World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Isolation of acetic acid bacteria with biotechnological uses from wild fruit *Diospyros mespiliformis* picked in the north Côte d'Ivoire

Souleymane Soumahoro [1], Moussa Konate [2, *], Maimouna Liliane Kouame [1], Attawa Campbelle Dongo [1], Abdoulaye Toure [3] and Yadé René Soro [2]

[1] *Laboratory of Biochemistry, Microbiology and Valorization of Agroresources, Agropastoral Management Institute, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire, Korhogo BP 1328.*
[2] *Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources (LBAVBR), Faculty of Biosciences, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire, 22 BP 582 Abidjan 22.*
[3] *Laboratory of Biotechnology and Valorization of Agroresources and Natural Substances, Faculty of Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire, Korhogo BP 1328.*

World Journal of Advanced Research and Reviews, 2025, 27(01), 2672-2685

Publication history: Received on 22 June 2025; revised on 27 July 2025; accepted on 30 July 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.1.2820

Abstract

Despite its nutritional value, *Diospyros mespiliformis* is an underexploited fruit in Ivory Coast. This study was conducted with the aim of enhancing this fruit. Thus, according to the analysis, the precise quantities of fruits were collected in Korhogo (northern Côte d'Ivoire) and the physico-chemical properties determined. Endogenous acetic acid bacteria from the fruit were then isolated on selective medium and identified using phenotypic and biochemical methods. Their acidification capacity and resistance to different stresses were tested using reference methods. The strains with the best technological potential were selected by the ascending classification technique. The results showed that the fruit has an acidic pH (2,85 ± 0,01) with a soluble dry extract of 5°Brix, high water content (74,97 ± 0,86 %) and vitamin C (5,06 ± 2,16 mg/100 g). Out of a set of thirty-two (32) isolates of acetic bacteria, nine (09) or (28.1%) showed a strong ability to produce acetic acid in solid media. However, among these nine (09) isolates, four (04) named BAD11, BAD14, BAD23 and BAD24 presented the best resistance to different stresses. Thus, these four (04) isolates identified as Acetobacter sp. with highest acetic acid productions and best other physiological properties could be used in biotechnological processes, particularly in the vinegar industry.

Keywords: Isolation; Influence; Acetic acid; Acetic acid bacteria; *Diospyros mespiliformis*; Korhogo

1. Introduction

Fruits and vegetables are an essential part of a healthy diet. Indeed, a diet rich in fruits and vegetables is likely to reduce the risk of cardiovascular disease and protect against certain types of cancer [10]. Thus, the consumption of 400 to 600 g of fruits and vegetables per day is recommended by the World Health Organization and the Global Research Fund to combat cancer, [1, 27].

Côte d'Ivoire, recognized as one of the leading fruit producers in West Africa, plays a crucial role in food supply and regional economy [11]. The country's climatic and geographical diversity promotes the cultivation of a variety of tropical fruits, which are not only intended for local consumption but also for export to international markets. Among the most popular fruits, we find mango, pineapple and banana, which contribute significantly to farmers' incomes and the country's trade balance.

* Corresponding author: Moussa Konate

Indeed, mango is the third fruit exported by the country behind banana and pineapple [35]. In addition, a large proportion of the fruit grown is still destined for the local market, where it is consumed fresh or processed. This local consumption dynamic is essential to support rural economies and ensure food security [22].

In addition, Côte d'Ivoire is also home to a wealth of easily accessible wild fruits [25] and only for local consumption without resulting gainful activity. Indeed, these wild fruits are consumed directly at the place of picking. Also, these fruit species are traded because of their uses in the agro-food and pharmaceutical industries around the world [17].

Among these fruits, *Diospyros mespiliformis*, of the family of the Ebenaceae still known as African Ebenier [6]. It is a species of tree that produces fruits whose pulp is very sweet and very appreciated not only by animals that ensure its dispersion in the natural environment, but also by rural populations [3]. Indeed, its edible fruits are used as food supplements during lean periods [7] and are also used to produce juice and alcoholic beverages [9].

Diospyros mespiliformis fruits are rich in sugar. Due to the high carbohydrate content in the pulp, which gives it a sweet taste, they could be an appropriate substrate for research into a fermentative flora of interest for the food industry. Fermentation is the transformation of organic matter by ferments, which leads to changes in the organoleptic properties of a food [5]. Acetic bacteria classified in the Acetobacteria group of the Alpha-Proteobacteria class [14, 23] are microorganisms known for their ability to produce acetic acid, an essential process in vinegar production.

Acetic bacteria are distinguished by their ability to oxidize sugars, alcohols into organic acetic acids, ketones and aldehydes through a process known as oxidative fermentation [21]. They are widespread in nature and can be isolated from various sources such as fruits, flowers, sugars and fermented beverages [16]. However, they are considered tedious due to their difficult growth on conventional growing media. Their culture is often weak and highly irregular [33]. However, the isolation of acetic bacteria capable of producing large amounts of acetic acid with better resistance to alcohol has always been of interest. The general objective is to contribute to enhance *Diospyros mespiliformis* fruits technological value.

2. Material and methods

The biological material used in this study consists of *Diospyros mespiliformis* fruits (Figure 1), still known as African Ebony, harvested at the site of the Peleforo Gon COULIBALY University. Then it is sent to the laboratory of the university for physico-chemical and microbiological analyses.

Figure 1 *Diospyros mespiliformis* fruits

2.1. Determination of physico-chemical parameters of the fruit

2.1.1. Water and dry matter content

The AOAC method [2] was used to determine water and dry matter. It consists in dehydrating the samples by drying them in an oven until a constant mass is obtained. 5 grams of pulp is weighed into a glass capsule of known mass (m0). The capsule with the sample (total mass m1) is placed in an oven at 105 °C for 24 hours, then cooled in a desiccator.

The moisture content (Mc) expressed as a percentage of wet sample mass is determined by the following relationship:

$$Mc\ (\%) = ((m_1-m_2) \times 100) / (m_1-m_0)$$

m_0: crucible mass with lid (g)
m_1: mass of the crucible with its lid and containing the sample before drying (g)
m_2: mass of the crucible with its lid and containing the sample after drying (g).

The dry matter content (Dm), expressed as a percentage of the wet sample mass, is calculated as:

$$Dm\ (\%) = 100 - Mc$$

Dm: sample dry matter content (%); Mc: sample moisture or water content (%)

2.1.2. pH

The pH was determined by the AOAC [2] method, using a pH meter with an electrode sensitive to hydrogen ions (H+). Ten (10) grams of pulp were homogenized in 100 mL of distilled water, and the mixture was filtered through filter paper (WHATMAN). The pH meter glass electrode (HANNA) was immersed in the filtrate. The pH value was determined on the screen of the pH meter, previously calibrated.

2.1.3. Titratable acidity

The titratable acidity was determined by AOAC [2] method that involves measuring the acidity of a product by titrating it with sodium hydroxide solution (NaOH) in the presence of phenolphthalein as a color indicator. Ten (10) grams (me) of the fruit pulp were crushed and homogenized in 100 mL of distilled water. After filtration on Whatman paper, 10 mL (V_0) of the filtrate was transferred in an Erlenmeyer to which 3 drops of phenolphthalein were added. A solution of NaOH (0.1N) contained in a burette was then added drop by drop to the mixture until a persistent pink color was obtained. The volume of NaOH (V_1) added up to the turning point has been noted on the burette scale. This volume allowed the acid concentration in the initial sample to be calculated, expressed as a percentage of the corresponding acid.

The titratable acidity (**Tac**) was expressed in milliequivalent (meq) per 100 g of fresh matter by the following relation:

$$Tac = (N \times V_1 \times 10^4) / (mp \times V_0)$$

$\mathbf{V_0}$: volume (mL) of the test portion; $\mathbf{V_1}$: Volume of NaOH added; **mp**: mass (g) of fresh pulp sample; **N= 0.1** (normality of sodium hydroxide solution)

2.1.4. Soluble dry extract

The soluble dry extract expressed in degree brix was measured using a digital refractometer as recommended by the device manufacturer. Ten (10) grams of fruit pulp were put into the extractor to obtain the juice. A drop of this juice was placed on the plate of the prism of the refractometer. The value was obtained directly.

2.1.5. Ethanol content

The percentage ethanol content was measured with a digital refractometer as recommended by the device manufacturer. Ten (10) grams of fruit pulp were crushed in the extractor. The regrind was used to obtain the pulp juice. A drop of this juice was placed on the plate of the prism of the refractometer. The value is obtained directly.

2.1.6. Vitamine C content

The method used to determine vitamin C content was that described by Pelletier [26]. The principle of this method is to stabilize vitamin C by metaphosphoric acid/acetic acid 2, then oxidize with reduced 2,6-dichlorophenol indolphenol. A mass of 10 g of fruit pulp was solubilized in 40 mL of metaphosphoric acid/acetic acid (2%; w/v). The mixture was centrifuged at 3000 tr/min for 20 min. The supernatant is recovered in a 50 mL flask and filled to the gauge with distilled water boiled and cooled off from air. A volume of 10 mL of the contents of the flask was taken and placed into an erlenmeyer (test portion). The test portion is titrated with a 0.5 g/L solution of 2,6-DCPIP (2,6-dichlorophenol indophenol) until it turns persistent pink for 30 s. The 2,6-DCPIP solution is pre-calibrated with a 0.5 g/L vitamin C solution. Either V (mL), the volume of 2.6 DCPIP paid to equivalency. The vitamin C content as a percentage of fresh sample mass is determined by the following relationship:

$$\text{Vitamine C content} = [2(V_C - V_O) / (V_E - V_O)] * 100$$

VO: volume (mL) of 2,6-DCPIP solution poured into the blank; **VE:** volume (mL) of solution used for 2.6 DCPIP solution calibration; **VC**: volume (mL) of 2,6-DCPIP solution used for the test portion.

2.2. Isolation and group distinction test of acetic bacteria

2.2.1. Isolation

25 g of the pulp of *Diospyros mespiliformis* are taken aseptically into a stomacher bag to which 225 ml of buffered peptonic water are added. The whole was thoroughly mixed by manual stirring giving the stock solution. The purpose of this stirring is to dissolve germs. In test tubes containing 9 mL of tryptone salt (TS), decimal dilutions of the stock solution were performed from 10^{-1} to 10^{-5} under aseptic conditions. To do this, 1 mL of the stock solution was taken and placed in a tube containing 9 mL of TS, resulting in 10^{-1} dilution. Subsequently, 1 mL of this dilution was taken and placed in another tube containing 9 mL of TS, corresponding to 10^{-2} dilution. The dilutions are thus carried out in the same way, one after another. 100µL of each dilution range were inoculated by spreading on the agar surface. The seeded media were incubated in a 30 °C oven for 72 hours under aerobic conditions.

Biochemical tests for the identification of acetic bacteria are Gram stain, catalase test, oxidase test and respiratory type.

2.2.2. Group distinction test for acetic bacteria

The group distinction test was performed according to the method described by Soumahoro *et al*. [32]. The purpose of this test is to subdivide isolates into two groups: those that oxidize the acetic acid into CO_2 + H_2O and those that are unable to do it.

The principle of this group distinction test is based on the ability of strains to deacidify the medium containing acetic acid. Thus, the HS broth was prepared with bromocresol green and placed in a flask. After sterilization at 121°C and cooling to 45°C in a water bath, an amount of acetic acid (1%) as the carbon source was added to the broth. The addition of acetic acid causes the colored pH indicator to turn from green to yellow. The HS broth is distributed in 5 mL hemolysis tubes at a rate of 3 mL per tube. Seeding a colony of the strain to be tested is done in broth with a sterile Pasteur pipette. The seeded tubes are then incubated for 5 days in aerobic conditions at 30 °C. The ability of the strain to use acetic acid results in a deacidification of the broth, causing the medium to go from yellow to green.

2.3. Study of the potential technological properties of acetic bacteria isolates

2.3.1. Analysis of the acidification ability

The ability of isolates to acidify the medium was demonstrated using the method proposed by Aydin and Aksoy [4]. The purpose of this method is to evaluate the acidification power of isolated strains. Thus, colonies were inoculated by spot on pre-poured agar in petri dishes. The seeded media were incubated for 5 days at 30 °C in aerobic conditions. During the growth of acetic bacteria, acidification of the medium results in the presence of a clear halo around the colony. Two trials for each isolated strain were carried out on the same container.

2.3.2. Ethanol resistance

To evaluate the effect of ethanol resistance, 100 µL of a suspension of isolates (OD_{600}= 0.5, optical density at 600 nm) previously prepared in Tryptone Salt (TS) were inoculated into 10 mL of HS broth, then the culture broths were incubated at 30 °C for 48 hours. Bacterial growth was determined by reading the turbidity in the culture broth with a spectrophotometer at 600 nm.

2.3.3. Influence of temperature on isolates growth

100 µL of a bacteria suspension of DO_{600} = 0.5 previously prepared in Tryptone Salt (TS) were inoculated into each tube. Then the tubes were incubated for 48 hours in an aerobic environment at different temperatures (30 °C, 35 °C, 40 °C and 45 °C). Bacterial growth was determined by reading turbidity in the culture broth using a spectrophotometer at 600 nm.

2.3.4. Influence of pH on isolates growth

This method is based on the ability of isolates to resist pH variations. Evaluation of pH resistance allows the selection of isolates capable of resisting the influence of pH. Eight hundred (800) mL of the HS broth was prepared and divided

into 8 vials due to 100 mL per vial. Sterilization to 121°C and cooling to 45°C were performed and ethanol was added at a final concentration of 4% in each 100 mL vial. This influence was achieved but the HS broth was adjusted to different pH: 2, 3, 4, 5, 7, 8, 10 and 12.

2.3.5. Influence of glucose on isolates growth

The principle of this method is based on the ability of isolates to resist osmotic stress. The osmotic stress resistance evaluation allows to select isolates capable of growing under the influence of a glucose concentration. Six hundred (600) mL of the HS broth was prepared and divided into 5 vials at 100 mL per vial. Sterilization to 121°C and cooling to 45°C is performed. Each vial containing the medium is poured into test tubes at a rate of 10 mL per tube. This influence was performed as described above but the HS broth was adjusted to the following different glucose concentrations: 0%, 4%, 6%, 8%, 10% and 12%.

2.3.6. Acetic acid production, growth kinetics and pH evolution of isolates in liquid medium

The acidity produced by selected strains was quantified. This assay was done by titration with sodium hydroxide solution, 0.1 N (NaOH 0.1 N) and phenolphthalein as a color indicator (Nanda *et al.* [24]). For this, the HS medium was used. A 24h bacterial culture of all selected isolate was previously carried out. Then, for each of them, 10 mL of culture medium was introduced into a test tube and inoculated with 100 µL of the different suspensions. Incubation was carried out under agitation at 160 tr/min. Every day, acetic acid production was quantified until constant values are observed while growth was determined by measuring the optical density (OD) at 600nm and pH evolution using pH meter. To determine the amount of acid produced, 3 drops of phenolphthalein were added to a beaker containing 2ml of culture medium. A volumetric dosage with NaOH 0.1 N contained in a burette, made it possible to quantify the production of acetic acid. Acetic acid production (AAP) per liter is given by the following relation:

$$AAP = (N \times V_b \times 1000 \times M) / Va$$

Va: Test volume (mL); N = 0.1 : NAOH Normality ; Vb: NAOH Volume (mL); M: Acetic acid molar mass

2.4. Data processing

The analysis of variances (ANOVA) followed by the Tukey test with a significance level of 5% was performed using the XLSTAT software version 2016. This software allowed to calculate the means, the distances of the microbiological parameters.

3. Results

3.1. Physico-chemical parameters

The physico-chemical parameters studied are pH, moisture content, dry matter, alcohol content, vitamin C content and soluble dry extract. The results are recorded in table 1.

Table 1 Physico-chemical parameters of *Diospyros mespiliformis* fruits

Parameters	Values
Ph	2,85 ± 0,01
Moisture content (%)	74,97 ± 0,86 %
Titratable acidity (meq/100 g)	3,38 ± 0
Dry matter (%)	25,03 ± 0,86 %
Alcohol content (%)	2,5 ± 0
Vitamin C content (mg/100 g)	5,06 ± 2,16
Soluble dry extract (°B)	5 ± 0

3.2. Acetic acid bacteria isolated and identified

3.2.1. Isolated bacteria

Macroscopic and microscopic observations allowed the selection of acetic bacteria. Acetic bacteria are small, flat colonies with smooth contours of beige color. They are also short bacilli. The biochemical tests carried out showed short Gram-negative bacilli, strict aerobic, positive catalase and negative oxidase (Figure 2). A total of thirty-two (32) strains were isolated and named with codes ranging from BAD1 to BAD32.

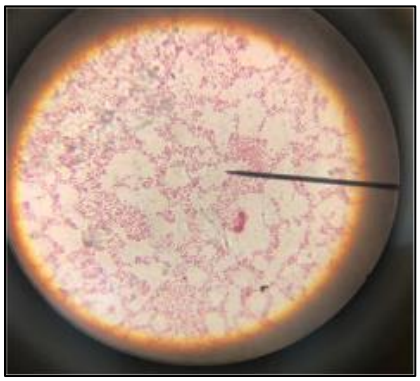

Figure 2 Microscopic observation of acetic acid bacteria isolated

3.2.2. Acetic acid bacteria groups identified

The group distinction test was to identify, among the 32 acetic bacteria isolates, those capable of using acetic acid as a carbon source. Thus, the culture media were prepared with this acetic acid as the sole carbon source. Results showed a yellow to green turn or no turn of strains (Figure 3). Of the 32 isolates tested, 23 (71.87%) showed a yellow to green turn indicating that these isolates have the ability to degrade acetic acid. However, 9 isolates (28.13%) could not degrade acetic acid. Isolates capable of degrading acetic acid belong to the Acetobacter group and those that do not have this ability belong to the Gluconobacter group.

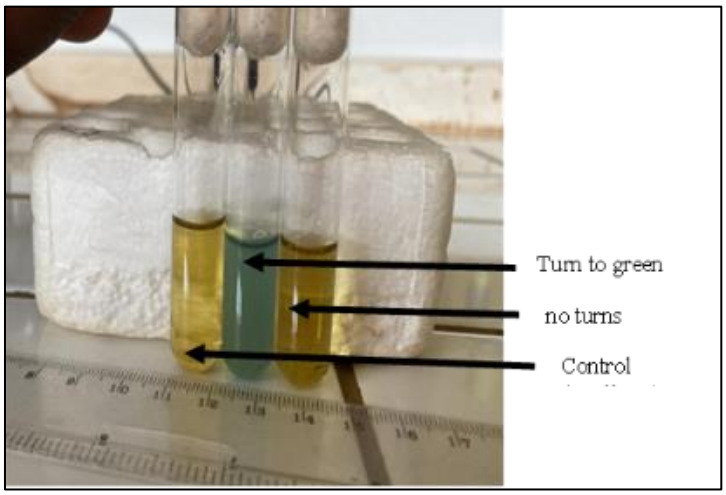

Figure 3 Tubes with or without a turn of the color indicator

3.3. Potential technological properties of isolated acetic bacteria

3.3.1. Acidification ability in solid medium of isolated acetic bacteria

A total of thirty-two (32) isolates were screened. Results show that 24 isolates (75%) were able to acidify the medium and that 8 isolates (25%) were not able to acidify the medium. The isolates with the ability to acidify the medium show transparent halos around spots and those without the ability to acidify the medium did not do it (Figure 4).

Depending on the diameter of measured halos, acidifying isolates can be classified into different groups:

- High acidifiers with diameters ranging from 20 to 29 mm (39.14%);
- Medium acidifiers with diameters ranging from 11 to 19 mm (30.43%);
- Low acidifiers with a diameter ranging from 3 to 9 mm (30.43 %).

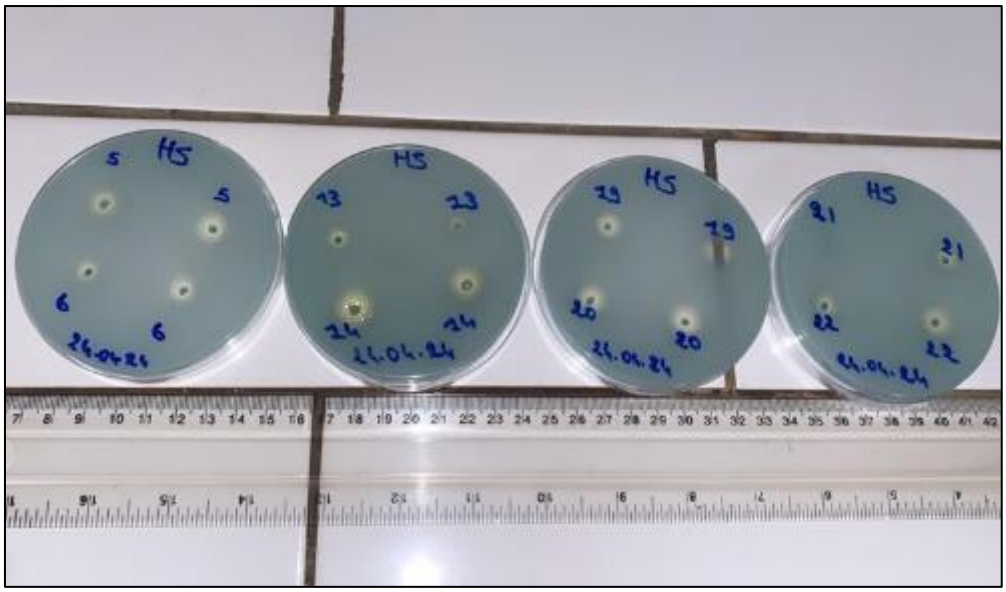

Figure 4 Transparent halos of acidifying isolates

3.3.2. Ethanol resistance of isolates

The ethanol resistance study assessed the ability of 9 isolates with best acidification to tolerate high alcohol concentrations (Figure 5). Thus, the culture media were prepared with varying proportions of ethanol (4%, 8%, 10%, 12%). In general, ethanol variation influenced the growth of individual isolates. All isolates had their best growth when the medium contained 4% ethanol. Above 4%, a decrease in growth is observed for all isolates studied. At 4% ethanol, isolate BAD5 had the best growth with OD_{600} = 0.987 while isolate BAD23 had the lowest growth with a OD_{600} = 0.658. At 12%, most isolates decrease until they cancel.

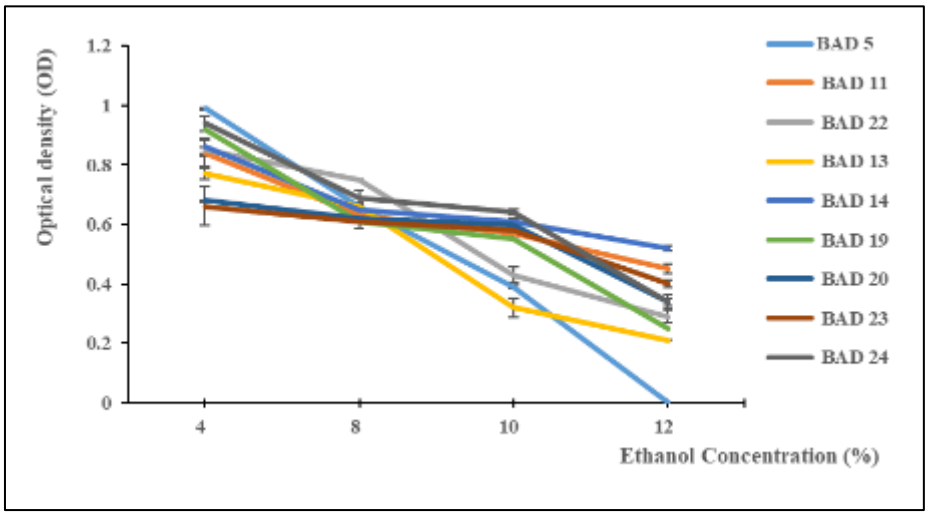

Figure 5 Ethanol influence on isolates growth

3.3.3. Temperature influence on isolates growth

Figure 6 shows the influence of temperature on bacteria growth. The selected bacteria had growth that varied at different temperatures. In general, the growth of all isolates studied decreases as temperature increases. It should be noted that all isolates had their best growth at 30°C. At this temperature, isolate BAD24 had the best growth (OD_{600} =

0.983) and isolate BAD20 had the lowest growth (OD_{600} = 0.565). At 45°C, growth ranged from 0.055 to 0.524 and isolate BAD13 was the most resistant with OD_{600} = 0.524 and isolate BAD11 was the least resistant (OD_{600} = 0.055).

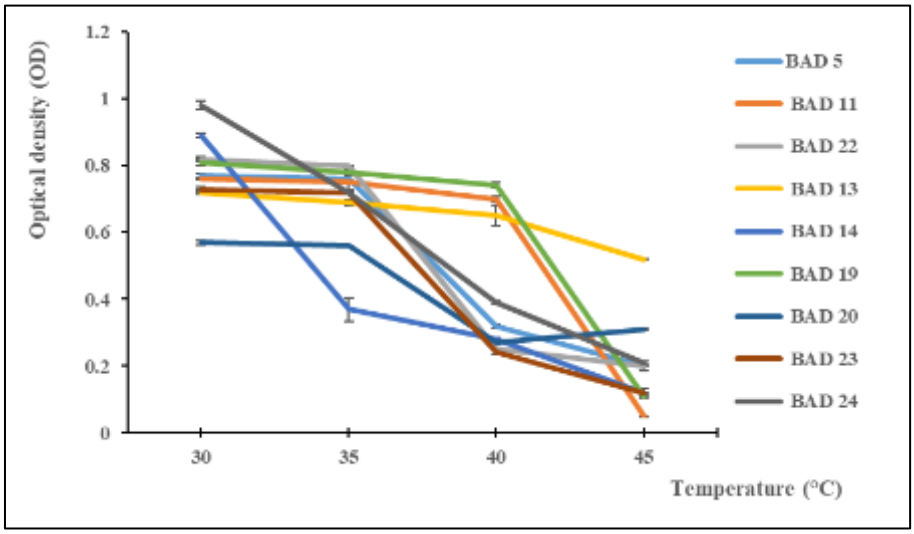

Figure 6 Temperature influence on isolates growth

3.3.4. pH influence on isolates growth

Growth of selected bacteria varies with the pH concentration in the medium (Figure 7). It should be noted that for the nine (9) isolates studied, growth increases to a peak above which it decreases. Thus, growth peaks varied from one isolate to another. Most isolates, eight in total had their growth peak at pH3 while isolate BAD20 had its growth peak at pH4. For growth values of all isolates tends to cancel.

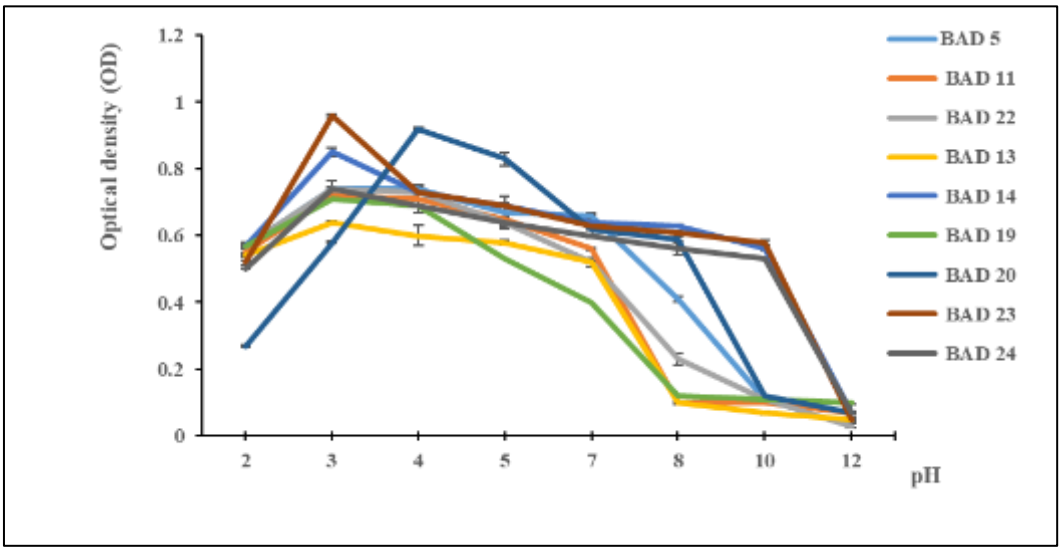

Figure 7 pH influence on isolates growth

3.3.5. Glucose influence on isolates growth

The selected isolates showed good growth at different glucose concentrations (Figure 8). The growth of all isolates studied follows a Gaussian curve with a growth peak when the medium contains 4% glucose. Above 4% glucose in the medium, growth stabilizes slightly for eight (8) isolates (BAD5, BAD11, BAD13, BAD14, BAD19, BAD20, BAD23, BAD24) before dropping. Isolate BAD22, which had the best growth at 4% glucose (OD_{600} = 0.958), has a lower growth rate above this glucose concentration but still remains higher than the other eight (8) isolates. At a concentration of 8% glucose, the growth of isolate BAD22 is reversed while that of isolate BAD13 is reversed with 10% glucose in the medium. Growth of isolates BAD20 and BAD23 was nil at 12% glucose. Only isolate BAD14 had growth at 12% glucose (OD_{600} = 0.191).

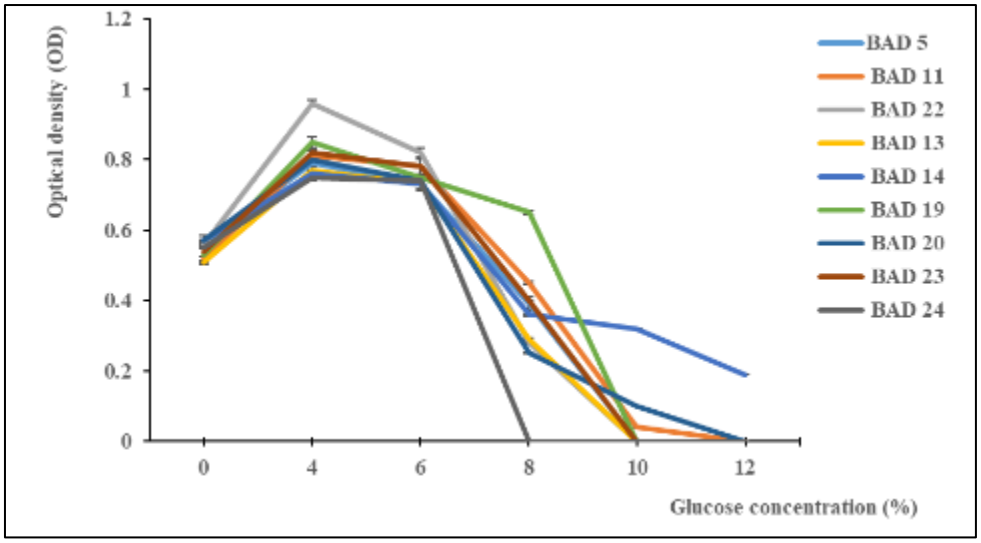

Figure 8 Glucose influence on isolates growth

3.3.6. Acetic acid influence on isolates growth

Figure 9 shows the influence of acetic acid on bacteria growth. All isolates studied had their best growth when the medium did not contain acetic acid. However, given the pace of the different curves, acetic acid has little effect on growth in the isolates studied (OD_{600} greater than 0.5 for all isolates). With an acetic acid concentration of 4% in the medium, isolate BAD22 (OD_{600} = 0.547) had the best growth while the other isolates have similar growth.

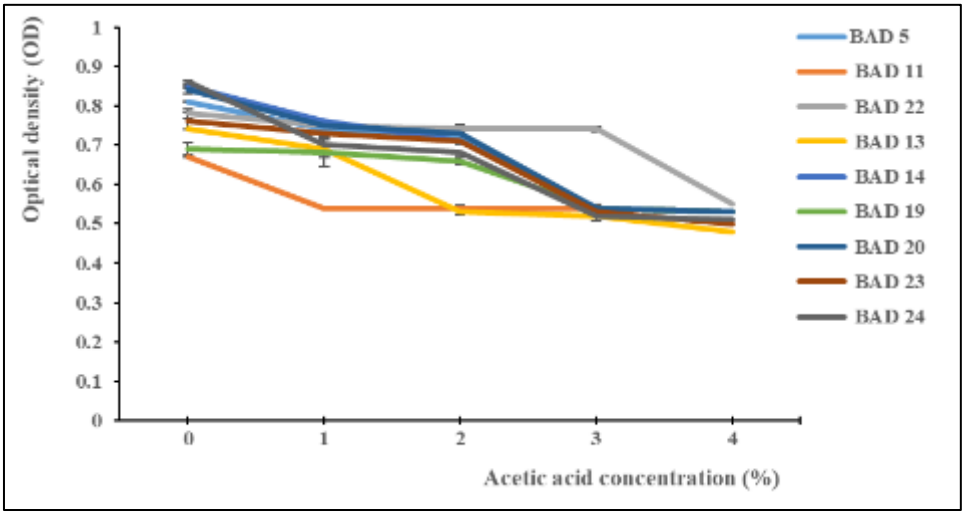

Figure 9 Acetic acid influence on isolates growth

3.3.7. Acetic acid production kinetics of selected isolates

The curves obtained show a similar pace. From D1 to D2, acetic acid production increases regularly before reaching its peak at D2. From D2 to D3, production is constant. From D3 to D4, production drops. Beyond D4, acetic acid production is stable towards the end. BAD24 is the best producer (Figure 10).

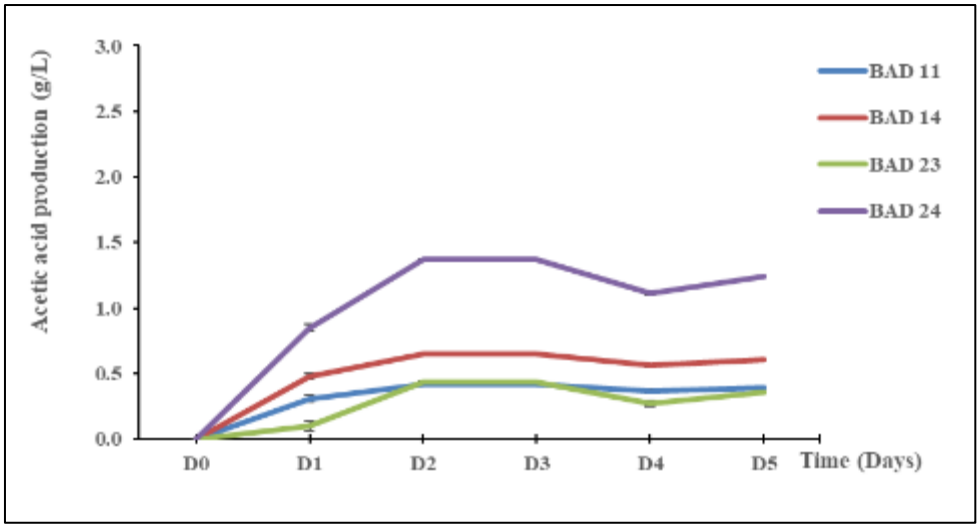

Figure 10 Acetic acid production kinetics of isolates

3.3.8. Growth kinetics of selected isolates during acetic acid production

In terms of growth, the isolates also show a similar pace. From D0 to D4, growth increases steadily until it peaks on D4. Beyond D4, it is constant (Figure 11).

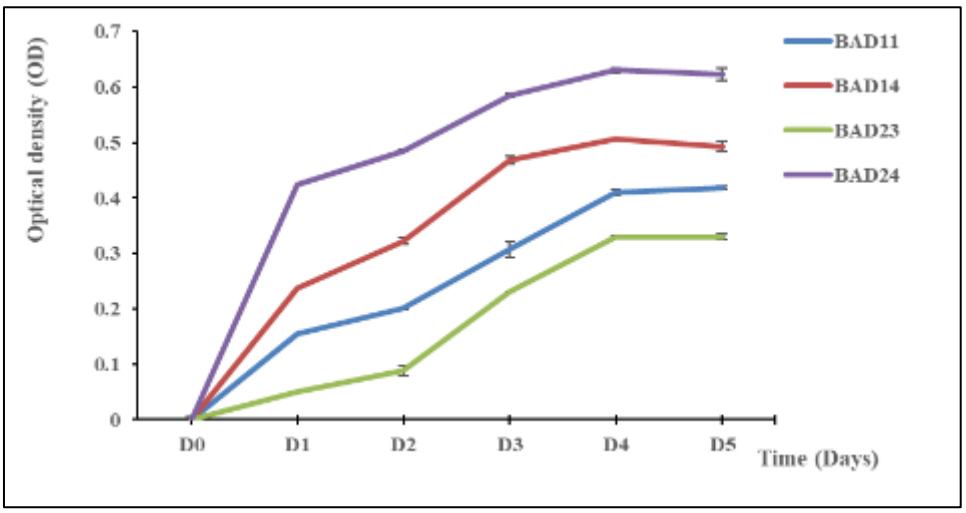

Figure 11 Growth kinetics of isolates during acetic acid production

3.3.9. Change in pH of the medium during acid production

As regards the change in pH, the curves obtained show a similar pace. From D0 to D2, growth increases until it peaks on D2. Then it decreases until D3 before staying constant (Figure 12).

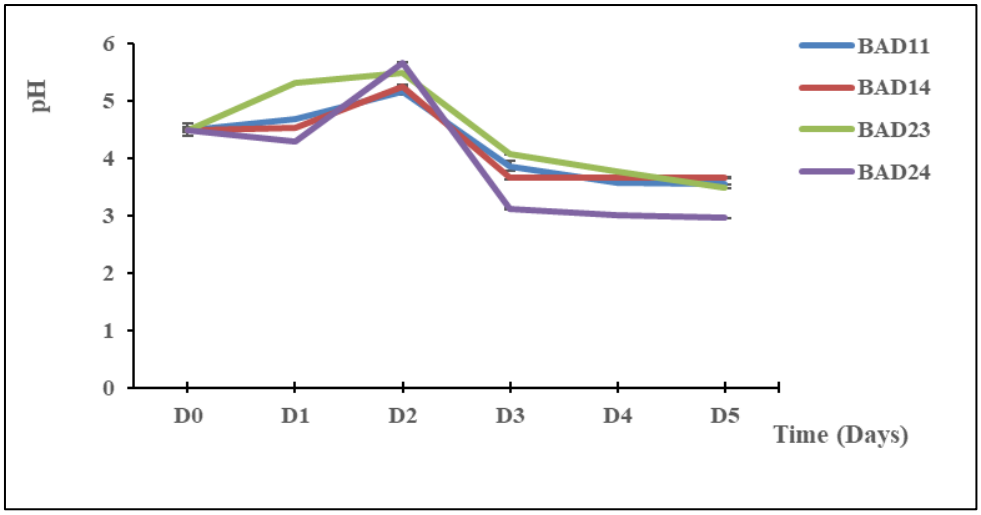

Figure 12 pH evolution during acetic acid production

4. Discussion

This study determined the physico-chemical parameters of *Diospyros mespiliformis* fruits. It shows that the acidity of fruits is relatively high, with a pH of 2.85 ± 0.01. The titratable acidity of 3.38 meq/100 g is an important measure of the total acid composition of the fruit, influencing its taste and preservation properties. These results are consistent with Lee *et al*. [20]. Acidic pH is characteristic of many tropical fruits and plays a crucial role in their conservation by inhibiting the growth of many pathogenic microorganisms, this is in line with the results of Gould [15] which compares it with other tropical fruits, thus revealing similar values, reinforcing the idea that this acidity contributes to the stability and shelf life of the fruit. The moisture content of 74,97 ± 0,86 % indicates that *Diospyros mespiliformis* is a very juicy fruit. These results are consistent with those of Smith [31], which indicate that *Diospyros mespiliformis* is similar to other tropical fruits such as papaya and mango Alcohol content is 2.5%. These results are consistent with those of Williams [35] who claim that this could be the result of natural fermentation of the sugars in the fruit.

This spontaneous fermentation is common in tropical fruits rich in sugar when storage conditions are inadequate. *Diospyros mespiliformis* has a vitamin C content of 5.06 ± 2.16 mg/100 g. These results are different from those of Duncan [8] who worked on citrus. Soluble dry extract, measured at 5°B, this is consistent with the work of Johnson [18] which indicates that such a significant concentration of soluble sugars contributes to the sweet taste of the fruit. The group distinction test identified two genera (Acetobacter, 71.87% and Gluconobacter, 28.13%). Acetobacter are known to oxidize ethanol into acetic acid and continue this oxidation to use acetic acid as a carbon source (Sievers [30]). This ability is due to the presence of specific enzymes, such as aldehyde dehydrogenase and acetaldehyde dehydrogenase, which facilitate the breakdown of acetic acid (Gullo and Giudici [16]). Gluconobacter, on the other hand, have a limited ability to use acetic acid because they lack some key enzymes needed for this degradation (Kersters [19]). Regarding acetic acid productions in liquid medium, the four strains that proved to be the best had their highest productions ranging from 0.421 g/L for strain BAD11 to 1.374 g/L for strain BAD24 after two days of incubation. These values then decrease until the fifth day before stabilizing. This is due to the fact that these four strains are of the genus Acetobacter, capable of oxidizing the acetic acid they produce into CO_2 and H_2O when the medium becomes low in alcohol (Sievers [30]).

The influence of ethanol on isolate growth shows that ethanol has a significant influence on isolate growth. At 4% ethanol, the majority of isolates had their best growth, with optical densities (OD_{600}) ranging from 0.658 to 0.987. Isolate BAD5 showed the best growth (OD_{600} = 0.987), indicating a high tolerance to this ethanol concentration. In contrast, isolate BAD23 showed the lowest growth (OD_{600} = 0.658). These results are consistent with previous studies by Gullo and Giudici [16] and Trcek and Teuber [33] which have shown that acetic bacteria can tolerate moderate concentrations of ethanol and often show optimal growth at concentrations around 4-6%. At higher concentrations (8%, 10% and 12%), growth of isolates generally decreases due to the inhibitory effects of ethanol on cell membranes and bacterial enzymes (Raspor and Goranovic [28]). For example, at 12% ethanol, growth of isolates was very limited, with OD ranging from 0 to 0.399. Isolate BAD23 showed the best growth (OD_{600} = 0.399), while isolate BAD5 showed no growth (OD_{600} = 0), indicating a total inability to tolerate this high ethanol concentration. This observation is consistent with

Williams [36] reporting that very few acetic bacteria can survive at such high levels of ethanol, often due to the toxicity of ethanol at these levels.

At 30°C, all isolates reached optimal growth with OD_{600} values ranging from 0.565 to 0.983. Isolates BAD24 and BAD14 showed their best growth at this temperature, with BAD24 reaching a DO_{600} of 0.983, indicating optimal adaptation to this thermal condition. Sievers [30] report that 30°C is a favorable temperature for the growth of many acetic bacteria, maximizing their enzymatic activity and fermentation capacity. At higher temperatures (35°C and 40°C), a gradual decrease was observed. At 45°C, growth of isolates was minimal, with ODs_{600} ranging from 0.055 to 0.524. This temperature seems to be beyond the thermal tolerance of the acetic bacteria isolates studied, corroborating the results of Kersters [19] where temperatures above 40°C are often inhibitory for most acetic bacteria. Isolate BAD23 nevertheless showed some residual tolerance at this temperature, with a OD_{600} of 0.524, which could indicate a unique genetic or physiological variation allowing for better heat resistance.

For the influence of pH on the growth of acetic bacteria, isolates showed a significant influence, with marked variations between isolates as a function of the pH of the medium. The results showed that the majority of isolates achieved their best growth at pH 3, underlining the importance of acidity for these microorganisms. These results are consistent with the work of Sharafi [29] which showed that acetic bacteria prefer acidic environments for optimal growth. At pH 3, the optical density (OD_{600}) values ranged from 0.605 to 0.920, with BAD20 being the highest value. On the other hand, at higher pH (8%, 10% and 12%), growth gradually decreases due to the fact that acetic bacteria in general are not optimized for highly basic environments. These observations are consistent with the findings of Ganzle *et al*. [13], which show that the ability of acetic bacteria to grow at high pH levels is often limited by protein and enzyme denaturation.

For the influence of glucose, all isolates reached their best growth at 4% glucose with OD_{600} ranging from 0.753 to 0.958. Isolate BAD22 showed the best growth (OD_{600} = 0.958), while isolate BAD24 showed the lowest growth at this concentration (OD_{600} = 0.753). These results are consistent with previous studies that demonstrated that moderate levels of glucose promote optimal growth of acetic bacteria by providing an easily metabolized energy source (Sievers [30]). However, with increasing glucose concentrations (6%, 8%, 10% and 12%) the growth of isolates decreased. These results are in agreement with those of Gullo and Giudici [16], who reported that high levels of glucose can inhibit the growth of acetic bacteria due to the formation of toxic metabolic products.

The influence of acetic acid concentration on growth of acetic bacteria strains shows that all isolates reached their optimal growth in the absence of acetic acid (0% acetic acid). At 1% acetic acid, growth of isolates decreased, with OD_{600} ranging from 0.538 to 0.757. Isolate BAD14 showed the best growth (OD_{600} = 0.757), while isolate BAD11 showed the lowest growth (OD_{600} = 0.538). At this concentration, Sievers [30] indicated that acetic acid begins to have an inhibitory effect on bacterial growth, but some strains still show significant tolerance. At 2% acetic acid, growth of isolates ranged from 0.534 to 0.742. Isolate BAD22 showed the best growth (OD_{600} = 0.742), while isolate BAD13 showed the lowest growth (OD_{600} = 0.534). These results are in agreement with those of Trcek and Teuber [33], which indicate that some isolates can tolerate moderate concentrations of acetic acid, which could be beneficial for fermentation processes requiring higher levels of this compound. At 3% acetic acid, growth of isolates further decreased from 0.515 to 0.735 with isolate BAD22 showing the best growth (OD_{600} = 0.735). At 4%, growth ranged from 0.481 to 0.547, with isolate BAD22 still showing the best growth (OD_{600} = 0.547) and isolate BAD13 the weakest (OD_{600} = 0.481). These acetic acid concentrations appear to exceed the optimal tolerance of many isolates, exerting a significant inhibitory effect on their growth, states Kersters [19].

The kinetic analysis of acetic acid production in liquid shows variations in the production dynamics among isolates. The growth of isolates is due to the fact that the isolates adapt to the environment and use the available substrates efficiently. These results are consistent with those of Gänzle [12] who claim that acetic bacteria can grow rapidly when in a nutrient-rich environment. The decrease in acetic acid production in liquid may be explained by the fact that isolates are less effective due to factors such as slower growth rate, increased sensitivity to inhibitory products (Trcek *et al*., [33]).

5. Conclusion

At the end of this study, it should be recalled that the general objective is to promote the fruit *Diospyros mespiliformis.* Thus, the results of physico-chemical analyses showed that the pH of the fruit is acidic (2.85) with a moisture content of 74.97 %, a high vitamin C content (5.06 mg/100 g) and an extract soluble (5°B). Furthermore, microbiological analyses yielded a population of acetic bacteria of 2.73.105 CFU/g. Indeed, the isolation of acetic bacteria allowed to list thirty-two (32) pure isolates belonging to the genus Acetobacter (71.87%) and the genus Gluconobacter (28,13%). Of these 32 isolates, 9 showed high acetic acid production in solid medium. The different influence tests allowed 4 isolates

(BAD11, BAD 14, BAD 23 and BAD24) identified as Acetobacter sp. to be selected from the 32 isolates as possessing high technological properties.

Compliance with ethical standards

No conflict of interest to be disclosed.

References

[1] Adjrah Y, Karou DS, Djéri B, Anani K, Soncy Ameyapoh Y, De Souza C, Gheassor M. Hygienic quality of commonly consumed vegetables, and perception about disinfecting agents in Lomé. International Food Research Journal. 2011; 18(4):1499-1503.

[2] AOAC. Official methods of analysis. Association of Official Analytical Chemists**.** Ed., Washington DC. 1990. p. 684.

[3] Arbonnier M. Trees, shrubs and lianas of dry areas in West Africa. CIRAD (Montpellier) and the National Museum of Natural History (Paris). 2002. p. 574.

[4] Aydin A, Aksoy D. Isolation of cellulose producing bacteria from wastes of vinegar fermentation. Proceedings of the world congress on Engineering and Compunter Science. 2009; 1: 20-22.

[5] Burillard L, Daumas V, Glaz M, Kouyoumdjian L, Lobrot S, Loger N, Maillot N, Marchand C. Food ferments. Final summary report bibliography. 2016. 63 p.

[6] Daanon A, Padonou EA, Akakpo BA, Houinato M. Variability in the uses of *Diospyros mespiliformis* Hochst according to sociodemographic factors in Northern Benin. Tropical Woods and Forests.2021. 347: p. 29-40

[7] Diarra N, Togola A, Denou A, Willcox M, Daou C, Diallo D. Ethnobotanical study of food plants used during the lean season in the southern regions of Mali. International Journal of Biological and Chemical Sciences.2016; 10 (1): 184-197.

[8] Duncan R, Blanc L. Nutritional value of tropical fruits. Nutrition research. 2019. 58. p. 45-52.

[9] El-Kamali HH. *Diospyros mespiliformis* Hochst. Ex A. DC. Record from PROTA4U. Lemmens, R.H.M.J. Plant Resources of Tropical Africa. 2011. 23. p. 45-48.

[10] FAO. Promotion of Fruit and Vegetables for Heathl. Human nutrition meetings Oceania nutrition education fruit vegetables public health. Rome, Italy. 2015. p. 94.

[11] FAO. Fruits and vegetables for health. Report of the joint investigator FAO/OMS, Japon. 2020. p. 52.

[12] Gänzle MG. Lactic Acid Bacteria and Their Role in Fermented Foods. Microbiology Spectrum. 2015; 4: 1-11.

[13] Gänzle MG, Loponen J, Gobbetti M. Proteolysis in sourdough fermentations: mechanisms and potential for improved bread quality. Trends in Food Science and Technology. 2008; 19: 513–521.

[14] Gao L, Wu X, Zhu C, Jin Z, Wang W, Xia X. Metabolic engineering to improve the biomanufacturing efficiency of acetic acid bacteria: advances and prospects. Critical Reviews in Biotechnology. 2020; 2: 1-17.

[15] Gould H. Acidity and microbial stability of tropical fruits. International Journal of Food Microbiology. 2021; 87: 123-130.

[16] Gullo M, Giudici P. Acetic acid bacteria in traditional balsamic vinegar: functional roles and microbiological properties. International Journal of Food Microbiology. 2018; 125(1): 46-56.

[17] Guy-Alain. Wild edible fruits of the Guinean savannas of Côte d'Ivoire: state of knowledge by a local population, the Malinkés. Biotechnology Agronomy and Sociology. 2001; 5(1): 43-58.

[18] Johnson P. Sugar content in tropical fruits: Comparative analysis. International Journal of Food Properties. 2019; 22: 120-130.

[19] Kersters K. The genus Gluconobacter and its applications. Applied microbiology and biotechnology. 2021; 104: 5329-5343.

[20] Lee J, Lee Y. Does online Shopping Make Consumers Feel Better? Exploring Online Retail Therapy Effects on Consumers' Attitudes towards Online Shopping Malls. Asia Pacific Journal of Marketing and Logistics. 2019; 31: 464-479.

[21] Lynch KM, Emanuele Z, Stuart W, Luk D, Elke KA. Physiology of Acetic Acid Bacteria and Their Role in Vinegar and Fermented Beverages. Comprehensive Reviews in Food Science and Food Safety. 2019; 18(3): 587-625.

[22] Mamadou TSD, Mahmoud C, Mastan YD, Alpha SB, André MP, Lanciné T. Towards a fight against *citrus cercosporiosis* in Guinea. Fruits. 2003; 58:p. 329-343.

[23] Naloka K, Yukphan P, Matsutani M, Matsushita K, Theeragool G. Komagataeibacter Diospyros sp. nov., a novel species of thermotolerant bacterial nanocellulose-producing bacterium. International Journal of Systematic and Evolutionary Microbiology. 2020; 70: 251- 258.

[24] Nanda K, Taniguchi M, Ujike S, Ishihara N, Mori H, Ono H, Murooka Y. Characterization of acetic acid bacteria in traditional acetic acid fermentation of rice vinegar (komesu) and unpolished rice vinegar (kurosu) produced in Japan. Applied and Environmental Microbiology Journal. 2001; 67: 986-990.

[25] Oibiokpa FI, Adoga GI, Saidu AN, Shittu O. Nutritional composition of *Detarium microcarpum* fruit. African Journal of Food Science. 2014; 8: 342-350.

[26] Pelletier O. Vitamin C, (L-Ascorbic and Dehydro-L-Ascorbic Acids), Methods of Vitamin Assa, (Wiley, New york). 1985; 303-347.

[27] Pollard C, Miller M, Woodman RJ, Meng R, Binns C. Changes in Knowledge, Beliefs, and Behaviors Related to Fruit and Vegetable Consumption among Western Australia Adults from 1995 to 2004. American Journal of Public Health. 2009; 99 (22): 35-61.

[28] Raspor P, Goranovic D. Biotechnological applications of acetic acid bacteria. Critical examinations in biotechnology. 2018; 29: 101-124.

[29] Sharafi H, Rasooli I, Beheshti-Maal K. Isolation characterization optimization of indigenous acetic acid bacteria and evolution of their preservation methods. Iranian Journal of Microbiology. 2010; 2: 41-48.

[30] Sievers M. Metabolic pathways and regulations in Acetobacter species. Journal of Industrial Microbiology and Biotechnology. 2020; 5: 43-47.

[31] Smith T. Water content and texture of tropical fruits. Food quality and preferences. 2020; 34: 77-85.

[32] Soumahoro S, Ouattara HG, Goualié BG, Koua G, Doue G, Niamke SL. Occurrence of High Acetic Acid-Producing Bacteria in Ivorian Cocoa Fermentation and Analysis of Their Response to Fermentative Stress. American Journal of Bioscience. 2015; 3(3): 70-79.

[33] Torija MJ , Vegas C , Mateo E , González Á , Jara C , Guillamón JM , Poblet M, Mas A. Population dynamics of acetic acid bacteria during traditional wine vinegar production. International Journal of Food Microbiology**.** 2010; 138, 130-136.

[34] Trcek J, Teuber M. The biochemistry of acetic acid bacteria. Journal of food science. 2020; 83: 563-573.

[35] Virgine P. The mango in Côte d'Ivoire. Country profile producer, fruitrop. 2018. 255. p. 78-83.

[36] Williams R. Fermentation of tropical fruits and impact on alcohol content. Journal of food science. 2018; 85: 567-576.