021.1910520

[10] Oort VM. Enzymes in bread making. In: Whitehurst RJ, van Oort M, eds. Enzymes in Food Technology. 2nd ed. Chichester: Wiley-Blackwell; 2010. p. 103-143

[11] Buzzini P, Martini A. Extracellular enzymatic activity profiles in yeast andyeast-like strains isolated from tropical environments. J Appl Microbiol 2002, 93: 1020-1025.

[12] Lakshmi SS, Mahesh CH, Gayatri K, Manisha P, Aishwarya K. Statistical optimization of amylase production and its purification from a palm wine isolate Bacillus sp., Q-164. Biocat Agri Biotechnol. 2920, 29: 101820. https://doi.org/10.1016/j.bcab.2020.101820

[13] Gopinath SCB, Anbu P, Arshad MK, Lakshmipriya T, Voon CH, Hashim U, Chinni SV. Biotechnological processes in microbial amylase production. BioMed Res. 2017, 1–9.

[14] Liu J, Zhang Z, Zhu H, Dang H, Lu J, Cui Z. Isolation and characterization of α-amylase from marine Pseudomonas sp, K6-28-040. Afr. J. Biotechnol. 2011, 10: 2733–2740.

[15] Sukumaran RK, singhania RR, Pandey A. Microbial cellulases-production, applications and challenges. J. Sci. Industr. Res. 2005, 64:832-844.

[16] Sumbhate S, Satish N, Damodar G, Ajay T, Rajesh SJ. Colorimetric Method for the Estimation of Ethanol in Alcoholic-Drinks. J Analytical Technique. 2012, 1: 1-6

[17] Wood JP, Elliston A, Ryden P, Bancroft J, Roberts JN, Waldron K.W. Rapid quantification of reducing sugars in Biomass hydrolysates,; Improving the speed and precision of the dinitrosalicylic acid assay. Biomass Bioenergy 2012, 44:117-121.

[18] Afolabi FT, Owoola AT. Microbiological and Physicochemical Changes in Palm Wine Subjected To Spontaneous Fermentation During Storage. Int J Biotechnol. 2019, 8(1): 48-58.

[19] Suh SO, Zhang N. Nguyen N. Gross S, Blackwell M. Lab manual for yeast study, 2008, 7–21. Mycology Lab.

[20] Karamoko D, Deni NT, Moroh J-LA, Bouatenin KMJ-P, Dje KM. Biochemical and Microbial Properties of Palm Wine: Effect of Tapping Length and Varietal Differences. Food Nutrition Sci. 2016, 7: 763-771

[21] Amoikon TLS, Aké FM, Djéni TN, Grondin C, Casaregola S, Djè MK. Diversity and enzymatic profiles of indigenous yeasts isolated from three types of palm wines produced in Côte d'Ivoire. J. Appl MicrobioL. 2018, doi: 10.1111/jam.14154

[22] Perkins V, Vignola S, Lessard M-H, Plante P-L, Corbeil J, Dugat-Bony E, Frenette M, Labrie S. Phenotypic and Genetic Characterization of the Cheese Ripening Yeast Geotrichum candidum. Front Microbiol, 2020, 7(11):737 DOI: 10.3389/fmicb.2020.00737

[23] Akcan N. High level production of extracellular α-amylase from Bacillus licheniformis ATCC 12759 in submerged fermentation. Rom. Biotechnol. Lett. 2011, 16: 6833–6840.

[24] Elechi DP, Ogugbue CJ, Akaranta O, Oji A. Optimization of B. cereus PW4 alpha amylase production by OVAT technique. GSC Biological and Pharm Sci. 2022, 20(01): 083–090

[25] Amaeze NJ, Okoliegbe IN, Francis ME. Cellulase production by Aspergillus niger and Saccharomyces cerevisiae using fruit wastes as substrates. Int. J. Appl. Microbiol. Biotechnol. Res. 2015, 3:36-44

[26] Saraswati B, Ravi Kumar M, Mukesh Kumar DJ, Balashamugam P, BalaKumaran MO, Kalaichevan PT. Cellulose production by Bacillus subtilis isolated from cow dung. Archives Appl Sci Res. 2012, 4 (1): 269-279.

[27] Caritas UO, Humphrey CN. Effect of acid hydrolysis of Garcinia kola (bitter Kola) pulp waste on the production of CM-cellulase and β-glucosidase using Aspergillus niger. Afr. J. Biotechnol. 2006, 5(10): 819-822.