

Fermentative Potential of Yeasts Isolated from the Fruits of the African Ebony Tree (*Diospyros mespiliformis*) in Côte d'Ivoire for Bioethanol Production

Souleymane Soumahoro¹, Maimouna L. Kouame¹, Wilfried K. Yao^{2*}, Gédéon W. Coulibaly¹, Abdoulaye Toure³, Yadé R. Soro²

¹Laboratory of Biochemistry, Microbiology and Valorization of Agrosources, Agropastoral Management Institute, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire, Korhogo BP 1328

²Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, Faculty of Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire, 22 BP 582 Abidjan 22

³Laboratory of Biotechnology and Valorization of Agrosources and Natural Substances, Faculty of Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire, Korhogo BP 1328

DOI: <https://doi.org/10.36347/sajb.2025.v13i03.006>

| Received: 08.02.2025 | Accepted: 11.03.2025 | Published: 15.03.2025

*Corresponding author: Wilfried K. Yao

Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, Faculty of Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire, 22 BP 582 Abidjan 22

Abstract

Original Research Article

This study, which aims to contribute to the valorization of *Diospyros mespiliformis* fruits, was conducted at the laboratory of Peleforo GON COULIBALY University in Korhogo (Ivory Coast). It aimed, on the one hand, to analyze some physicochemical parameters (moisture content, dry matter, total soluble solids, pH, titrable acidity, and vitamin C content) of *Diospyros mespiliformis* pulp and, on the other hand, to select potential yeast starters for bioethanol production. Thus, the microbiological analyses of the pulp consisted of testing their fermentative capacity, then subjecting the isolates with high fermentative capacity to the influence of some fermentative parameters (sodium chloride, glucose, and ethanol) on their growth. Physico-chemical parameters revealed that the pulp of *Diospyros mespiliformis* has a high moisture content (74.97%), an acidic pH of 3.85, a titrable acidity of 1.41%, a vitamin C content of 12.35 mg/100 g, and a soluble dry extract of 5 °B. All these characteristics make the fruits of *Diospyros mespiliformis* an interesting source of nutrients. Furthermore, microbiological analyses allowed for the isolation and identification of eighty-six (86) yeasts based on their macroscopic and microscopic characteristics. Among these isolates, six showed a strong fermentative capacity and exhibited good microbial growth under the influence of the studied fermentative parameters. Thus, these six (6) isolates could be proposed as potential starters in biotechnological applications to standardize and control certain fermentation processes such as bioethanol production.

Keywords: *Diospyros Mespiliformis*, Physico-Chemical Analysis, Yeasts, Techno-Functional Properties, Potentials Starters.

Copyright © 2025 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

1-INTRODUCTION

Fruits play an important role in the daily lives and well-being of many people. People prefer cultivated fruits to wild ones in many countries (Chakravarty *et al.*, 2016). However, the importance of wild edible fruits lies in the fact that some have a higher nutritional value compared to cultivated fruits (Burlingame, 2000). Wild fruit are defined as all ligneous fruit species growing in the wild (Kini *et al.*, 2008). These fruit species are essential to the daily lives of rural populations. Their use in the pharmaceutical and agro-food industries around the world does, in fact, reflect this popularity (Daanon *et*

al., 2021). These fruit species are essential to the daily lives of rural populations. The majority of these wild fruits is eaten on plantations, while a low percentage of these species is endangered during deforestation and commercialized because of their high rentability (Ambé, 2001). In Côte d'Ivoire, populations primarily consume wild fruits locally without producing any profitable activity. Yet, processing these wild fruits could provide a significant source of income (Alira, 2004). So processing these wild fruits is a more appropriate strategy for adding value to them. The processing of fruit and vegetables involves operations and techniques designed to convert relatively bulky and perishable raw

Citation: Souleymane Soumahoro, Maimouna L. Kouame, Wilfried K. Yao, Gédéon W. Coulibaly, Abdoulaye Toure, Yadé R. Soro. Fermentative Potential of Yeasts Isolated from the Fruits of the African Ebony Tree (*Diospyros Mespiliformis*) In Côte d'Ivoire for Bioethanol Production. Sch Acad J Biosci, 2025 Mar 13(3): 340-348.

materials into more useful, edible and pleasant-tasting foods or drinks that can be stored for a long time (FAO, 2009).

Diospyros mespiliformis is one of the many wild fruit trees that grow in the West African nation of Côte d'Ivoire. Known as the African ebony tree, it is a wild fruit in the *Ebenaceae* family (Daanon *et al.*, 2021). *Diospyros mespiliformis* is a type of herbaceous plant with a variety of uses. Indeed, all of its components (fruit, leaves, bark, and roots) are used for a variety of purposes (Ado *et al.*, 2017). Unfortunately, Côte d'Ivoire populace underutilizes and knows very little about this fruit from a nutritional and industrial standpoint, despite its economic potential. This species produces fruits with highly sweet pulp that is valued by both rural communities and animals that ensure its spread across the natural environment. Due to the high amount of sugars in its pulp, *Diospyros mespiliformis* could be a good substrate for the hunt for an intriguing fermented microflora for the food industry. Indeed, bacteria, yeasts and moulds dominate the microflora of fruits and vegetables (Desbordes, 2003). These microbial organisms selected as starters and frequently used in the food fermentation industry (Ribéreau-Gayon *et al.*, 2006). Strains used as starters are typically isolated from food substrates or processes in which they are used. The selection criteria for strains vary depending on the desired characteristics. However, factors like competitiveness, viability, resistance to pathogenic microorganisms, acid and alcohol production rate, organoleptic changes, primary metabolites, degradation of anti-nutrition factors, and probiotic characteristics must also take into account when choosing starting cultures. Ideally, we would be to obtain a strain that

fulfils each of these functions (Ribéreau-Gayon *et al.*, 2006). In this sense, the fermentation process benefits largely from the starters (Ayad, 2009). Indeed, they are used in the food fermentation industry to improve flavour and other desirable qualities, such as organic acids linked to digestibility and palatableness (Kolawole *et al.*, 2017), add new nutritional value to foods, and preserve product quality as in the case of yeasts. Yeast inoculation to obtain a product of predictable quality, including in the production of alcoholic beverages, has been widely used in the food industry (Ribéreau-Gayon *et al.*, 2006). Nevertheless, research efforts are concentrated on improving the fermentation step, in particular the study on yeast strains that are more resistant to the various stresses encountered during alcoholic fermentation, i.e. temperature, high concentration of glucose and ethanol (Samagaci *et al.*, 2014). Thus, the aim of this study is to valorise the fruits of *Diospyros mespiliformis* through research into ethanol-producing yeast strains that are resistant to fermentative stress.

2-MATERIELS AND METHODS

2-1-Study Materials

The biological material studied is known as African ebony or *Diospyros mespiliformis* (Figure 1). The fruits were harvested at maturity from smallholder plantations in the town of Korhogo (9° 27' 41" N, 5° 38' 19" W) in northern Côte d'Ivoire. The fruit collected were transported directly to Laboratory of biochemistry, microbiology and agroresources at the PELEFORO Gon Coulibaly University of Korhogo. Samples (Fruits) were submitted to physico-chemical and microbiological analysis.



Figure 1: Unripe (A) and ripe (B) fruits of *Diospyros mespiliformis*

2-2-METHODS

2-2-1-Physico-Chemical Evaluation of Africa Ebony Fruits

2-2-1-1-Measurements of Moisture Content and Dry Matter

African ebony fruit was evaluated for moisture content and dry matter using AOAC method (1990). After weighing five (5) grams of *Diospyros mespiliformis* and placed in a glass capsule of mass (m_0), the capsule-sample (m_1) completely was dried for twenty-four hours at 105°C within oven. Once the capsule-sample completely had cooled in the desiccator, it was weighed again (m_2). The moisture content (H) of the African ebony fruit as a percentage of mass was calculated using the formula below:

$$H(\%) = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100$$

Dry matter (DM) content was calculated using the following formula and represented as a percentage of the sample raw mass:

$$DM(\%) = 100 - H(\%)$$

2-2-1-2-pH and titrable Acidity of *Diospyros Mespiliformis* Fruits

pH and titrable acidity were determined using AOAC method (1990). Ten (10) grams of *Diospyros mespiliformis* pulp crushed were homogenized in 100 ml of distilled water. Next, the pHmeter (HANNA, Germany) was partially immersed in the solution under agitation. Finally, the pH value displayed on the pHmeter screen was read directly. Ten (10) mL of the previously obtained filtrate were mixed with three drops of phenolphthalein (1%) indicator. The homogenized mixture is added with a dropwise addition of a NaOH (0.1N) solution from a burette until a pink hue is achieved. Titrable acidity is expressed in percentage of citric acid (%).

2-2-1-3-Total Soluble Solids (TSS) of *Diospyros Mespiliformis* Fruits

To measure TSS, which was evaluated in Brix degree (°B), a drop of Africa ebony juice was put on the plate and tested using an ATC refractometer (Erb 32, ERMA, Tokyo). The refractometer's ocular was used to read the measurement (AOAC, 2005).

2-2-1-4-*Diospyros Mespiliformis* Vitamin C Content

Vitamin C content of *Diospyros mespiliformis* pulp was assessed using Pongracz *et al.*, (1995) methodology. To 40 ml of 2% w/v metaphosphoric acid/acetic acid, 10 g of crushed pulp (m_e) were added. For 20 minutes, the resulting solution was centrifuged at 3000 rpm and the supernatant was collected in a 100 mL flask and completed with boiled distilled water. Ten (10) mL of the cooled contents were removed and placed in an Erlenmeyer flask. To get a persistent pink colour, the assay sample was titrated against a 0.5 g/L solution of 2,6-DCPIP. Previously, a 0.5 g/L vitamin C solution was

used to calibrate the 2,6-DCPIP solution. Hence, V (mL) is the volume of 2,6-DCPIP that was poured to neutralise vitamin C. A fresh sample vitamin C content was calculated using the following formula.

$$\text{Vit C } (\%) = \frac{(0.5 \times V \times 10^{-3}) \times 5}{m_e}$$

2-2-2-Microbiological Study of *Diospyros Mespiliformis* Fruits

2-2-2-1-Yeast Isolation and Biochemical Characterization

In order to isolate yeast, MYGP agar (3 g/L malt extract, 5 g/L bactopectone, 3 g/L yeast extract, 10 g/L glucose and 15 g/L agar) enhanced with 100 mg/L of chloramphenicol was streaked with a stock solution made up of 25 g of crushed Africa ebony pulp in 225 mL of peptone water solution. For 48 hours, MYPG agar was incubated at 30°C. In addition to macroscopic and microscopic characteristics, the yeasts were biochemically identified after incubation using standard Biolog Identification System Techniques (Biolog, 1993).

2-2-2-2-Yeast Isolated from *Diospyros Mespiliformis* and Their Technological Characteristics

2-2-2-2-1-CO₂ Production by Yeasts Strains

Methodology described by Koffi *et al.*, (2018) with slight modifications was used to measure the amount of CO₂ produced by yeast strains in liquid medium. For CO₂ production, the durham cloche was replaced by hemolysis tubes. Indeed, the quantity of CO₂ produced by the yeasts strains correlates with the production of ethanol. Thus, a yeast suspension (OD 600 nm = 0.7) prepared in tryptons salt (TS) was used to inoculate YPG broth and incubated at 30°C for 48 hours without shaking. After incubation period, the height of the gas generated in the hemolysis tube was measured. The relationship following provides the amount of CO₂ emitted by yeasts strains.

$$\text{Volume of CO}_2 \text{ released (cm}^3\text{)} = \pi r^2 h$$

r: radius of the hemolysis tube

h: height of gas in the hemolysis tube

2-2-2-2-2-Environmental Stress Conditions (Glucose, Ethanol and Sodium Chloride) On Yeasts Growth

The effects of glucose, ethanol and sodium chloride (NaCl) on the growth of yeast isolates were evaluated using a liquid medium that contained 0.3% casein peptone and 0.05% yeast extract (Samagaci *et al.*, 2014). A glucose rate of 5 to 30% was added at liquid media. Ethanol concentrations ranging from 5 at 14% and NaCl of 2.5 at 10% were inserted into the broth medium. One hundred (100) µL of yeast preculture (OD₆₀₀=0.7) was used to inoculate 10 mL of liquid media contains to a test tube, and then incubated for three days at 30°C. A spectrophotometer (Pioway Medical Labs, Singapore) was used to measure the optical density

at 600 nm following the incubation period in order to evaluate the yeast's growth.

2-3-Statistical Analysis

Following triplicate analysis of the samples, the data were presented as mean $\pm$ standard deviation. Analysis of variance (ANOVA) was performed using SPSS Statistics 20.0 software, and significant differences between means were determined using Duncan's test at the 5% level.

3-RESULTS

3-1-Monitoring the Physico-Chemical Parameter of *Diospyros Mespiliformis* Fruits

Physico-chemical parameters of *Diospyros mespiliformis* fruits are summarised in Table I. The moisture content of *Diospyros mespiliformis* fruits is very high with a value of 74.97% with a dry matter of 25.03%. This parameter shows that African ebony fruits are rich in water and have a reasonable dry matter content. African ebony fruit pulp has an acidic pH of 3.85, which is associated with a titrable acidity of 1.41% citric acid. According to our research, the pulp of African ebony fruits has a Brix value of 15°B. The percentage of Total soluble solids in a fruit's juice is known to be its brix degree. The result of the vitamin C content is shown in Table I. African ebony fruits contains vitamin C with a content of 12.35 mg/100 g.

Table I: Physico-chemical characteristics of *Diospyros mespiliformis* fruit

Parameters	Unit	Values
Moisture content	%	74.97 $\pm$ 0.42
Dry matter	%	25.03 $\pm$ 0.86
pH	-	3.85 $\pm$ 0.01
Titrable acidity	%	1.41 $\pm$ 0.15
Vitamine C	mg/100 g	12.35 $\pm$ 0.08
Total soluble solid	°B	15 $\pm$ 0

3-2-Isolation and CO₂ Production of Yeasts

According to the observed macroscopic and microscopic characteristics, forty-six (46) presumptive yeasts were isolated from the pulp of African ebony fruits. Waiting confirmation by the molecular approach, the yeast strains numbered YD 01 to YD 46 were stored in PDA broth supplemented with 15% glycerol at -20°C. The production of CO₂ varies depending on the yeast isolates tested. According to the volume of CO₂ released

on a scale ranging from 0 to 6 cm³, three groups were distinguished (Table I). Three (3) isolates produced CO₂ strongly with values ranging between 4 and 6 cm³, accounting for 6.52% of the strains. Six (6) isolates produced CO₂ moderately with values ranging between 2 and 4 cm³, accounting for 13.04%. Thirty-seven (37) isolates produced CO₂ weakly with values less than 2 cm³, accounting for 80.44% of the tested yeasts.

Table II: CO₂ production by yeasts strains isolated from *Diospyros mespiliformis*

Groups	Volume of CO ₂ (cm ³)	Number of isolates	Percentage (%)
Group 1	[4 – 7.2 cm ³]	03	6.52
Group 2	[2 – 4 cm ³]	06	13.04
Group 3	[0 – 2 cm ³]	37	80.44

3-3-Influence of Stress Conditions (Glucose, Ethanol and Sodium Chloride) On Yeasts Growth

3-3-1-NaCl Effect

The concentration of NaCl has an impact on the growth of the nine yeast strains (Figure 1). Indeed, a decrease in yeast microbial growth was observed with an increase in NaCl concentration. In the absence of NaCl

in the culture medium, the yeast strains exhibit good growth with optical densities at 600 nm ranging between 0.643 and 0.929. However, when the concentration of NaCl increases, a decrease in optical density was reported until it becomes zero for the isolates YD 07, YD 16, YD 19, YD 21, and YD 37 (Figure 2).

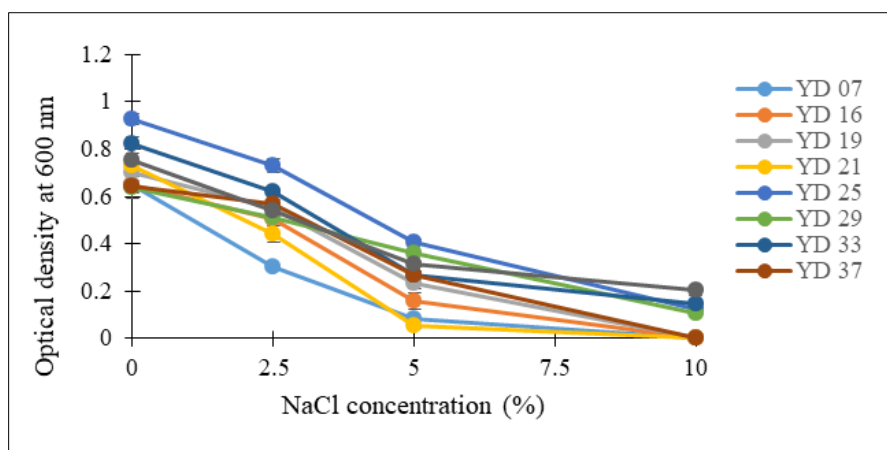


Figure 2: influence of NaCl concentration on the growth of yeast strains with high fermentative capacity isolated from *Diospyros mespiliformis* fruits

3-3-2-Glucose Effect

Figure 3 presents the effect of glucose concentration on the growth of yeasts strains with high fermentative capacity isolated from *Diospyros mespiliformis* fruits. All nine isolates showed good glucose tolerance with an optical density greater than 0.3 at 600 nm at 40% glucose. In fact, glucose acts as an activator of microbial growth for the nine isolates. Indeed, an increase in microbial growth in relation to an

elevation in optical density observed up to 10% glucose with optical densities ranging between 1.237 and 1.818. However, the isolates YD 16 and YD 33 reached their growth peak at 5 and 10% glucose with optical densities of 1.435 and 1.545 respectively. Beyond these various peaks obtained, a exponential decrease in microbial growth which is observed by huge drop on figure 3 up to 40% glucose.

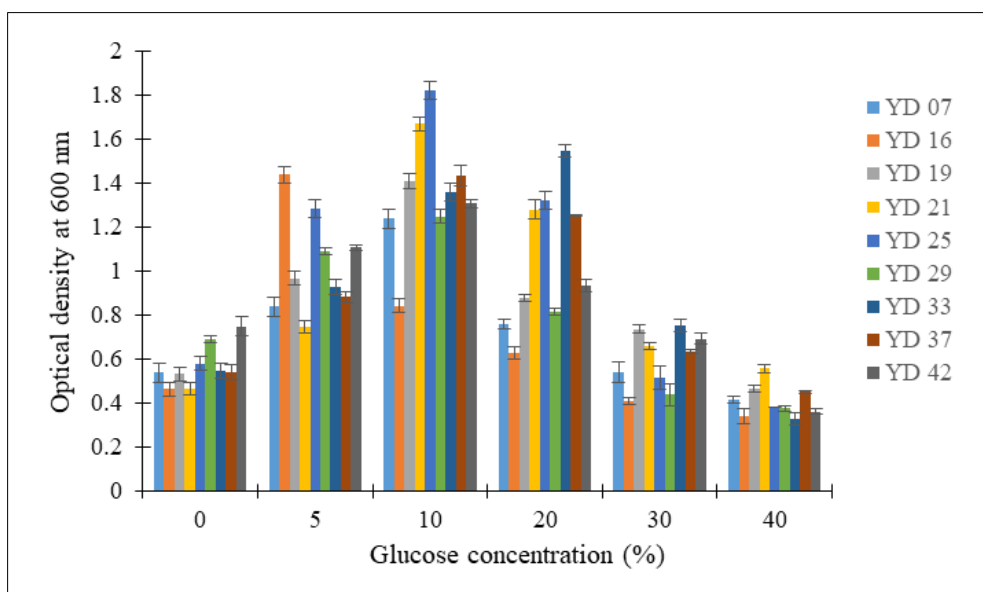


Figure 3: Impact of glucose concentration on the growth of yeast strains with high fermentative capacity isolated from *Diospyros mespiliformis* fruits

3-3-3-Ethanol Effect

The effect of ethanol concentration on the growth of the nine yeast strains is presented in Table III. All the isolates were able to grow under alcoholic stress up to 12%. Beyond this concentration, three strains (YD 19, YD 21, and YD 25) showed no microbial growth at 14% ethanol. However, ethanol acts differently on the

nine microbial strains. Indeed, two trends emerge. The first trend concerns the isolates YD 07, YD 19, and YD 25, where a decrease in optical density is correlated with an increase in ethanol concentration. The second recorded trend shows an increase in microbial growth by 5% for strains YD 29, YD 33, YD 37, and YD 42, and up to 8% for the isolates YD 16 and YD 21.

Table III: Effect of ethanol concentration on the growth of yeast strains with high fermentative capacity isolated from *Diospyros mespiliformis* fruits

Yeasts strains	Optical density per concentration of ethanol at 600 nm					
	0%	5%	8%	10%	12%	14%
YD 07	0.950±0.03a	0.762±0.01b	0.639±0.01c	0.545±0.03d	0.421±0.01e	0.219±0.02f
YD 16	0.685±0.03c	0.756±0.04b	0.968±0.02a	0.699±0.01c	0.524±0.02d	0.316±0.01e
YD 19	0.781±0.02a	0.729±0.02b	0.610±0.02c	0.345±0.03d	0.231±0.01e	0.000±0.00f
YD 21	0.530±0.01b	0.747±0.04a	0.762±0.01a	0.490±0.05b	0.358±0.00c	0.000±0.00d
YD 25	0.974±0.03a	0.952±0.03a	0.833±0.09b	0.723±0.02c	0.620±0.03d	0.000±0.00e
YD 29	0.672±0.05b	0.924±0.01a	0.876±0.05a	0.540±0.02c	0.117±0.00d	0.121±0.03d
YD 33	0.739±0.02bc	0.818±0.03a	0.773±0.02ab	0.701±0.07cd	0.663±0.02e	0.337±0.03f
YD 37	0.733±0.01c	0.974±0.01a	0.917±0.04b	0.593±0.03d	0.553±0.04d	0.415±0.03e
YD 42	0.730±0.03b	0.959±0.03a	0.750±0.03b	0.637±0.03c	0.441±0.04d	0.245±0.02e

Data are represented as means±SEM (n=3). Means with different letters in the same line are statistically different (p<0.05) according to Duncan's test.

4-DISCUSSION

Physico-chemical parameters of *Diospyros mespiliformis* fruits were determined. With regard to moisture content, the work of Muhammad *et al.*, (2024) showed that the fresh pulp of African ebony fruits has a moisture content of 65.2% and a dry matter of 34.8%, near to our observed results. Microbial activity during storage is increased by higher moisture content (Abdel *et al.*, 2007). Since there is a strong correlation between microbial presences and spoiling, the pulp of fresh whole fruit should be thoroughly dried before, being stored (Kari *et al.*, 2022). According to Ilouno *et al.*, (2021), the amount of moisture in fresh fruits affects how quickly they deteriorate and how long they last on the shelf. However, the presence of water in the fruit pulp could be an advantage for isolation a microbial flora whose water activity allows its growth (Ananias *et al.*, 2012). As for the dry matter that accounts for the composition of the fruits of the African ebony, it shows that the pulp of these wild fruits has a low concentration of macromolecules such as carbohydrates, lipids and proteins according to its dry matter content of 25.03%. Research by Muhammad *et al.*, (2024) showed that the pulp of African ebony fruits has a dry matter content of 34.8% with 1.41% lipids, 0.90% proteins and 31.72% carbohydrates. Despite a lower dry matter content in our study, African ebony contains a good concentration of carbohydrates appreciated by the populations.

The pH of the juice from the pulp of African ebony fruits has a value of 4.13 according to the work of Muhammad *et al.*, (2024). In addition, pH value from our study was similar to the 3.12-3.88 pH for baobab, pineapple, and blackplum fruit juice reported by Tawakalt *et al.*, (2020). The acidity of the pulp of African ebony fruits would be due to the presence of organic acids, mainly citric acid, which is the predominant acid in fruits worldwide (Silva *et al.*, 2002). This acidity helps limit the growth of pathogenic microorganisms but promotes the growth of fermentative microbial strains

such as yeasts. Indeed, these microorganisms can withstand acidity and ferment the sugars present (Salas-Navarrete *et al.*, 2023).

The result is quite similar to the 14.57 °B value found by Muhammad *et al.*, (2024). The percentage of solids in a fruit's juice is known to be its brix degree. This parameter generally indicates the sucrose concentration in a fruit (Akubor, 2017). Together with fruits like avocados, bananas, lemons and coconuts, the African ebony fruit's brix level (15°B) is in the excellent category (Boamponsem *et al.*, 2013). Indeed, this brix value indicates that the fruits of the African ebony are a good source of sugars that could be transformed into juice or fermented beverages.

African ebony fruits contain vitamin C with a content of 12.35 mg/100 g. Compared with several wild African edible fruits such as *Borassus aethiopum*, *Dovyalis caffra*, *Sclerocarya birrea* and *A. digitata* which have a vitamin C content ranging between 100 and 300 mg/100 g (Umaru *et al.*, 2007 ; Ali *et al.*, 2010). The value obtained in our study is low; however, out of all the vitamins specified in the recommended daily allowance (RDA) for humans, vitamin C is the one that is most essential, with a recommended daily intake of 75 mg. Even though, citrus fruits have long been known to be significant sources of vitamin C (Rudge *et al.*, 2012), but these results showed that other wild fruits, such *Diospyros mespiliformis*, also contain a not negligible quantity of vitamin C. In fact, vitamin C is a vital component that supports the immune system and prevents damage from free radicals, besides other processes that occur in the human body (Muhammad *et al.*, 2024).

Forty-six (46) yeasts were isolated from *Diospyros mespiliformis* fruits using the dependent culture technique. Yeasts have already been isolated from several wild fruits and constitute the major fermentative microflora (Nyanga *et al.*, 2007). Indeed, the acidic pH, high moisture content, and presence of sugars are favourable conditions for isolating yeasts from the pulp of most fruits (Tsegaye *et al.*, 2018).

The ability of yeasts to produce CO₂ in a liquid medium, which is corroborated by the production of ethanol, has been extensively covered in the scientific literature (Dung *et al.*, 2014; Koffi *et al.*, 2018; Nguyen *et al.*, 2022; Tadese *et al.*, 2024). The primary property recognized in yeasts is the production of ethanol from fermentable sugars (Koffi *et al.*, 2018). Thus, the yeast strains isolated from wild fruit *Diospyros mespiliformis* would be good candidates for industrial bioethanol production. Nine isolates with CO₂ levels equal to or greater than 2 cm³ were selected for further analysis.

NaCl effect has an impact on the growth of the nine yeast strains. At 15% NaCl, cell growth is null for all tested isolates (data not shown). This study shows that NaCl is a growth inhibitor at high concentrations and consequently a barrier to ethanol synthesis by yeasts. In fact, research by Logothetis *et al.*, (2010) revealed that 10% NaCl had a negative impact on cell viability and this concentration completely inhibited yeast growth. Four isolates were able to withstand this stress factor and exhibited optical densities ranging between 0.106 and 0.206. These isolates YD 25, YD 26, YD 33, and YD 42 correspond to the characteristics of potential starters.

The nine (9) isolates tested were able to resist up to 40% glucose with a growth peak at 10%. Our study's findings are consistent with Osho (2005), Ali and Khan (2017) research on yeasts sugar tolerance, which also showed that Yeasts have a maximum sugar tolerance at 20%. The ability of our isolates to tolerate high concentrations of glucose would be an asset for bioethanol production. These high concentrations could stimulate the microbial growth of yeasts and enable excellent ethanol production.

These nine isolates were screened for their ability to grow at different ethanol concentrations in liquid broth. This study also found that ethanol inhibited the growth of certain yeasts isolates by up to 14% in the growth medium. Research conducted by Ali and Khan (2014, and Nurcholis *et al.*, (2021) showed that yeasts are capable of resisting and growing under alcoholic stress in a culture medium with concentration ranging between 1 and 16%. Indeed, according to Dalawai *et al.*, (2017), increasing concentrations of ethanol can affect yeast tolerance to ethanol. The accumulation of ethanol in the medium can cause considerable stress on the yeast during process (Stanley *et al.*, 2010). High concentrations of ethanol can decrease resistance and inhibit the growth of yeast cells. Ethanol can also influence cellular metabolism and the biosynthesis of macromolecules by stimulating protein production. The ability of our microbial strains to withstand and grow in high concentrations of ethanol gives them potential as starters for bioethanol production.

5-CONCLUSION

Fruits of *Diospyros mespiliformis* exhibit interesting physicochemical characteristics. They have

an acidic pH (3.85) which limits the growth of certain pathogenic germs. A dry matter content of 25.03% that indicates its composition in nutrients such as sugars and vitamin C. The isolation of yeasts and the study of techno-functional properties have led to the selection of six (6) isolates that present themselves as ideal candidates for bioethanol production.

REFERENCES

- Abdel, M. S., Fatima, M. I., & Elamin, A. E. (2007). Quantitative determination of tannin content in some sorghum cultivars and evaluation of its antimicrobial activity. *Research Journal of Microbiology*, 2(3), 284-288.
- Adedokun, T. O., Matemu, A., Hoglinger, O., Mlyuka, E., & Adediji. A. (2022). Evaluation of functional attributes and storage stability of novel juice blends from baobab, pineapple, and black-plum fruits. *Heliyon*, 8, e09340.
- Ado, A., Bil-Assanou, I. H., Iro, D. G., Karim, T. D. A., Ali, M., & Mahamane S. (2017). Effect of pre-treatments, substrates and water stress on germination and initial growth of *Diospyros mespiliformis* Hochst. Ex A.DC. *European Scientific Journal*, 13(21), 251-268.
- Akubor, P. I. (2017). Quality characteristics and storage properties of squash prepared from pineapple (*Ananas comosus*) fruit juice. *Asian Journal Biotechnology Bioresource Technology*, 1(4), 1-8.
- Ali, A., Alhadji, D., Tchiegang, C., & Saïdou, C. (2010). Physico-chemical properties of *Palmyra palm* (*Borassus aethiopum* Mart.) fruits from Northern Cameroon. *African Journal of Food Science*, 4(3), 115-119.
- Ali, M. N., & Khan, M. M. (2014). Screening, identification and characterization of alcohol tolerant potential bioethanol producing yeasts. *Current Research in Microbiology and Biotechnology*, 2(1), 316-324.
- Alira, A. (2004). Commercialization of wild fruits and their products in the Mouhoun loop: case of the villages of Bissanderou, Bomborokuy, Soana, and the city of Dedougou. End-of-cycle dissertation, Bobo-Dioulasso, 110 pages.
- Ambé, G. A. (2001). Wild edible fruits of the Guinean savannahs of Côte d'Ivoire: state of knowledge by a local population, the Malinkés. *Biotechnology, Agronomy Society and Environment*, 5(1), 43-58.
- Ananias, K. R., de Melo, A. A. M., & de Moura C. J. (2013). Analysis of moisture content, acidity and contamination by yeast and molds in *Apis mellifera* L. honey from central Brazil. *Brazilian Journal of Microbiology*, 44(3), 679-683.
- AOAC (1990). Official methods of analysis of the Association of Official Analytical Chemists, Association of Official Analytical Chemists, 15th edition, volume 1, Washington DC, 771 p.

- AOAC (2005). Official Methods of analysis. Association of official Analytical Chemists, 17th Edition Washington, DC.
- Ayad, E. H. E. (2009). Starter culture development for improving safety and quality of Domiati cheese. *Food Microbiology*, 26(5), 533-541.
- Biolog (1993). YT Microplate: Instructions for Use. Hayward California: Biolog Inc.
- Boamponsem, G. A., Johnson, F. S., Mahunu, G. K., Stephen, F., & Awiniboya, S. F. (2013). Determination of biochemical composition of *Saba senegalensis* (Saba fruit). *Asian Journal of Plant Science and Research*, 3(1), 31-36.
- Burlingame, B. (2000). Wild nutrition. *Journal of Food Composition and Analysis*, 13(2), 99-100.
- Chakravarty, S., Bhutia, K. D., Suresh, C. P., Shukl, G., & Pala, N. A. (2016). A review on diversity, conservation and nutrition of wild edible fruits. *Journal of Applied and Natural Science*, 8(4), 2346-2353.
- Daanon, A., Padonou, E. A., Akakpo, B. A., & Houinato, M. (2021). Variability in the use of *Diospyros mespiliformis* Hochst. according to socio-demographic factors in North Benin. *The tropical woods and forests*, 347, 29-40.
- Dalawai, N., Karupa, K. N., Nadkarni, S., Bharani, S., & Harinikumar, K. M. (2017). Screening of efficient ethanol tolerant yeast strain for production of ethanol. *International Journal of Pure & Applied Bioscience*, 5(1), 744-752.
- Desbordes, D. (2003). Fruits and vegetables microbiology: microflora, spoilage, health care, sanitation. Bibliographic Research Report, National School of Information and Library Sciences, 47 pages.
- Dung, N. T. P., Tuong, N. H., & Phong, H. X. (2014). Study on Ethanol Fermentation Conditions from Molasses by Thermo-Tolerant Yeasts. *International Journal of Business and Applied Science*, 1(1), 13-22.
- FAO (2009). FAO International Technical Conference. Summary of the current situation and options regarding the application of biotechnologies to food processing and safety in developing countries, Guadalajara (Mexico), 52.
- Ilouno, L. E., Omaji, G. O., & Anthony, E. O. (2021). Proximate, Mineral and Anti-Nutritional Composition of Jackal Berry (*Diospyros mespiliformis*) Seeds. *FUDMA Journal of Sciences*, 2(1), 75-79.
- Kari, N. M., Ahmad, F., & Ayub, M. N. A. (2022). Proximate composition, amino acid composition and food product application of anchovy: a review. *Food Research*, 6(4), 16-29.
- Kini F., Saba A., Ouédraogo S., Tinguéri B., Sanou G., Guissou, I. P. (2008). Nutritional and therapeutic potential of some 'wild' fruit species from Burkina Faso. *Pharmacopoeia and Traditional African Medicine*, 15, 32-35.
- Koffi, O., Samagaci, L., Goualié, G. B., & Niamké, L. S. (2018). Screening of potential yeast starters with high ethanol production for a small-scale cocoa fermentation in Côte d'Ivoire. *Food environment safety*, 7(2), 113-130.
- Kolawole, M., Kayode, R., & Akinduyo, B. (2017). Proximate and microbial analyses of burukutu and pito produced in Ilorin, Nigeria. *African Journal of Biotechnology*, 6(5), 587-590.
- Logothetis, S., Nerantzis, E. T., Giouliti, A., Kanelis, T., Panagiotis, T., & Walker, G. (2010). Influence of sodium chloride on wine yeast fermentation performance. *International Journal of Wine Research*, 2, 35-42.
- Muhammad, H. S., Atiku, M. K., Basheer, D. H., Zubairu, I. K., & Muhammad, S. M. (2024). Nutritional and Phytochemical Evaluation of Kanya (*Diospyros mespiliformis*) Juice: A Potential Functional Beverage for Enhanced Food Security. *UMYU Scientifica*, 3(2), 62-67.
- Nguyen, P. V., Nguyen, K. H. V., Nguyen, N. L., Ho, X. T. T., Truong, P. H., & Nguyen, K. C. T. (2022). Lychee-Derived, Thermotolerant Yeasts for Second-Generation Bioethanol Production. *Fermentation*, 8(515), 1-16.
- Nurcholis, M., Setiawan, A., Kusnadi, J., & Maligan, J. M. (2021). Isolation of thermo-tolerant and ethanol-tolerant yeast from local fermented foods and their potential as bioethanol producers. In IOP Conference Series: Earth and Environmental Science (Vol. 924, No. 1, p. 012077). IOP Publishing.
- Nyanga, L. K., Nout, M. J. R., Gadaga, T. H., Theelen, B., Boekhout, T., & Zwietering, M. H. (2007). Yeasts and lactic acid bacteria microbiota from masau (*Ziziphus mauritiana*) fruits and their fermented fruit pulp in Zimbabwe. *International Journal of Food Microbiology*, 120(1-2), 159-166.
- Osho, A. (2005). Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice. *African Journal of Biotechnology*, 4(7), 660-662.
- Pongracz, G., Weiser, H., & Matzinger, D. (1971). Tocopherols- Antioxydant. *Food Science and Technology*, 97(3), 90-104.
- Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2006). The microbiology of wine and winemaking. Dunod, Bordeaux, 497 pages.
- Rudge, H., Barros, D. M., Aparecida, T., De Castro, P., & Inés, M. (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food Chemistry*, 134(4), 1892-1898.
- Salas-Navarrete, P. C., Rosas-Santiago, P., Suárez-Rodríguez, R., Martínez, A., & Caspeta, L. (2023). Adaptive responses of yeast strains tolerant to acidic pH, acetate, and supraoptimal temperature.

Applied Microbiology and Biotechnology, 107(12), 4051-4068

- Samagaci, L., Ouattara, H. G., Goualié, B. G., & Niamké, S. L. (2014). Growth capacity of yeasts potential starter strains under cocoa fermentation stress conditions in Ivory Coast. *Emirates journal of food and agriculture*, 26(10), 861-870.
- Silva, B. M., Andrade, P. B., Mendes, G. C., Seabra, R. M., & Ferreira M. A. (2002). Study of the Organic Acids Composition of Quince (*Cydonia oblonga* Miller) Fruit and Jam. *Journal of Agricultural and Food Chemistry*, 50(8), 2313-2317.
- Stanley, D., Bandara, A., Fraser, S., Chambers, P. J., & Stanley, G. A. (2010). The Ethanol Stress Response and Ethanol Tolerance of *Saccharomyces cerevisiae*. *Journal of Applied Microbiology*, 109(1), 13-2.
- Tadesse, T., Dase, D., Koricha, A. D., & Bacha, K. (2024). Optimization of Bioethanol Production Using Response Surface Methodology for Stress-Tolerant Wild Yeasts Isolated from Natural Forests. *International journal of energy research*, 1, 1-21.
- Tsegaye, Z., Tefera, G., Gizaw, B., & Abatenh, E. (2018). Characterization of Yeast Species Isolated from Local Fruits used for Bakery Industrial Application. *Journal of Applied Microbiological Research*, 1(1), 21-26.
- Umaru, H. A., Adamu, R., Dahiru, D., & Nadro, M. S. (2007) Levels of antinutritional factors in some wild edible fruits of Northern Nigeria. *African Journal of Biotechnology*, 6(16), 1935-1938.